The Discovery of Small-Molecule Inhibitors of ERK5 for the Treatment of Cancer

Lauren Molyneux

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Declaration

The work contributing to this thesis was conducted between October 2009 and September 2013 in the Medicinal Chemistry laboratories, Bedson Building, Northern Institute for Cancer Research at the Newcastle Cancer Centre, Newcastle University, Newcastle upon Tyne, NE1 7RU. The research was conducted in collaboration with scientists at Cancer Research Technology Discovery Laboratories, The Cruciform Building, Gower Street, London, WC1E 6BT, the Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow, G61 1BD and the Babraham Institute, Babraham Research Campus, Cambridge, CB22 3AT.

All of the research presented in this thesis is original in context, and does not include any material or ideas previously published or presented by other authors except where due reference is given in the text. Any figures included without reference were generated using Microscoft Powerpoint and were drawn from knowledge .

No part of this thesis has been previously submitted for a degree, diploma or any qualification at any other university.

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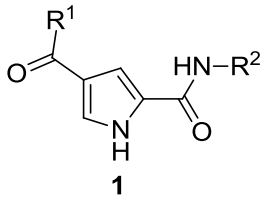
Abstract

Mitogen-activated protein kinases (MAPKs) play an essential role in the transduction of extracellular signals to cytoplasmic and nuclear effectors. MAP kinase kinases (MEKs/MAPKKs) represent protein kinases upstream of MAPKs, critically controlling cellular proliferation and apoptosis. Mitogen/extracellular signal regulated kinase kinase-5 (MEK5) specifically activates extracellular signal-rlated kinase-5 (ERK5), also known as Big MAP kinase 1 (BMK1). Studies conducted in Newcastle have shown that abnormal MEK5/ERK5 signalling is present in certain types of prostate cancer and correlates with shorter disease-specific survival. ERK5 is over-expressed in 20% of breast cancers and is also thought to promote cell growth in hepatocellular carcinoma (HCC) by regulating mitotic entry. In addition, ERK5 has been shown to interact with, and inactivate promyleocytic leukaemia (PML) protein. This prevents activation of its downstream effector, tumour suppressor p21. An ERK5 inhibitor based on a series of isoform selective polo kinase inhibitors was reported in the literature. XMD8-92 displayed selectivity for ERK5 when tested against a panel of 402 kinases in an ATP-site competition binding assay (ERK5; IC_{50} = 300 nM) and the compound showed reasonable activity in HeLa cells (GI_{50} = 1.5 µM). XMD8-92 was also shown to inhibit growth in two human tumour xenograft models (HeLa cells and Lewis lung cells), highlighting that inhibition of ERK5 is a valid target for anti-cancer therapy. Encouragingly, both *in vitro* and *in vivo* activities of XMD8-92 have been tested and confirmed in our assays.

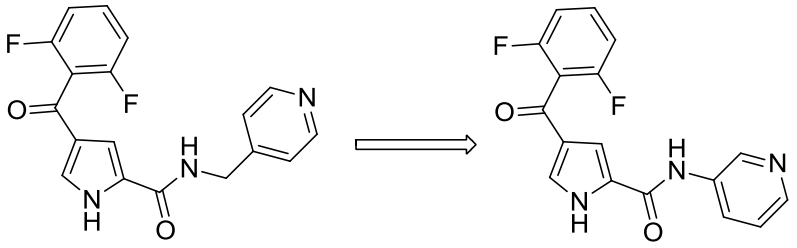
A high throughput screening (HTS) campaign was conducted using an immobilised metal affinity polarisation (IMAP^{TM}) format assay at Cancer Research Technology-Discovery Laboratories (CRT-DL), identifying three distinct chemical series for further investigation. A pyrrole carboxamide series (**1**) has undergone hit validation by re-synthesis and re-testing of the original HTS hits along with synthesis of closely related series.



1

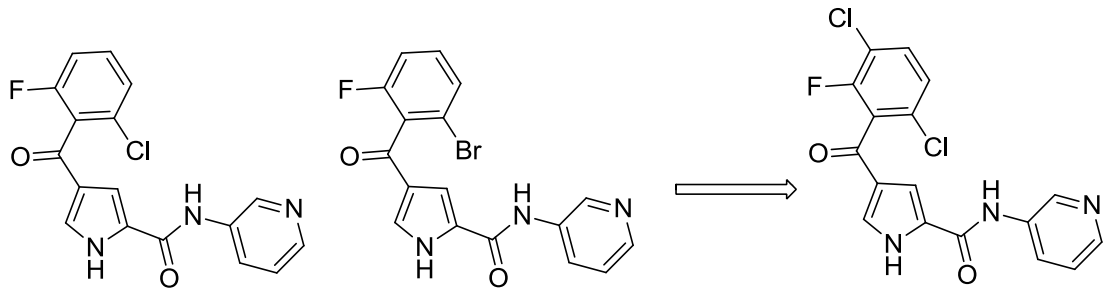
Initial hit-to-lead studies focussed on modification of R^1 in structure **1**, and the best activity against ERK5 was observed when R^1 is an aromatic ring disubstituted with a

halogen at the 2,3- or 2,6-positions (e.g. **2**, ERK5; IC_{50} = 2.3 μM), with the R^2 substituent being a pyridyl moiety linked to the amide *via* a methylene group. Further structure-activity relationship studies (SARs) revealed that removal of the methylene group led to an increase in potency (**3**, ERK5; IC_{50} = 1.1 μM) and more importantly, in more than 100-fold selectivity for ERK5 over the closely related MAPK, p38α.



2 **3**

Introduction of a different halogen atom into the 2-position of the aromatic ring conferred good potency and retained selectivity for ERK5 over p38α (e.g. **4**, ERK5; IC_{50} = 0.6 μM and **5**, ERK5; IC_{50} = 0.8 μM). Inhibitor **5** was selected for *in vitro* and *in vivo* pharmacokinetic (PK) studies with promising results [*in vitro* PK; solubility >100 μM, PPB = 94% and *in vivo* PK; oral bioavailability = 68% and $t_{1/2}$ = 65 min (p.o. mice)]. More recently, introduction of a 2,3,6-tri-substituted aromatic ring led to an increase in potency against ERK5, identifying the first compound with an ERK5 IC_{50} <100 nM, **6** (ERK5; IC_{50} = 0.07 μM).



4 **5** **6**

Modification of R^2 has also been investigated in order to improve CYP_{450} inhibition profiles. Synthesis of a series of compounds with various substituted pyridyl, pyrimidyl or pyridazyl rings was conducted. The SARs revealed compounds with a pyrimidyl moiety to be potent inhibitors of ERK5 (IC_{50} <5 μM) with excellent CYP_{450} inhibition profiles when tested against a panel of five CYP_{450} isoforms (IC_{50}>25 μM). Further lead optimisation studies are currently being undertaken in order to find an inhibitor suitable for clinical evaluation.

Abbreviations

Abl	-	Abelson kinase
ADEPT	-	Antibody Directed Enzyme Prodrug Therapy
ADMET	-	Absorption, Distribution, Metabolism, Excretion and Toxicology
ALL	-	Acute lymphoblastic leukaemia
ATP	-	Adenosine triphosphate
bad	-	Bcl-2-associated death promoter
bcl-2	-	B-cell lymphoma 2
BMK1	-	Big MAP kinase 1
Boc	-	*tert*-Butoxycarbonyl
Brk	-	Breast cancer kinase
Caco-2	-	Human epithelial colorectal adenocarcinoma cell line 2
cat.	-	Catalytic
CDI	-	1,1-Carbonyldiimidazole
CDK	-	Cyclin dependent kinase
cLogP	-	Partition coefficient (calculated)
CML	-	Chronic myelogenous leukaemia
CRT	-	Cancer Research Technology
CREB	-	cAMP response elemene binding protein
CRT-DL	-	Cancer Research Technology – Discovery Laboratories
CTGF	-	Connective tissue growth factor
CYP450	-	Cytochrome P450 enzyme
DCC	-	*N,N'*-Dicyclohexylcarbodiimide
DCM	-	Dichloromethane
DHT	-	Dihydrotestosterone
DMF	-	Dimethyl formamide
DMPK	-	Drug metabolism and pharmacokinetics
DMSO	-	Dimethyl sulfoxide
DPPA	-	Diphenyl phosphoryl azide
DTT	-	Dithiothreitol

EI	-	Electron Ionisation
EGF	-	Epidermal growth factor
EGFR-TK	-	Epidermal growth factor receptor – tyrosine kinase
EGTA	-	Ethyleneglycol tetraacetic acid
ELSD	-	Evaporative light scattering detection
EMT	-	Epithelial mesenchymal transition
ERK	-	Extracellular signal related kinase
(ES^+)	-	Electron spray positive mode
(ES^-)	-	Electron spray negative mode
FAK	-	Focal adhesion factor kinase
FGF	-	Fibroblast growth factor
FTIR	-	Fourier Transform Infrared
G_1	-	Cell Cycle Gap 1
G_2	-	Cell Cycle Gap 2
GDEPT	-	Gene Directed Enzyme Prodrug Therapy
GIST	-	Gastrointestinal stromal tumour
GnRH	-	Gonadatropin releasing hormone
Grb2	-	Growth factor receptor-bound protein 2
GTP	-	Guanosine triphosphate
HAC	-	Heavy atom count
HBTU	-	O-Benzotriazole-*N*,*N*,*N*',*N*'-tetramethyl-uronium-hexafluoro-phosphate
HCC	-	Hepatocelullar carcinoma
HEK293	-	Human embryonic kidney cell line
HeLa	-	Human cervical cancer cell line
HER2	-	Human epidermal growth factor receptor 2
hERG	-	Human ether-a-go-go related gene
HGF	-	Hepatocyte growth factor
HPLC	-	High Performance Liquid Chromatography
HRMS	-	High resolution mass spectrometry
HSP	-	Heat shock protein
HTS	-	High throughput screen

IC_{50}	-	Concentration of inhibitor required for 50% inhibiton
Id1	-	Inhibitor of differentiation 1
IL	-	Interleukin
Ile	-	Isoleucine
IMAP	-	Immobilised metal affinity polarisation
IP	-	Intraperitoneally
IR	-	Infrared
IV	-	Intravenously
J	-	Coupling constant
JAK	-	Janus activated kinase
JNK	-	c-Jun N-terminal kinases
Ki	-	Inhibition constant
λ_{max}	-	Wavelength for an absorption maximum
Lad	-	Lck-associated adaptor
Lck	-	Lymphocyte-specific protein tyrosine kinase
LC-MS	-	Liquid Chromatography-Mass Spectrometry
LE	-	Ligand efficiency
LHRH	-	Luteinising hormone releasing hormone
LL	-	Lewis lung
MAPK	-	Mitogen activated protein kinase
MAPKK	-	Mitogen activated protein kinase kinase
MAPKKK	-	Mitogen activated protein kinase kinase kinase
MEF2	-	Myocyte enhancer factor 2
MEK	-	Mitogen activated protein kinase kinase
MMP	-	Matrix metalloproteinase
MPLC	-	Medium pressure liquid chromatography
MS	-	Mass Spectrometry
MW	-	Microwave
NBS	-	*N*-Bromosuccinimide
NCS	-	*N*-Cholorosuccinimide

ND	-	Not determined
NFκB	-	Nuclear factor kappa-light-chain-enhancer of activated B-cells
NGF	-	Nerve growth factor
NIS	-	*N*-Iodosuccinimide
NLK	-	Nemo-like kinase
NLS	-	Nuclear localisation sequence
NMR	-	Nuclear Magnetic Resonance
NSCLC	-	Non-small cell lung cancer
PBS	-	Phosphate buffered saline
PC3	-	Prostate cancer cell line 3
PD	-	Pharmacodynamic
P-gp	-	Permeability glycoprotein
PI3K	-	Phosphatidylinositol 3-kinase
PK	-	Pharmacokinetic
PLK	-	Polo-like kinase
PMB	-	*para*-Methoxybenzyl
PML	-	Promyelocytic leukaemia protein
PO	-	Orally
POC	-	Proof of concept
PPB	-	Plasma protein binding
PSA	-	Prostate specific antigen
Raf	-	Rapidly accelerated fibrosarcoma
Ras	-	Rat sarcoma
RCC	-	Renal cell carcinoma
Rf	-	Retention factor
rps6	-	Ribosomal protein S6
RSK	-	Ribosomal s6 kinase
RT	-	Room temperature
S	-	Cell cycle DNA synthesis phase
SAPK	-	Stress activated protein kinase
Sap 1a	-	Secondary ternary complex factor 1a
SAR	-	Structure activity relationship
SGK	-	Serum/glcocoticoid regulated kinase

siRNA	-	Small interfering RNA
SNU449	-	Human hepatocellular carcinoma cell line
SoS	-	Son of sevenless
Stat3	-	Signal transducer and activator of transcription
TAD	-	Transactivation domain
TBAF	-	Tetrabutyl ammonium fluoride
TERT	-	Telomerase breast cancer cell line
TFA	-	Trifluoroactetic acid
TFE	-	Trifluoroethanol
TIPS	-	Triisopropylsilyl
TPSA	-	Topological polar surface area
UPLC	-	Ultra performance liquid chromatography
UV	-	Ultraviolet
Vdss	-	Volume of distribution
VEGF	-	Vascular endothelium growth factor

Contents

Chapter 1. Overview of drug discovery and cancer

1.1 Introduction to drug discovery

The typical stages in drug discovery are summarised in Figure 1, with the process being broadly divided into two stages: preclinical proof of principle and clinical development.[1]

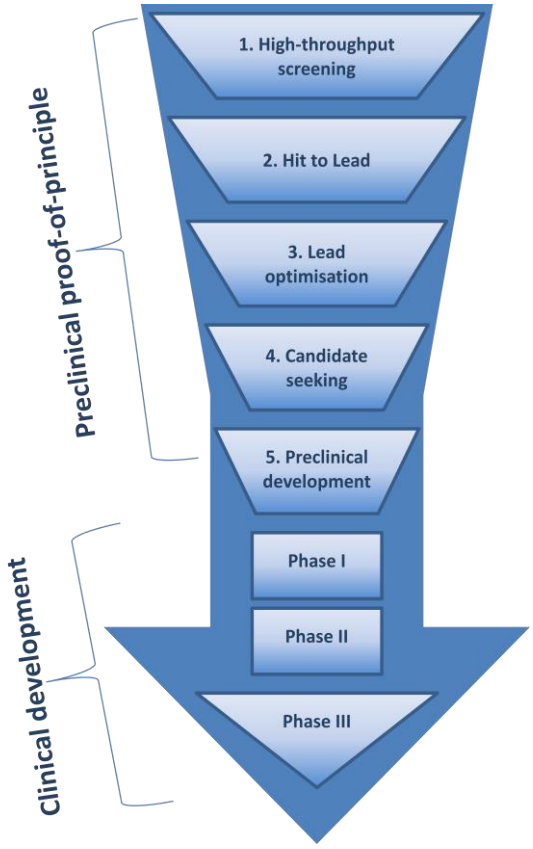


Figure 1. Summary of the drug discovery process.

The preclinical proof of principle phase commonly commences with a high-throughput screening (HTS) initiative whereby a library containing thousands of compounds is screened for activity against the biological target of interest. An IC_{50} value will be determined for each compound using a specifically designed assay and only compounds with moderate to good inhibitory activity or 'hit compounds' will then be taken forward for hit validation *via* re-synthesis and testing. Once validated, hit compounds are progressed to the hit-to-lead stage of the process. By this point, structure-activity relationship studies (SARs) can be undertaken based on potency. Physicochemical property assessment and selectivity studies will also be undertaken during the hit-to-lead phase. The Lipinksi 'rule of five' may also be considered at this stage if it is

essential for the drug to be orally active.[2-3] The 'rule of five' outlines specific rules for physicochemical properties believed to be required for a drug to be orally bioavailable and are: 1. the molecular weight should be below 500 amu, 2. the logP should be below 5, 3. the number of H-bond donors should be below 5, 4. the number of H-bond acceptors should be below 10. If one or more of the criteria are not met, compounds would be expected to have poor absorption and cell permeation and not be orally active.[2] Compounds which comply with Lipinski's rules and are both potent and selective would be tested in a range of *in vitro* ADME (absorption, distribution, metabolism, elimination) studies. Based on the results of these studies, dozens of compounds would be selected for lead optimisation, where the *in vivo* efficacy would be tested using DMPK (drug metabolism and pharmacokinetic) studies. *In vivo* clearance, volume of distribution, bioavailability and half-life determinations can be performed in animal models during the lead optimisation stage. One or two compounds could then be selected as pre-clinical candidates and subsequently assessed for *in vivo* toxicology during pre-clinical development before being progressed to the three phases of clinical trials.

1.2 Introduction to cancer

Cancer is a complex disease that arises due to genetic malfunction. Causes of this malfunction can be endogenous factors, including hormones or metabolism, or exogenous factors such as exposure to carcinogens. Such factors can bring about mutations in either tumour suppressor genes or oncogenes. Once a single mutation remains unchecked, accumulation of further genetic mutations is able to occur, and this cannot be controlled by the usual DNA repair mechanisms. Normal cells undergo a cell cycle process that is tightly regulated, and in which they have a controlled rate of growth, become organised, and are programmed to carry out a specific function. They also die in a predetermined way (apoptosis). In cancer cells, a number of these controls are lost, leading to aberrant cell growth without apoptosis. This results in the proliferation of mutant (malignant) cells that no longer have a specific physiological function, and build up into a tumour.[4]

In 2000, Hanahan and Weinberg proposed six common features observed in the vast majority of cancer types; 1. self-sufficiency, 2. insensitivity to anti-growth signals, 3. evasion of apoptosis, 4. sustained angiogenesis, 5. tissue invasion and metastasis and 6.

sustained angiogenesis. These were termed them 'the Hallmarks of cancer.'[4] In 2011 these 'hallmarks' were updated to include newly emerging capabilities of cancer cells for tumour progression (Figure 2).[5]

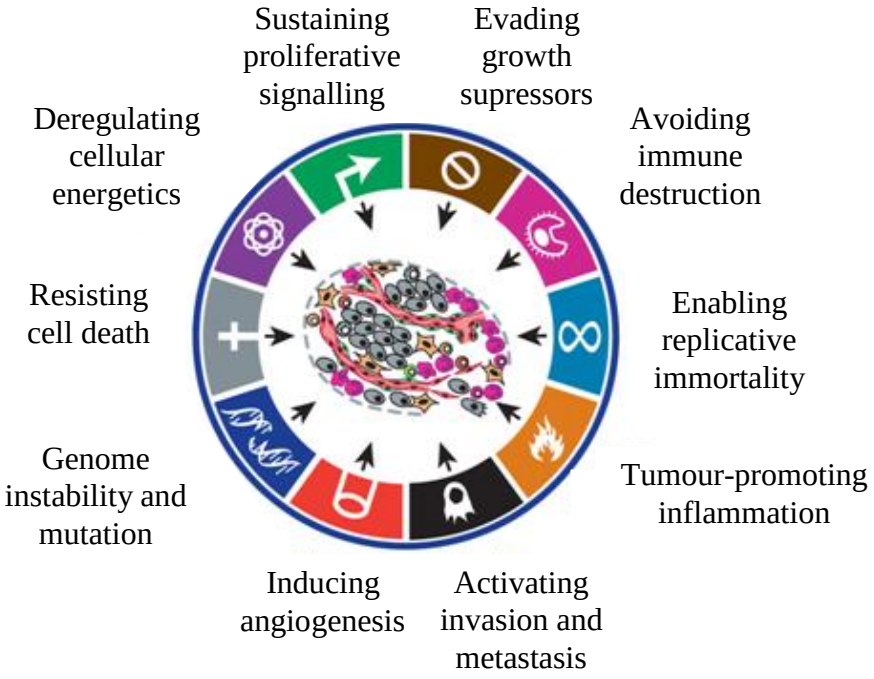


Figure 2. The updated 'Hallmarks of Cancer' Adapted from ref 5.

Each of the characteristics is induced by different mechanisms, depending on the specific type of cancer. These traits are the reason anticancer therapy can be so difficult, due to the vast variation in which cancer can manifest.

Despite the difficulties outlined, significant progress has been made in research into new cancer therapeutics. This is exemplified by recent statistics, which show that the number of people reaching five-year survival has increased. The graph in Figure 3 highlights the five-year survival rates for the most common cancer types in men and women in England between 2005 and 2010. The largest observed increase in five-year survival for males is for prostate cancer, where the percentage of people surviving for five years post-diagnosis has increased by 50% since the early 1970s (from 31% to 81%). For female breast cancer, the percentage of people reaching the five-year survival point has increased by 33% over the same time period (from 52% to 85%).[6]

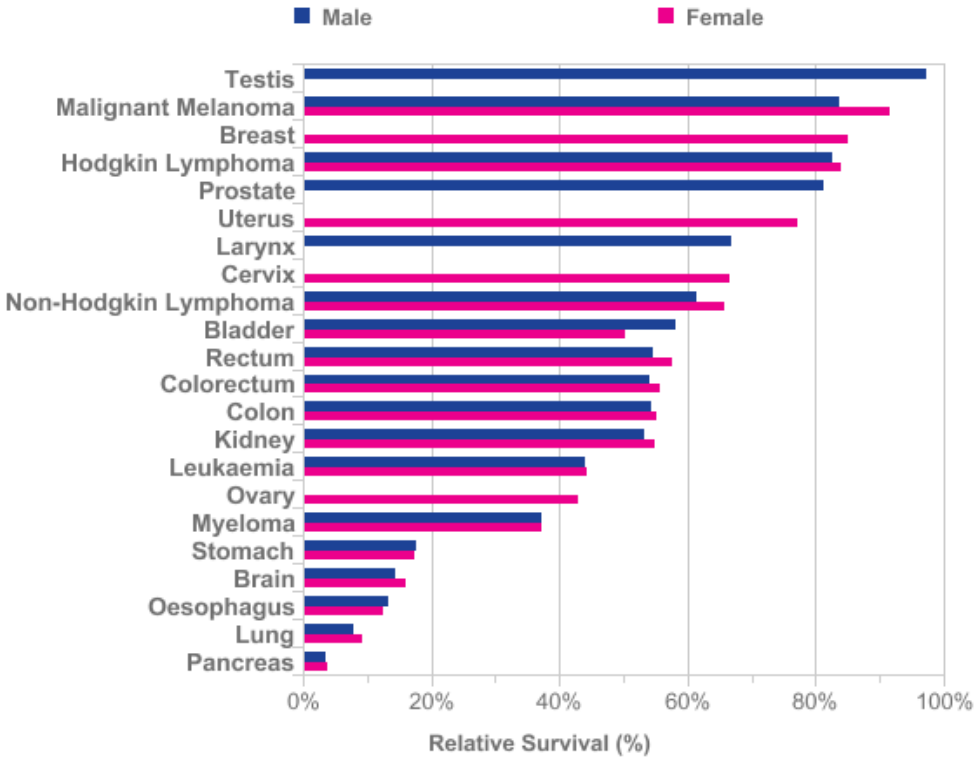


Figure 3. Age-standardised 5-year cancer survival rates for male and female adults (15-99 years) in England.[6]

One reason for the increase in five-year survival rates is the improvement in cancer therapy. This is due in part to a new generation of more specific targeted therapies that have a greater affinity for cancer cells over healthy cells.

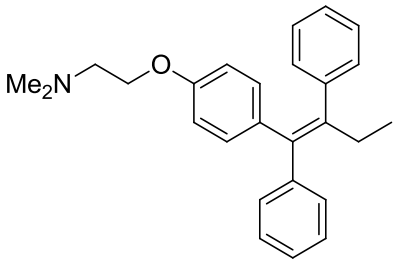
1.3 Targeted cancer therapies

Previous cancer therapies have involved the use of highly toxic chemotherapeutic agents. One example is the alkylating agents, which cause inter- and intra-strand DNA cross links. This results in prevention of DNA strand separation, miscoding of bases, and finally double strand breaks. Platinum complexes, such as cisplatin, also covalently bind to DNA, with similar results. The overall action of such drugs is cytotoxicity, with cellular necrosis and apoptosis being observed. The main problem with classes of drugs such as these is there is no selectivity between healthy cells and cancer cells. This, in turn, results in high levels of toxicity.[7]

Antibody Directed Enzyme Prodrug Therapy (ADEPT) and Gene Directed Enzyme Prodrug Therapy (GDEPT) were among some of the first strategies developed towards

more targeted, less toxic therapies, using either an antibody or a section of DNA along with a prodrug to direct treatment to the site of a tumour.[8]

A major breakthrough was the relationship discovered between hormones and certain cancers, and how this could be exploited to produce drugs to target hormone receptors. This approach was exemplified with the approval of Tamoxifen (**1**) in 1977 for patients with advanced breast cancer, acting as an antagonist of the oestrogen receptor. Tamoxifen is now used as the first-line treatment for advanced breast cancers that are positive for the oestrogen receptor, highlighting the importance of targeted therapies for cancer treatment.[9]



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1.4 Protein kinases and cancer therapy

Protein kinases play an integral role in the move towards more targeted therapies for cancer treatment. They are involved in many cell signalling processes, and are key regulators of processes such as proliferation and invasion. There have been 518 distinct kinases identified, and research has shown that the genes coding for these proteins are often mutated in cancer cells. Protein kinases are structurally related and categorised by their catalytic domains (approximately 250-300 amino acids in size). There are two main sub-divisions; tyrosine kinases and serine/threonine kinases.[10] Protein kinases catalyse the transfer of a terminal γ-phosphate group from ATP to either serine, threonine or tyrosine depending on which sub-division they belong to (Figure 4). These residues form part of a target protein substrate, which may be either activated or inactivated as a result of the phosphorylation. Structural conformation changes in the protein can also occur as a consequence of phosphorylation, and this can change the binding and recognition properties of the protein. These conformational changes can induce a variety of responses within the cell, and stimulate a signal cascade that ceases

on removal of the phosphate group. This dephosphorylation is conducted by a phosphatase enzyme, which returns the protein to its original state.[10]

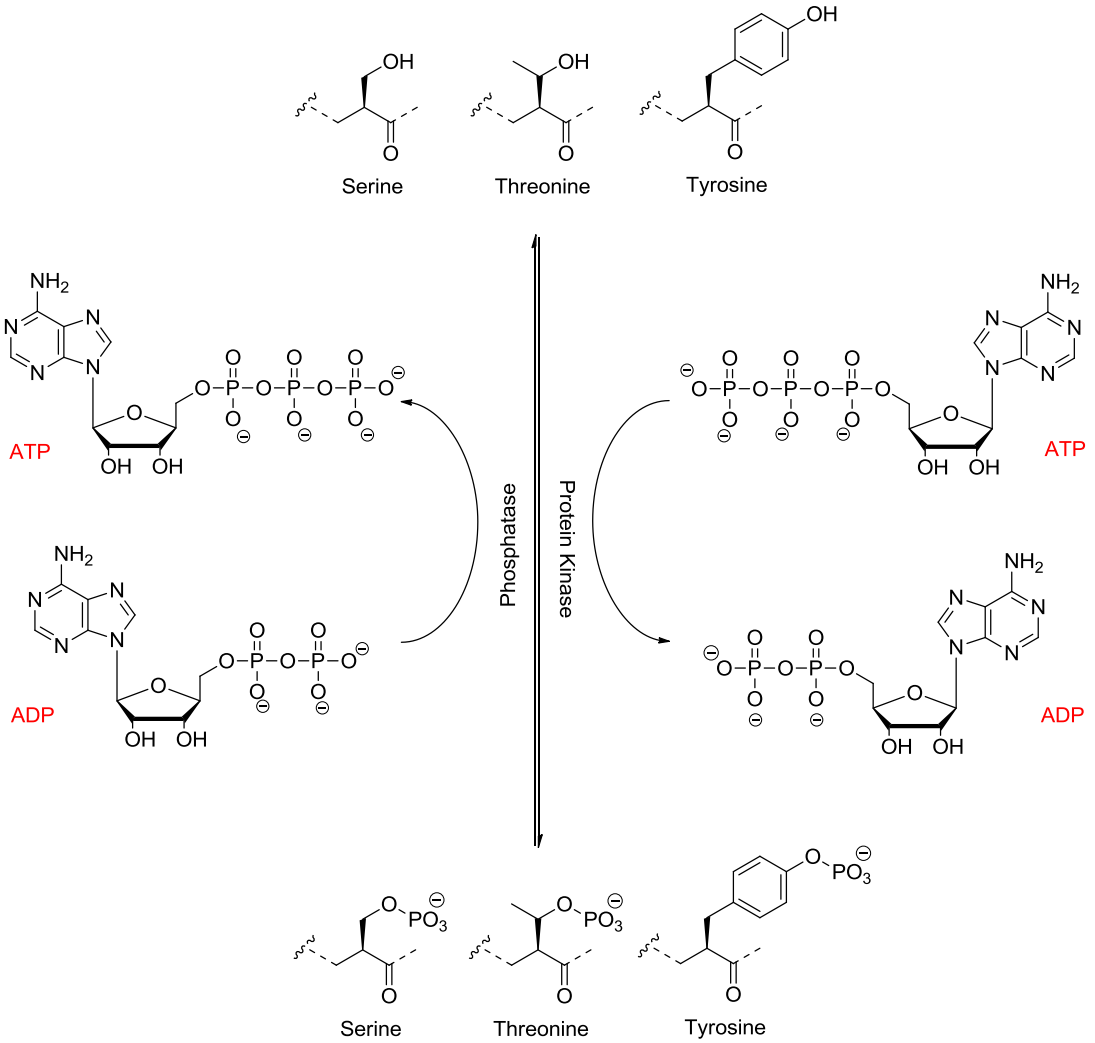


Figure 4. The phosphorylation of the hydroxyl groups in serine, threonine and tyrosine mediated by protein kinases, and their subsequent dephosphorylation by phosphatases.

In order to facilitate the transfer of the terminal γ-phosphate group from ATP to another protein, ATP binds to a specific binding domain on the kinase, through hydrogen bond interactions with amino acid residues. Recent research has centred on the development of effective mimics of ATP to competitively inhibit the phosphorylation process through binding to the active site of the kinase. Competitive inhibitors aim to mimic the hydrogen bond interactions of ATP within the binding sites of the mutated kinases that drive proliferation of cells in cancer. Inhibition of phosphorylation should then prevent the subsequent signalling cascade and hence prevent uncontrolled activation of

proliferation. Each kinase has a unique ATP binding domain with a highly conserved amino acid sequence. The differences in binding domain may be utilised in order to develop an inhibitor that binds selectively to the kinase of interest.[10]

1.4.1 Protein kinase inhibitors

The development of ATP-competitive inhibitors of protein kinases has proven to be a valid approach. The Bcr-Abl oncogene encodes a constitutively active tyrosine kinase in chronic myelogenous leukaemia (CML). The Bcr-Abl oncogenic protein arises from reciprocal translocation of genetic material from chromosomes 9 and 22 and its formation results in unregulated cell proliferation.[10-11] Imatinib is an ATP-competitive inhibitor of the Abl protein kinase and was approved in 2001 for the treatment of CML (marketed as Glivec in Europe). Figure 5 shows the crystal structure of imatinib (**2**) bound in the ATP binding site of the Abl protein kinase. Imatinib has also been shown to be an effective treatment for gastrointestinal stromal tumour (GIST) as it was found to be an inhibitor of protein kinase c-Kit, which is elevated in patients with GIST.[12]

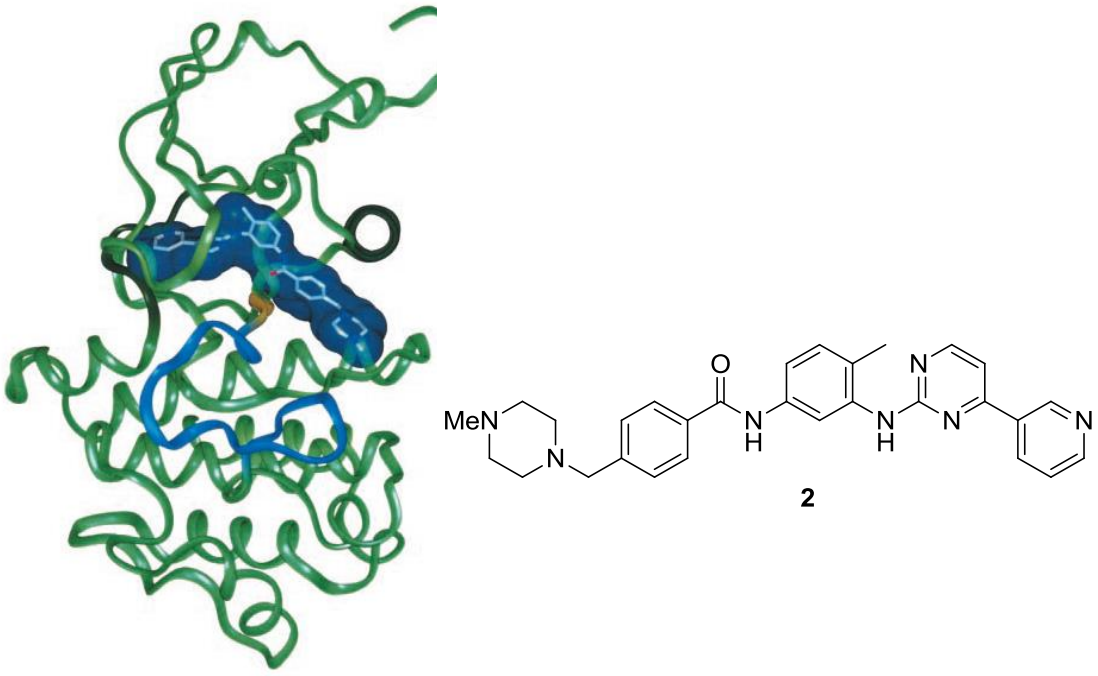


Figure 5. Imatinib bound in the ATP binding site of the Abl tyrosine kinase.[11]

Due to the success of imatinib, interest in developing inhibitors of protein kinases has increased and over the past 11 years a number of kinase inhibitors have been approved for clinical use (Table 1).

Table 1. Kinase inhibitors in the clinic for the treatment of cancer.[13-15]

Name	Marketed name	Year approved	Kinase target	Clinical use
Imatinib	Glivec (Novartis)	2001	Bcr-Abl fusion	CML, GIST
Gefitinib	Iressa (AZ and Teva)	2003	EGFR-TK	Non-small cell lung cancer (NSCLC)
Sorafenib	Nexavar (Bayer and Onyx)	2005	Raf1 (Multi-kinase inhibitor)	Renal cell carcinoma (RCC), hepato-cellular carcinoma (HCC)
Sunitinib	Sutent (Pfizer)	2006	Multi-kinase inhibitor	RCC, imatinib resistant GIST
Erlotinib	Tarceva (Roche)	2010	EGFR-TK	Metastatic NSCLC
Dasatinib	Sprycel (BMS)	2010	Bcr-Abl fusion (Multi-kinase inhibitor)	CML
Nilotinib	Tasigna (Novartis)	2010	Bcr-Abl fusion (Multi-kinase inhibitor)	CML
Vandetanib	Caprelsa (AZ)	2011	VEGFR/EGFR	Medullary thyroid cancer
Vemurafenib	Zelboraf (Plexxikon and Genentech)	2012	BRaf (V600E)	Malignant melanoma
Crizotinib	Xalkori (Pfizer)	2012 (USA) Exp. 2013 (UK)	ALK, ROS1	NSCLC

Chapter 2. Mitogen Activated Protein Kinases (MAPKs) and cancer

2.1 MAPK pathways

The mitogen activated protein kinases (MAPKs) are a specific family of protein kinases which have been identified as a useful target for cancer therapy as they control a number of fundamental cellular processes including cell survival, proliferation, motility, differentiation and inflammatory responses.[16-17] MAPKs regulate cellular signal pathways, which relay, amplify and integrate signals from a variety of stimuli including growth factors and cellular/extracellular responses such as osmotic and oxidative stress.[17-18] Growth factor signals are transmitted *via* transmembranal receptors, which, upon stimulation, can initiate several signalling pathways, leading to a variety of cellular responses. These signalling pathways are mediated *via* a series of dual phosphorylation reactions occurring on serine/threonine residues.[19]

In mammalian cells 14 highly conserved MAP kinases have been identified and categorised into seven groups. Four groups are known as the conventional MAPKs; Extracellular signal-related kinase (ERK) 1/2, the p38 isoforms, c-Jun amino (*N*)-terminal kinase (JNK) isoforms and ERK5. ERKs 3, 4, 7 and 8 and Nemo-like kinase (NLK) are classified as atypical MAPKs.[20] The TXY amino acid sequence present in the activation loop of conventional MAPKs varies between the four sub-families (ERK, TEY; p38, TGY; JNK TPY).[20] Of the conventional MAPKs, ERK5 differs most greatly from the other members. ERK5, or 'Big MAP kinase 1' (BMK-1) contains an *N*-terminus extension, which contains transactivation domain (TAD) and nuclear localisation sequences (NLS).[20] Figure 6 shows a representation of the structural domains of conventional and atypical MAPKs, which have highly conserved regions..

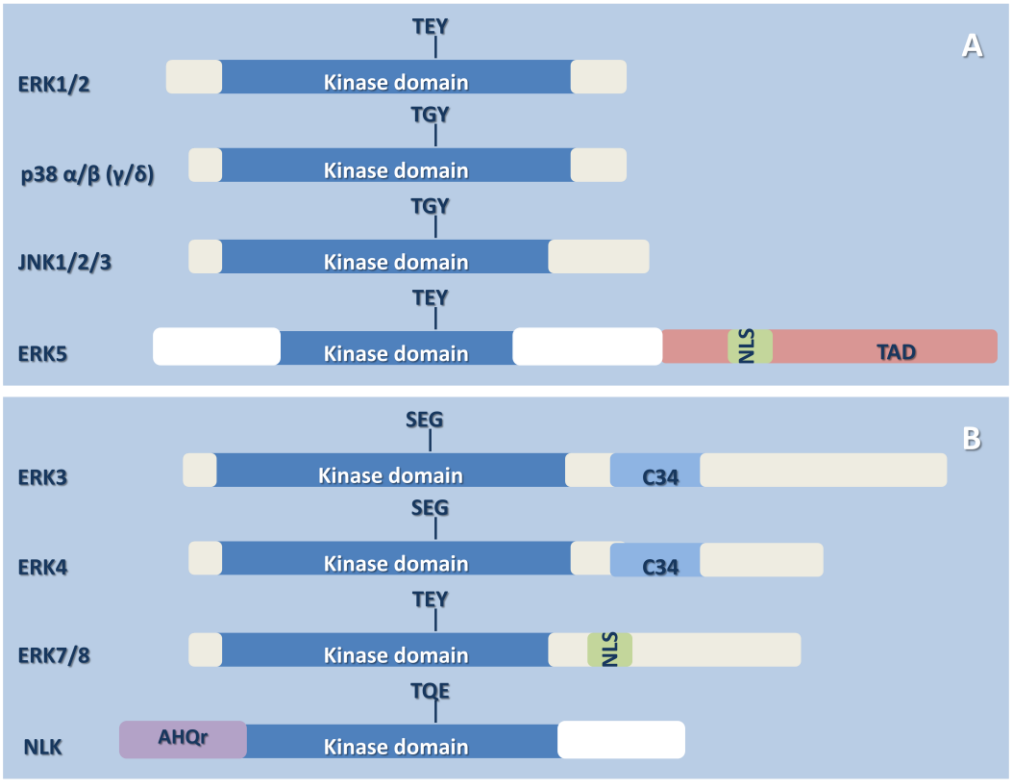


Figure 6. Representation of the structures of (A) conventional and (B) atypical MAPKs. TAD: transactivating domain, NLS: nuclear localisation sequence, C34: conserved region in ERKs 3 and 4, AHQr: Ala, His and Glu rich domain. Adapted from ref 20.

For selective signalling of a specific pathway, three docking domains have been identified; the D (δ) domain, the CD (C-terminal common docking) domain and the DEF (Asp-Glu-Phe) domain.[20] The CD domain is present in ERK, p38 and JNK subfamilies and is located outside the catalytic site. The CD domain forms electronic interactions with the D domain of a specific downstream target to ensure selectivity for the correct kinase in the signalling pathway. The DEF domain (identified by a conserved Phe/Tyr-Xaa-Phe-Pro sequence) is involved in efficient binding of downstream targets and is essential for phosphorylation of the correct downstream effector.[20]

Each MAPK cascade involves no fewer than three enzymes, which are activated in series; a MAPK kinase kinase (MAPKKK), a MAPK kinase (MAPKK) and a MAP kinase (MAPK).[17] The Ras/Raf/MEK/ERK signalling cascade is the most studied classical MAPK cascade. Following stimulation of the transmembranal receptor, through binding of a growth factor, cytokine or hormone, levels of Ras-GTP (guanosine

triphosphate) increase. Increased levels of Ras-GTP promote activation of the EGF-receptor tyrosine kinase, which leads to the recruitment of the adaptor protein, Grb2 (growth factor receptor-bound protein) and the guanine nucleotide exchange factor, SoS (Son of Sevenless), which form a complex with the activated receptor.[17] Ras-GTP acts as a 'molecular switch' and is able to hydrolyse GTP to GDP. SoS binds to inactive Ras-GDP, and forces the delocalisation of GDP so that Ras is able to bind GTP and become activated once again. Activated Ras-GTP then activates MAPKKK, Raf. There are three isoforms of the Raf kinase, A-Raf, B-Raf and C-Raf, which are activated when Ras-GTP binds and delocalises Raf to the plasma membrane where Raf is phosphorylated either by Ras-GTP, or by autophosphorylation. The sequence then continues with membrane bound Raf assembly of a signalling complex consisting of MAPKK, MEK and MAPK, ERK. Raf then phosphorylates MEK, which in turn phosphorylates ERK. Activated ERK then dissociates from the complex and translocates to the cell nucleus, where the kinase is able to activate a number of transcription factors. This leads to changes in gene expression, promotion of growth, differentiation, mitosis and angiogenesis. Activated ERKs may also transmit signals to a variety of cytoplasmic effectors, and therefore play a vital role in numerous cellular processes.[17, 19, 21]

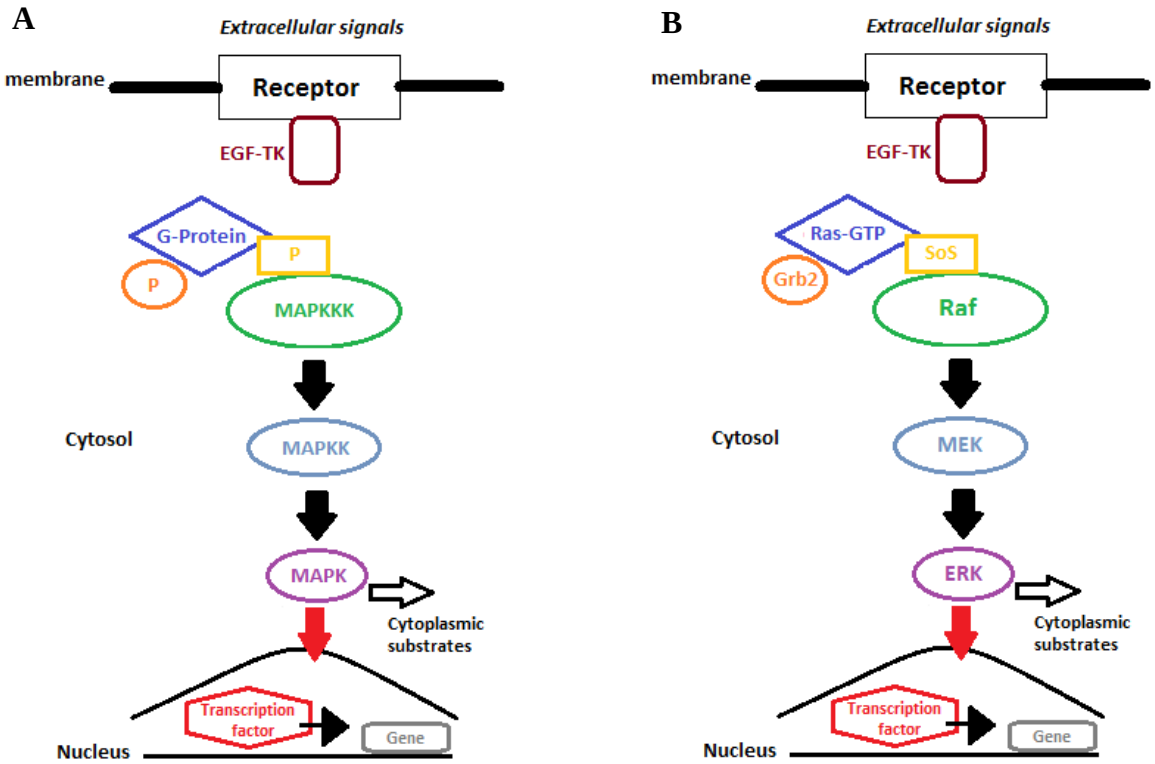
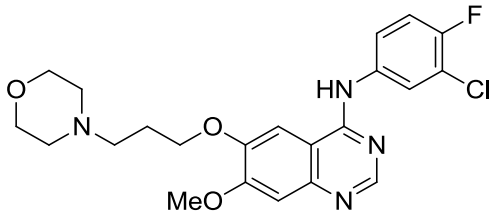


Figure 7. **A)** General MAPK signalling pathway **B)** Classical MAPK pathway.

Disruption of MAPK signalling pathways can result in cellular transformation or altered proliferation.[17] The Ras/Raf/MEK/ERK pathway is a key oncogenic pathway implicated in a variety of human cancers. The main cause of over-activation of the pathway is a mutated or over-expressed protein in the signal cascade. For example, Ras over-expression is commonly observed, with Ras being locked in a permanently 'switched on' or activated state, leading to a constitutively active signalling pathway. Over expression of Raf, particularly B-Raf, has also been identified as a common mutation in around 70% of human carcinoma. An upregulation of trans-membrane growth factor receptors has also been observed (e.g. HER2) as a mutation that can lead to disrupted cell signalling, and, hence, over proliferation of cells.[21-23]

2.2 Targeting the Ras/Raf/MEK/ERK pathway with inhibitors

Inhibition of the Ras/Raf/MEK/ERK pathway has been attempted by targeting the extracellular receptors. Examples are the EGFR-TK inhibitors gefitinib (**3**) (EGFR-TK; IC_{50} = 220 nM) and erlotinib (**4)** (EGFR-TK; IC_{50} = 2 nM).[24-25]



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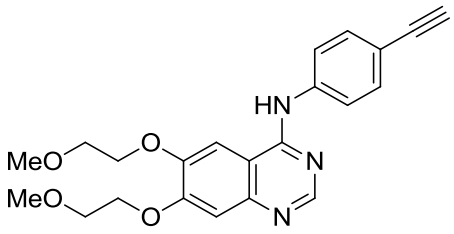

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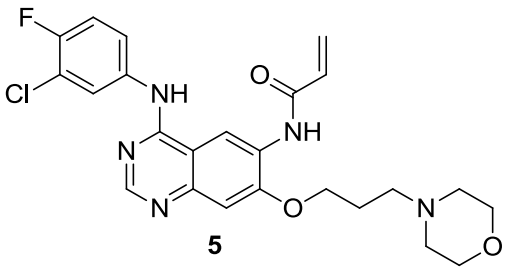
One major problem with drugs that target the EGFR-TK is the development of drug resistance, due to redundancy of the inhibited pathway. A single secondary mutation in the EGFR, with substitution of a methionine residue at position 790 for threonine (T790M) is the most common cause of resistance to EGFR-TK inhibitors.[26] Development of second generation inhibitors which target the T790M variant of the EGFR-TK is in progress for the treatment of patients who no longer respond to gefitinib or erlotinib treatment. Canertinib (**5**) is a highly potent irreversible inhibitor of wild type EGFR and $EGFR^{T790M}$ (EGFR; IC_{50} = 0.3 nM; $EGFR^{T790M}$; IC_{50} = 26 nM) and is currently being evaluated in Phase II clinical trials.[27]



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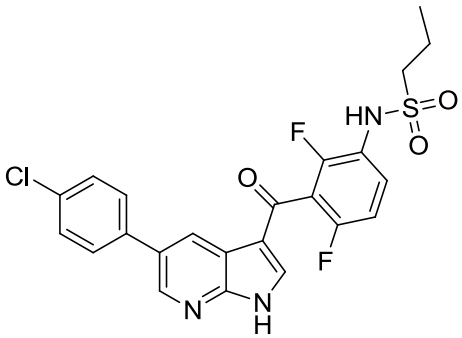
A novel mutation in BRaf (V600E) has been identified in human tumours including malignant melanoma. Vemurafenib (**6**) shows significant anti-tumour effects in cell lines possessing $BRaf^{V600E}$, but is inactive against cells containing wild type BRaf.[28] Drug resistance *via* secondary mutations highlights the necessity for continued genomic investigation and drug discovery for the development of new therapeutic agents.



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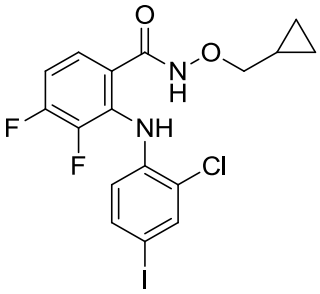
Ras oncoproteins activate signalling pathways such as the MEK/ERK cascade and, as such, play a key role in development of various cancers, with mutations in Ras present in approximately 30% of all human cancers.[29] Raf, MEK and ERK1/2 have also been shown to be over-expressed in many cancers and have, therefore, proven to be an attractive target for inhibition.

MEKs are an attractive target for inhibition as they have restricted substrate specificity and only phosphorylate a small number of downstream MAPKs, so their inhibition is not likely to disrupt any normally functioning cellular processes.[29] Pfizer developed the first MEK1 inhibitor to reach clinical trials, PD184352 (CI-1040, **7**) (MEK1; *Ki* = 17 nM). PD184352 has been shown to act as an allosteric inhibitor reducing the affinity of ATP at the ATP binding site, enabling specificity for MEK1 over structurally similar kinases such as p38α and JNK2. PD184352 was advanced to phase II for patients with metastatic breast cancer, non-small cell lung cancer and pancreatic cancer, but was

unsuccessful in producing a response following single agent dosing. As a result, second generation MEK1 inhibitor PD0325901 (**8**) (MEK1; IC_{50} = 1 nM) was developed. PD0325901 displays significantly improved *in vivo* efficacy and drug-like properties to PD184352.[21, 29]



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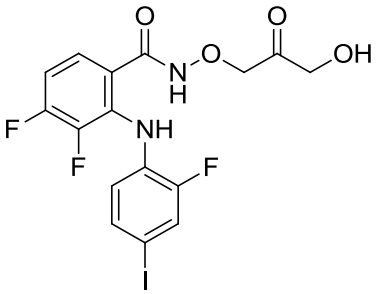

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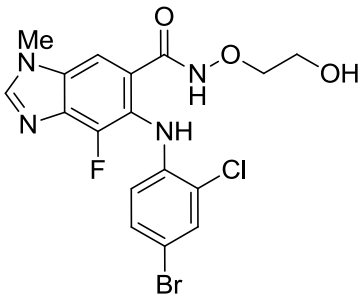
AZD6244 (**9**) (MEK1/2; IC_{50}= 14 nM) was developed by AstraZeneca as a second generation MEK inhibitor and is particularly effective in cell lines which display BRAF or Ras mutations.[30] Phase II clinical trials for AZD6244 have been completed for treatment of non-small cell lung cancer (NSCLC) and are ongoing for BRAF mutations.[30] Another MEK1 inhibitor, RO4987655 (**10**) is currently in Phase II clinical trials for the treatment of advanced solid tumours.[31]



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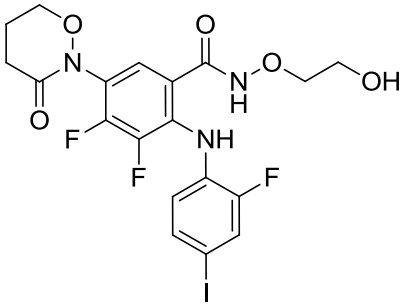

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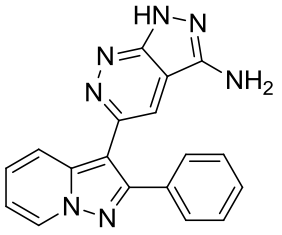
Inhibition of ERK1/2 by small molecules is less well investigated. FR180204 (**11**) was reported in 2005 an ATP competitive dual inhibitor of both ERK1 and ERK2 (IC_{50}; ERK1 = 0.51 μM; ERK2 = 0.33 μM). A cell-based assay of FR180204 revealed that dual inhibition of ERK1/2 results in inhibition of downstream effector, transcription factor AP-1. AP-1 transactivation is associated with the TGF-β pathway, which is involved in many cellular functions including cell growth and differentiation.[32]



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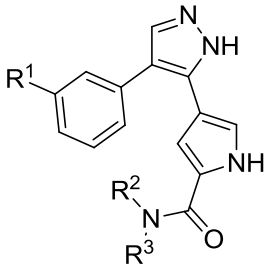
Potent pyrazolylpyrrole-based inhibitors of ERK1/2 have been identified by Vertex pharmaceuticals (**12**, *Ki*; ERK2 = 0.086 μM; **13**, *Ki*; ERK2 = 0.035 μM; *Ki*; **14**; ERK2 = 0.002 μM) and are in pre-clinical development.[33]



12; R^1 = Cl, R^2 = H, R^3 = benzyl

13; R^1 = Cl, R^2 = H, R^3 = hydroxyethyl

14; R^1 = Cl, R^2 = H, R^3 = (*R*)-1-(3-chloro,4-fluorophenyl)-hydroxyethyl

2.3 p38 and JNK signalling pathways

p38 and JNK are MAPK subfamilies and are known as stress-activated protein kinases (SAPKs) as their signalling pathways are activated by cellular stress. The p38 subfamily consists of four isoforms, α, β, γ and δ, with p38α being the most widely investigated as it is most abundantly expressed through cells. p38α is present in the majority of mammalian cells and is involved in the regulation of the immune response.[34] p38α mediates cellular responses to UV, heat, and osmotic shock. Neutrophils, monocytes, macrophages and other inflammatory cells synthesise the p38α isoform. Activation of the p38 MAPK pathway is associated with inflammatory diseases due to the production of pro-inflammatory cytokines (e.g. Interleukin-1 (IL-1).[35] The p38 pathway has been identified as a regulator of apoptosis and treatment with chemotherapeutic agents can induce p38-dependant apoptosis in certain cell lines.[36]

The JNK subfamily are activated by environmental stress primarily and Figure 8 shows that both p38 and JNK are activated by the same MAPKKKs following the stimulus (MEKK1-4, DLK, MLK2, Tpl-2, ASK1, TAK1 and TAO1/2).[37] The p38 isoforms are then phosphorylated by MAPKKs MEK3 and MEK6, resulting in the activation of a

number of cytoplasmic and nuclear targets (including cPLA2, MNK1/2, Tau, MEF2 and p53) regulating inflammatory response and inducing apoptosis. The JNK family are phosphorylated by MEK4 and MEK7, which leads to JNK-mediated activation of transcription factors including c-Jun, p53, Stat3 and c-Myc. Activation of the JNK pathway has been associated with tumour formation *via* disruption of cellular growth.[37]

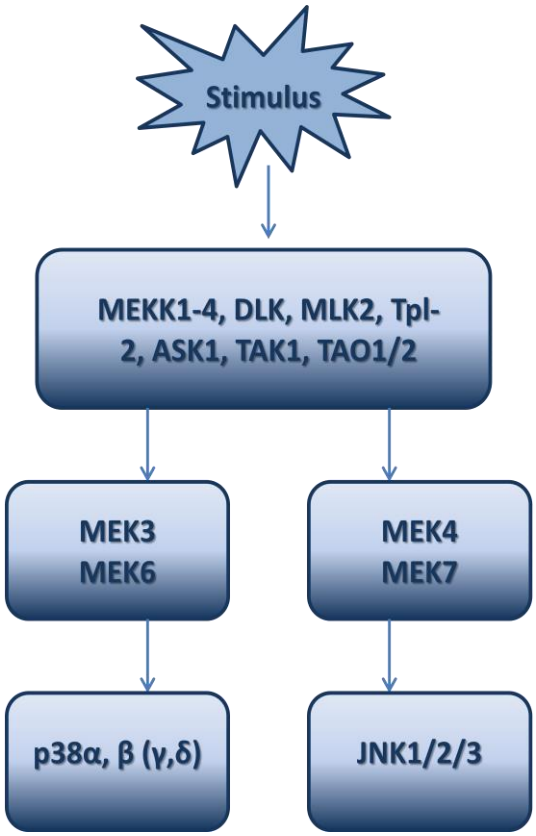
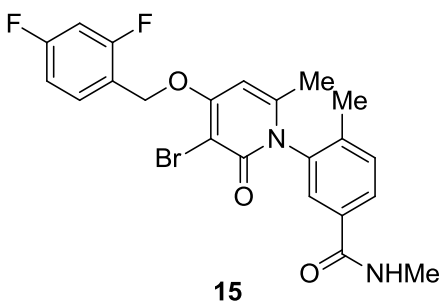


Figure 8. p38 and JNK signalling pathways.

2.3.1 Targetting the p38 and JNK pathways with small-molecule inhibitors

Inhibitors of p38α have been successful in entering clinical trials for diseases associated with inflammatory response, e.g. PH797804 (**15**) and GW856553 (**16**), which are currently in phase II clinical trials for treatment of rheumatoid arthritis and chronic obstructive pulmonary disease (COPD), respectively.[38-39]



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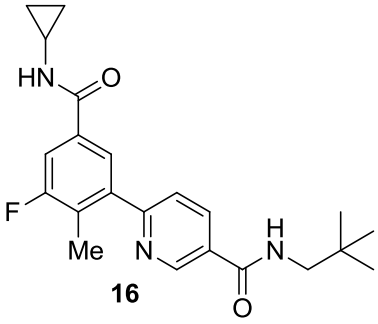

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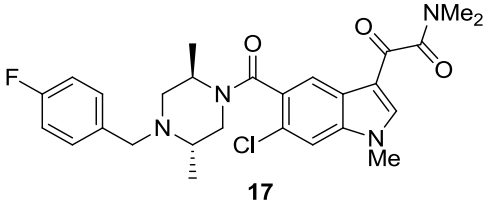
To date, only one inhibitor of p38α has entered clinical trials for the treatment of cancer, Talmapimod (SCIO-469, **17**). In 2009, Talmapimod entered clinical trials with single agent dosing in patients with multiple myeloma, but it was found to be ineffective. A second trial using Talmapimod in combination with proteasome inhibitor Velcade (**18**) produced a synergistic effect and cell proliferation was significantly affected.[40] p38 inhibition is thought to induce sensitisation to proteasome inhibitors through the reduction of MAPK-activated protein kinase-2 (MAPKAPK-2) and activation of the anti-apoptotic heat shock protein 27 (HSP27).[41] p38α inhibitors may be used clinically to increase sensitivity of resistant or unresponsive patients to existing anti-cancer therapies.



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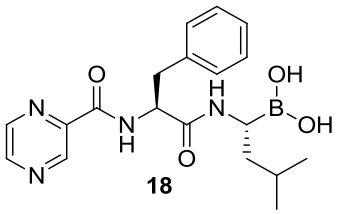

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Inhibitors of members of the JNK subfamily of MAPKs are also under investigation. As for p38, JNK is implicated in inflammatory diseases and cancer. JNK family members, may also play a role in the progression of neurodegenerative and metabolic disease. At present, there are no known JNK inhibitors under clinical evaluation.

2.4 Extracellular signal-related kinase 5 (ERK5)

Extracellular signal-related kinase 5 or ERK5 is almost twice the size of other MAPKs consisting of 816 amino acids and is also known as Big MAPK 1 (BMK-1).[42] The large size of ERK5 can be attributed to a unique C-terminal domain, which possesses a transcriptional activation domain and a MEF2 interacting domain. The ERK5 signalling pathway is associated with cell survival, proliferation and differentiation. Over-expression of ERK5 may lead to uncontrolled cell growth and tumourigenesis.[43-44]

ERK5 is part of a non-classical MAPK signalling cascade, which is independent of Raf activation (Figure 9).[45] The ERK5 signalling cascade is initiated by binding of growth factors, i.e. epidermal growth factor (EGF), nerve growth factor (NGF), vascular endothelium growth factor (VEGF) or fibroblast growth factor-2 (FGF-2) to receptors found on the cell surface, activating the EGFR-TK.[46-48] Activation of the signalling pathway can also occur as a result of cellular stresses, or cytokines as with the p38 and JNK signalling pathways. The EGFR-TK recruits Grb2 and SoS to activate Ras as in the classical pathway and then MAPKKKs, MEKK2/3 are then activated independently of Raf. MEKK2/3 activate MEK5, which has been identified as the sole activator of ERK5.[45] Following activation, ERK5 can translocate to the cell nucleus and activate transcription factors such as myocyte enhancer factors (MEF2A, 2C and 2D), which mediate cell survival, c-Myc, c-Jun, c-Fos, activating protein 1 (AP-1) and NFκB. Bcl-2 associated death promoter (Bad) and serine/threonine kinase (SGK) are also downstream targets of ERK5, along with the p90 ribosomal s6 kinase (RSK) involved in signal transduction. RSKs are themselves known to activate several cytosolic and nuclear substrates, and are implicated in the regulation of a number of cellular processes associated with the onset and progression of cancer, including cell proliferation, survival, and motility.[43-44, 49]

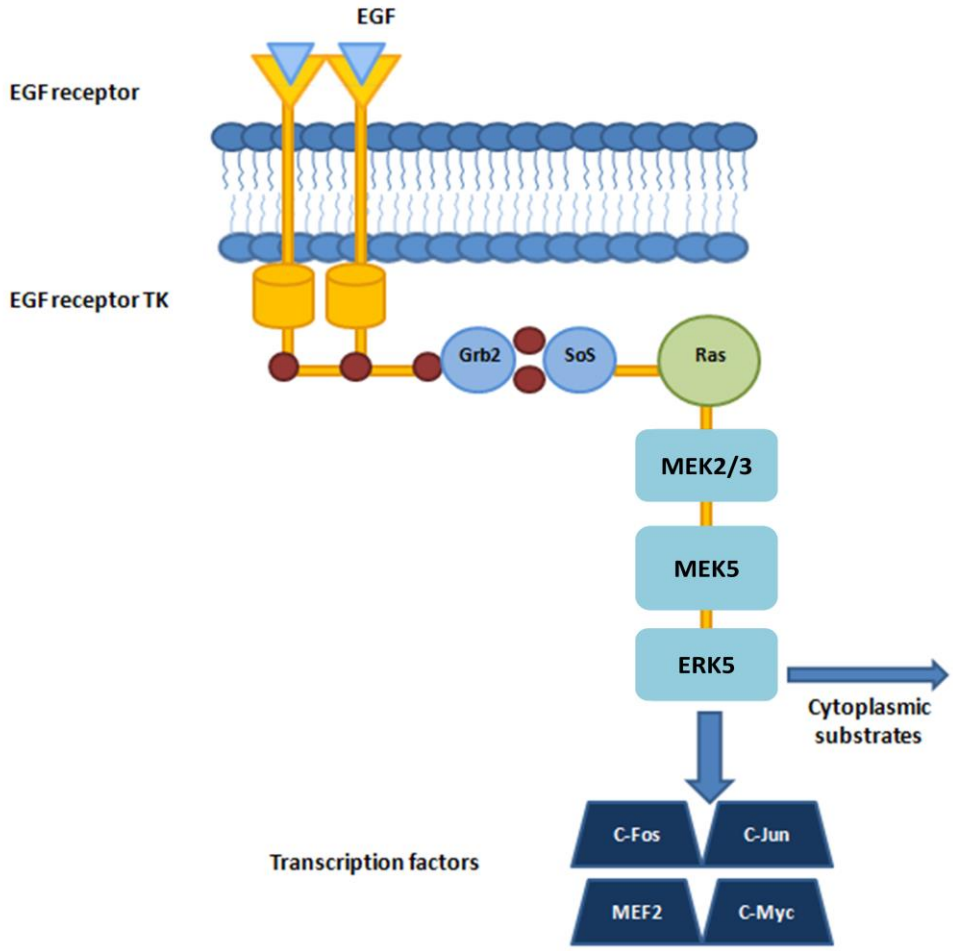


Figure 9. MEK5/ERK signalling cascade.

2.4.1 Adaptor and scaffold proteins in ERK5

In addition to docking domains, adaptor and scaffold proteins assemble signal cascade proteins in multi-enzyme complexes and ensure signal transduction to MAPKs.[46]

Scaffolding proteins are a novel class of proteins, which bring specific components of the MAPK cascade together in close proximity so that rapid and efficient phosphorylation of the downstream target can occur. Adaptor protein Lad (Lck-associated adaptor) promotes ERK5 activation by increasing the binding affinity between upstream kinases in the cascade, MEKK2 and MEK5.[50] MEKKs 2/3 contain a Phox/Bem1 (PB1) domain which may associate with other PB1 containing proteins *via* protein-protein interactions, and it is this interaction which mediates MEK5 activation. Interestingly, ERK5 is the only MAPK to have a PB1 domain and it has been demonstrated that MEK5 itself is able to act as a scaffold protein by tethering MEKK1/2 to ERK5 *via* its *N*- and *C*-terminal regions of the PB1 domain. The three-kinase complex is unique to the MAPK family.[51]

2.4.2 Structure of ERK5

ERK5 belongs to the conventional MAPK family and as such contains a TXY tripeptide motif in its activation loop (for ERK1, ERK2 and ERK5 X = E). Phosphorylation of the threonine and tyrosine residues of this tripeptide sequence activates ERK5 (and ERK1 and ERK2). Despite the conserved tripeptide motif, ERK5 differs from ERKs 1 and 2 due to a unique loop-12 structure and extended C-terminal domain containing around 400 amino acids (hence the alternative name, big MAPK 1, BMK-1). The C-terminus contains nuclear localisation and export sequences (NLS and NES) and a transcriptional activation domain that interacts with MEF2D.[46] The C-terminal domain is autophosphorylated at multiple sites by the kinase domain, leading to increased transcriptional activity. The C-terminal domain of ERK5 can also act in an autoinhibitory capacity by masking the catalytic domain and blocking substrates from binding.[52] It is only following phosphorylation of ERK5 by MEK5 that a conformational change occurs and the C-terminal domain no longer impedes binding of substrates.[52]

2.4.3 Role of ERK5 in cancer development

2.4.3.1 ERK5 and PML

2.4.3.1.1 ERK5-mediated reduction of p21 via interaction with PML

Mass spectrometric analysis has revealed that ERK5 interacts with and phosphorylates promyelocytic leukemia (PML) protein.[53] There are a number of isoforms of PML (I-VI) and ERK5 has been shown to interact with all isoforms except PML V.[54] PML acts as a tumour suppressor due to activation of downstream effector p21. It is known that upon activation of ERK5 by MEK5, ERK5 translocates to the cell nucleus from the cytoplasm and it is thought this is where the interaction between ERK5 and PML occurs. PML is able to activate p21 expression leading to tumour suppression. ERK5 phosphorylates PML on two phosphorylation sites, inactivating it and preventing activation of p21 (Figure 10). Inactivation of PML by ERK5 is usually a tightly controlled process. However, when there is an over-expression of ERK5, as observed in many tumour types, the levels of p21 are reduced so low that tumour suppression cannot be effected.[53]

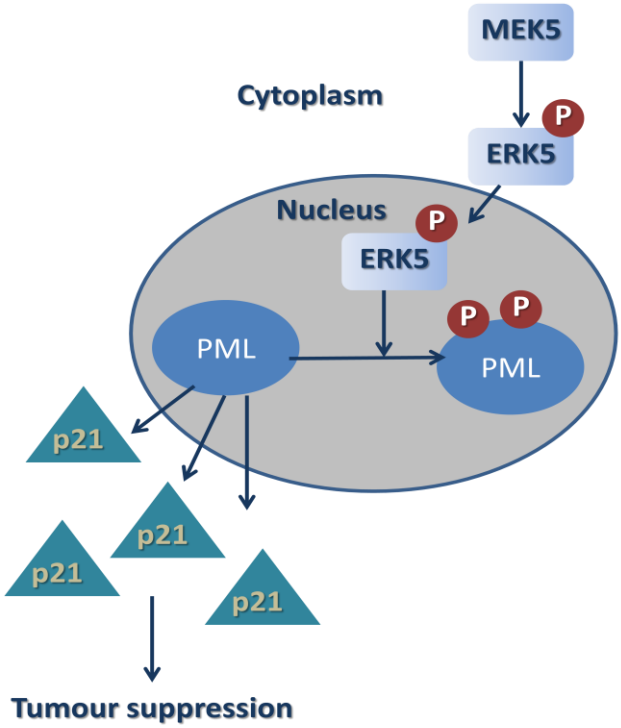


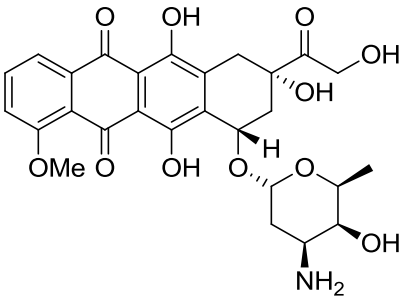
Figure 10. Regulation of p21 activation *via* phosphorylation of PML by ERK5.

Suppression of ERK5 using siRNA knockdown of MAPK7 (the gene encoding ERK5) induces expression of p21 and increases tumour suppression. For this reason, using an inhibitor to reduce levels of ERK5 would also be effective in inducing tumour suppression. An ERK5 inhibitor, XMD8-92 was shown to exert tumour suppression through increased p21 expression and is discussed in more detail in section 2.4.6.1 of this chapter.[53]

2.4.3.1.2 ERK5-mediated reduction of p53 via interaction with PML

More recently it has been shown that ERK5 has the highest affinity for the PML IV isoform. PML IV is able to interact with MDM2 and hence regulate levels of tumour suppressor, p53.[54] MDM2 is the major E3 ubiquitin ligase for p53, facilitating p53 ubiquitination and consequent degradation. Up-regulation of p53 can be achieved by disruption of the MDM2/p53 interaction. Doxorubicin (**19**) is a topoisomerase inhibitor and is able to induce p53 *via* nuclear sequestration of MDM2, which is thought to occur due to promotion of PML IV association with MDM2. Interaction of ERK5 with PML IV disrupts the MDM2-PML interaction leading to reduced levels of p53. It has been shown that inhibition of ERK5 with XMD8-92 (**20**) (section 2.4.6) or siRNA/shRNA

knockdown of ERK5 increases levels of cellular p53.[54] Mouse embryonic fibroblast (MEF) cells with $PML^{+/+}$ and $PML^{-/-}$ were used alongside A549 (lung cancer) and HeLa (cervical cancer) cells and were treated with doxorubicin, PD184352 (**7**) (a MEK1/2 inhibitor) and/or XMD8-92. Long-term treatment of the cells with XMD8-92 (32-40 h) showed significant synergy with doxorubicin and led to upregulation of p53 and tumour cell apoptosis. The synergistic effect was not observed in $PML^{-/-}$ MEF cells. Mouse HeLa and A549 xenograft models were condcted and in each case dosing with XMD8-92 and doxorubicin in combination led to tumour regression (Figure 11).[54]



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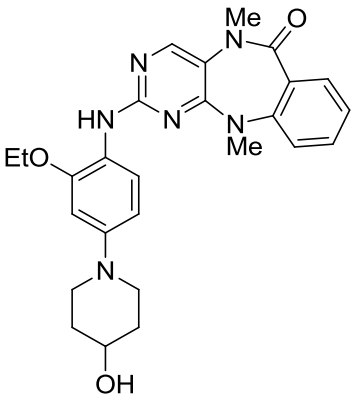

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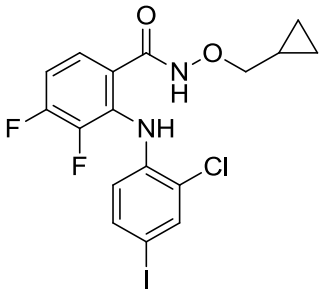

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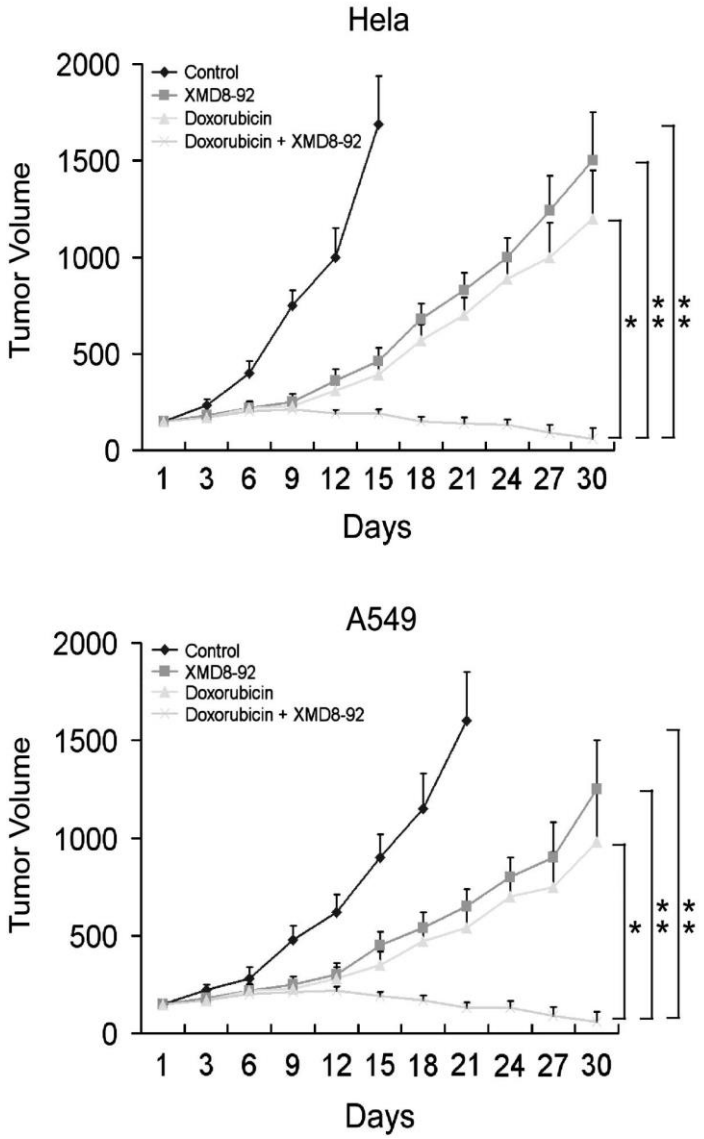


Figure 11. Mouse HeLa and A549 xenograft models treated with control, doxorubicin, XMD8-92 and doxorubicin + XMD8-92. Adapted from ref 53

This study indicates that the use of an ERK5 inhibitor may significantly enhance the anticancer effect of doxorubicin. The chemo-potentiation of doxorubicin in combination with XMD8-92 has been investigated in Newcastle using an SRB cytotoxicity assay in A549 cells. The cells were treated with doxorbubicin alone, or in combination with either 1 μM or 4 μM of XMD8-92. There is a reduction in cell growth for the cells treated with the combination therapy, validating the theory that an ERK5 inhibitor may enhance the effectiveness of doxorubicin treatment (Figure 12).

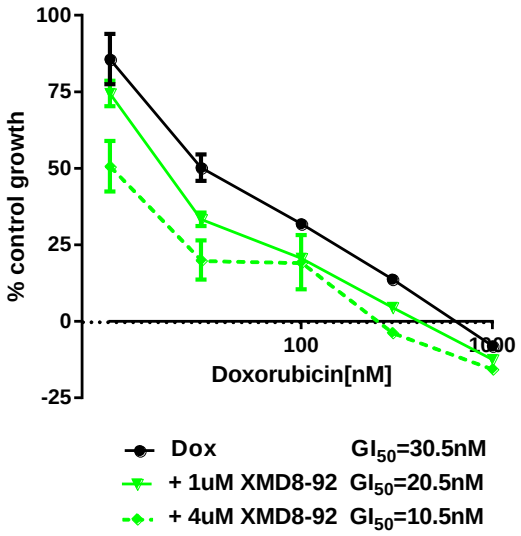


Figure 12. Percentage growth of A549 cells treated with doxorubicin alone or in combination with 1 μM or 4 μM of XMD8-92.

2.4.3.2 ERK5 and cell cycle progression

It is postulated that ERK5 may play a role in controlling progression through the cell cycle.[55] There is some dispute over which stage of the cycle ERK5 regulates as the hypothesis that it controls the G1/S transition may not be correct due to levels of activated ERK5 being negligible in HeLa cells arrested in the G1/S phase.[56] However, activated ERK5 levels have been shown to increase during the G2/M phase of the cell cycle. It has been shown that the phosphorylation of ERK5 during this phase is not conducted by MEK5 and does not occur on the TEY motif, but at several sites on the C-terminus.[55, 57] ERK5 was found to activate the transcription factor NFκB, mediated by RSK2 during the G2/M phase, which is essential for mitotic entry.[58]

It has been postulated that during mitosis, ERK5 phosphorylates Bim (a pro-apoptotic protein). However, in 2012 it was proven that it is CDK1 that mediates Bim phosphorylation rather than ERK5 as previously suggested.[59] Despite this discovery, it is clear that ERK5 does play a role in cell cycle progression, particularly in the entry to mitosis, and that an over-expression of ERK5 could lead to an uncontrolled increase in cell proliferation leading to tumourigenesis.

2.4.3.3 ERK5 and angiogenesis

ERK5 has been identified as a regulator of embryonic angiogenesis, since embryos without ERK5 cannot survive.[60] Tumours depend on angiogenesis in order to progress and therefore, a link between ERK5-mediated angiogenesis and tumourigenesis has been established.

Tumour growth and metastasis was shown to be reduced in studies using siRNA knockdown of MAPK7 (the gene encoding ERK5) in BI6F10 (melonoma) and LL/2 (Lewis lung) mouse xenografts and tumour vasculature was significantly affected.[60] ERK5 has been shown to activate ribosomal protein S6 (rpS6) following activation of the ERK5 signalling pathway by VEGF or FGF. rpS6 is involved in the RSK-rpS6 signalling pathway. This pathway regulates cell proliferation and survival, which are key processes in tumour angiogenesis.[61]

Interestingly, a link between ERK5 and Id1('inhibitor of differentiation') activation has been estabished. Id1 is a negative regulator of TSP1 (thrombospondin-1), which is a known inhibitor of angiogenesis, therefore over-activation of ERK5 can induce angiogenesis.[62]

2.4.3.4 ERK5 and migration, invasion and metastasis

ERK5 activation may occur *via* integrin-mediated focal adhesion kinase (FAK) signalling.[63] FAK signalling plays an important role in regulation of cell adhesion and motility. The integrins comprise a large family of cell-surface receptors that bind to, and control, the extracellular matrix. The extracellular matrix acts as a scaffold and mediates cellular organisation. Increased levels of ERK5 can lead to increased levels of matrix metallo-proteinase-9 (MMP-9), which leads to delocalisation of cancer cells *via* degradation of the extracellular matrix, increasing migration, invasion and metastasis.[63] It has been shown that prostate cancer cells transfected with a dominant negative mutant of FAK showed no ERK5 activation and cell migration, adhesion and motility was reduced as a result. ERK5 also activates the transcription factor AP-1, which can also promote several MMPs (including MMP-1, 3, 7, 9, 11, 13 and 19) to degrade the extracellular matrix, further implicating ERK5 in the development of tumour metastases.[63]

ERK5 has been linked to the HGF/Met/Brk signalling pathway, which has been associated with increased growth and migration in breast cancer cell lines. The levels of breast cancer kinase (Brk) are over-expressed in many breast cancer cell lines.[63-64] HGF (hepatocyte growth factor) activates the receptor tyrosine kinase Met, the upstream activator of Brk. Activated Brk is able to phosphorylate ERK5 *via* formation of a complex initiated by scaffolding proteins. Brk-mediated phosphorylation of ERK5 promotes HGF-induced migration of breast cancer cells.[64] These findings imply that an ERK5 inhibitor may be therapeutically useful for the prevention of metastatic breast cancer.

Interestingly, ERK5 has been shown to suppress epithelial-mesenchymal transition (EMT).[65] EMT plays a crucial role in the development of cancer metastasis and is generally promoted by MAPK signalling pathways (e.g. ERK, JNK and p38). ERK5 has been shown to upregulate levels of adherent junction proteins (e.g. E-cadherin and ZO-1) in the cell membrane leading to the development of close cell-cell contacts. The development of these contacts is a key indicator of mesenchymal-epithelial transition (MET), which is the reverse of EMT.[65] The expression of mesenchymal markers vimentin and N-cadherin was shown to be low in cells were ERK5 was upregulated and these cells showed slower serum-induced migration. Furthermore, knockdown of ERK5 in a number of cancer cell lines showed loss of close cell-cell contacts and cell scattering, both of which correlate with EMT.[65] It appears that ERK5 acts as a critical controller of EMT and metastasis. ERK5 down-regulation increases the accumulation of Snail, which was found to be modulated by Akt/GSK3β signalling *via* the modulation of mTOR by inhibitor DEPTOR.[65] Increased metastasis in patients undergoing treatment with EGFR inhibitors erlotinib and gefitinib has been observed and could be linked to the inhibition of downstream target, ERK5. Combination of EGFR antagonists with agents that effect the EMT process may enhance the sensitivity of more mesenchymal tumours to treatment, improving treatment strategies for metastatic cancer.[65] Combination studies are being conducted at Newcastle to determine if an ERK5 inhibitor has an effect on treatment with an EGFR inhibtor.

2.4.3.5 Cancer types where ERK5 signalling is deregulated

Deregulation of the MEK5/ERK5 signalling pathway has been associated with the development of a number of cancer types, usually resulting in more aggressive phenotypes and poor disease specific survival. An overview of the cancer types in which ERK5 has been implicated is provided herein demostrating the emerging requirement for develoment of ERK5 inhibitors as potential chemotherapeutics.

2.4.3.5.1 ERK5 and prostate cancer

Prostate cancer is the most commonly diagnosed cancer in men in the UK (Figure 13) and the second most frequent cause of cancer-related male deaths behind lung cancer.[6]

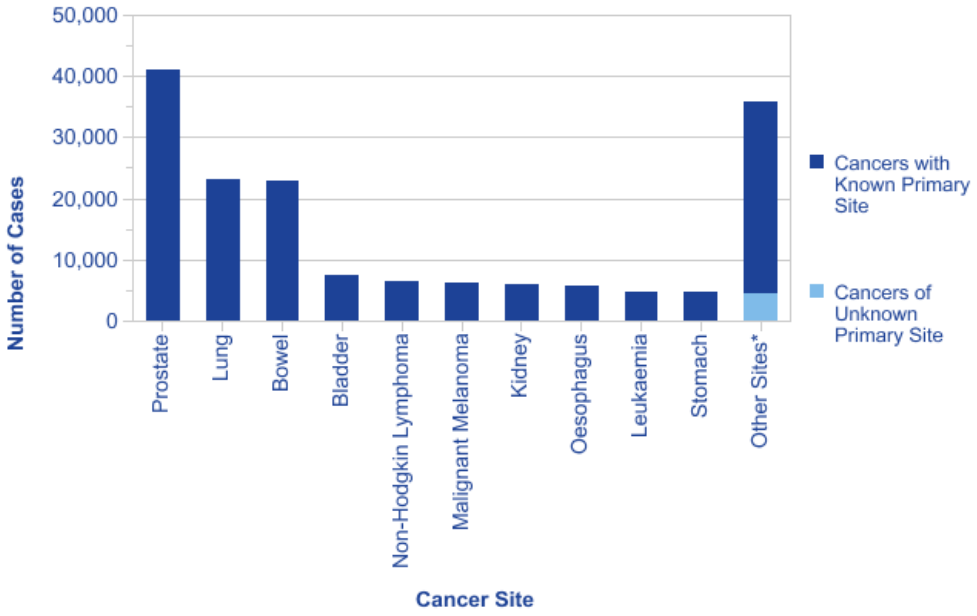


Figure 13. The 10 most commonly diagnosed cancers in males in the UK in 2010.[6]

There are several risk factors associated with the development of prostate cancer, the most prevalent being age. There may also be a genetic link to the development of prostate cancer. Men carrying germline mutations in the BRCA2 gene (associated with the onset of breast and ovarian cancers in females) may have an increased risk of developing prostate cancer.[66]

There is currently no national screening program for prostate cancer in the UK, but the prostate specific antigen (PSA) test is a useful diagnostic tool for the disease. PSA, also

known as P-30 antigen, is a glycoprotein produced by prostate cells. A high level of PSA in the blood is thought to be indicative of prostate cancer, and can be a good initial non-invasive test for the disease.[6] However, the PSA test is not a diagnostic measure, as elevated PSA levels may also be detected in non-cancerous conditions such as prostatitis, or benign enlargement of the prostate. If a high level of PSA is detected, a biopsy can be taken. The cells from the biopsy will be examined in order to deem how similar they are in appearance to healthy cells of the prostate. If the cells appear to be abnormal, as indicated by poor differentiation, a Gleason sum score can be determined. The Gleason score is based on the Gleason grading system, which is a helpful tool used to classify the stage of prostate cancer. Grading is on a scale of 1-5, based on the pattern of cell differentiation. Cells with a differentiation pattern similar to healthy prostate cells (i.e. small and uniform) will be graded as 1 on the Gleason grading scale, with cells that are poorly differentiated (i.e. lacking order) being graded as 5 (Figure 14). Grades from the two most common patterns seen in the biopsy sample are added together to give the overall Gleason score between 2 and 10, with a higher score, indicating more advanced and aggressive disease.[67-68]

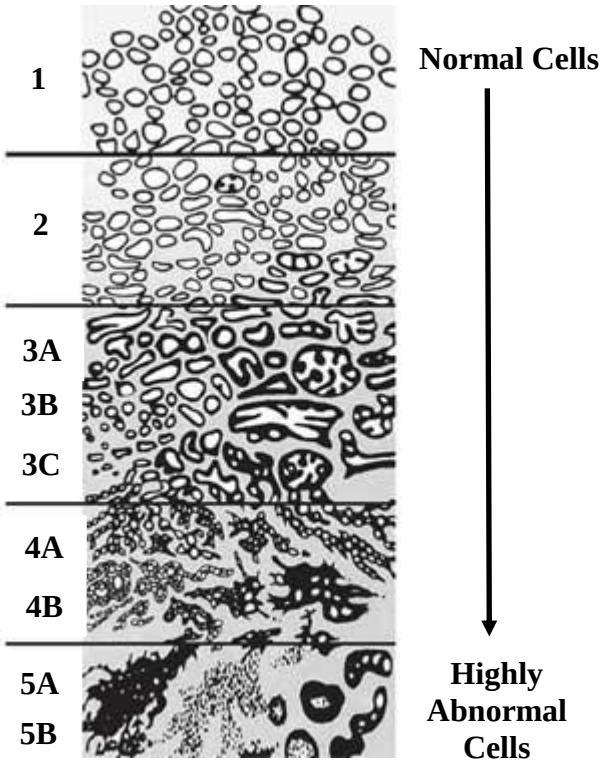
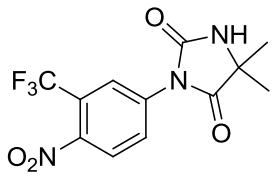


Figure 14. Gleason grading for prostate cancer cells. Adapted from ref 65.

The progression of prostate cancer predominantly relies on the androgens and, in particular, testosterone, which is required by the tumour for growth. It is estimated that 80-90% of diagnosed prostate cancers are dependent on androgens and the androgen receptor for their development, and expression of the androgen receptor is maintained during prostate cancer progression.[69]

Prostate cancer can be divided into two types, hormone sensitive (or dependent) and hormone insensitive (or independent), which is particularly more aggressive. Inhibition of androgen activity is particularly effective as a treatment against hormone dependent prostate cancer either *via* inhibition of the interaction between the androgen and its receptor or by androgen deprivation. There are currently several drug classes which suppress the action of androgens either by reducing serum testosterone and 5α-dihydrotestosterone (DHT) levels, or by directly inhibiting the androgen receptor. Nilutamide **21** and flutamide **22** are competitive inhibitors of the androgen receptor blocking the binding of testosterone/DHT and directly inhibiting the androgen signalling pathway.[70]



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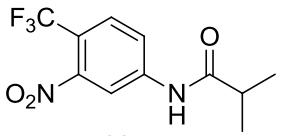

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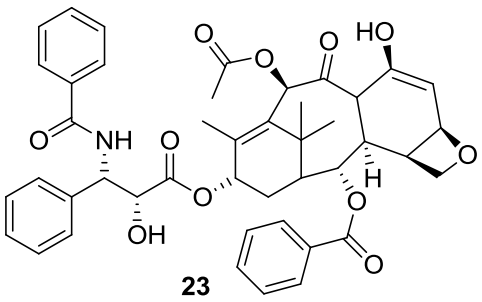
Another class of drugs used for the treatment of hormone dependent prostate cancer are Gonadotropin Releasing Hormone (GnRH) agonists, e.g. Leuprolide or Goserelin. These drugs work by reducing the release of luteinising hormone (LH). Luteinising hormone is responsible for androgen synthesis in the testes and use of GnRH agonists has been shown to reduce testosterone and DHT levels in serum and therefore slow the progression of prostate tumours.[69]

Most cases of prostate cancer are responsive to hormone therapies, and will show a positive response to androgen deprivation. However, around 20% of cases will show no response to this type of treatment. Along with the initially unresponsive patients, there is also a high possibility that responsive patients will develop a hormonal resistance to their treatment. This is very common after around 18-24 months of treatment, and

results in the development of the more aggressive hormone independent form of the disease, which can be ultimately fatal.[71-72]

Development of hormone independent cancer (also known as castrate-resistant) is linked to the anti-apoptotic gene, bcl-2, which can be over-expressed in prostate cancer cells, but not in healthy cells of the prostate. This is thought to prevent the cancerous cells from undergoing apoptosis independent of hormone deprivation and so resistance to hormonal therapies is initiated. Once this resistance has developed it is necessary to use secondary hormonal and chemotherapeutic agents in an attempt to treat the disease. Paclitaxel (Taxol, **23**) and Docetaxel (Taxotere, **24**) are from the taxane family of chemotherapeutic agents, and are currently used as the standard for initial treatment of hormone independent prostate cancer in combination with prednisone **25**, a corticosteroid. They are mitotic inhibitors and work by interrupting microtubule formation, causing apoptosis *via* preventing cells from entering mitosis.[73]



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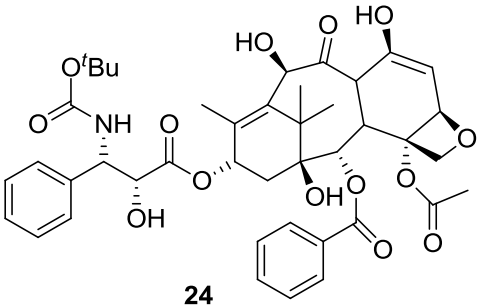

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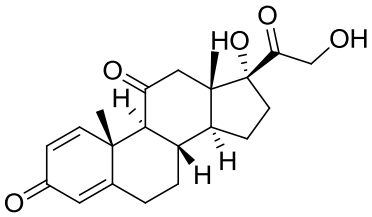

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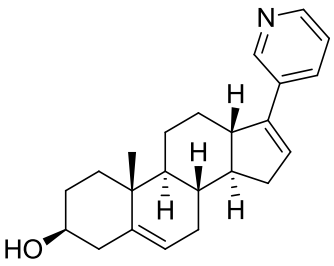
Steroid hormones dehydroepiandrostenedione (DHEA) and androstenedione can be converted into testosterone by enzymes found in the prostate and the androgen receptor's sensitivity to testosterone and DHT is often elevated in patients with hormone independent prostate cancer prostate cancer. In 2011, Abiraterone (**26**) was approved for use as a life-prolonging agent for patients suffering from terminal prostate cancer. Abiraterone works as a17α-hydroxylase and $C_{17\text{-}20}$ lyase inhibitor, which prevents androgen biosynthesis. The prevention of extra-testicular androgen synthesis by Abiraterone has provided a novel therapy for patients suffering advanced prostate

cancer which is no longer responsive to the traditional anti-androgen drugs.[74] Orteronel (**27**) is also in phase III clinical trials as an inhibitor of C_{17-20} lyase.[75]



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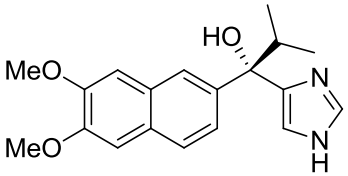

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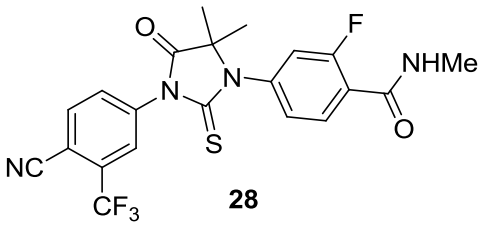
The development of second-generation anti-androgen treatments is in progress for the treatment of patients resistant to first-line hormonal therapy. One mechanism of resistance is the up-regulation of the androgen receptor. Enzalutamide (**28**) was developed as an inhibitor of the androgen receptor following a screen of non-steroidal compounds and is up to 8-fold more potent than common 1st line treatments.[76] Enzalutamide also impairs nuclear translocation, and recruitment of co-activators of the androgen receptor ligand-receptor complex, and has recently been approved for the treatment of patients with hormone independent prostate cancer.[76]



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The MEK5/ERK5 pathway has become a potential target for the treatment of homone independent prostate cancer since expression of ERK5 is up-regulated in prostate cancer cells. Studies conducted at Newcastle university show that ERK5 over-expression correlates with a high Gleason sum score as shown by the graph in Figure 15. The ERK5 staining intensity is semi-quantitative, scored as either absent (0), weak (1), moderate (2) or strong (3).[42]

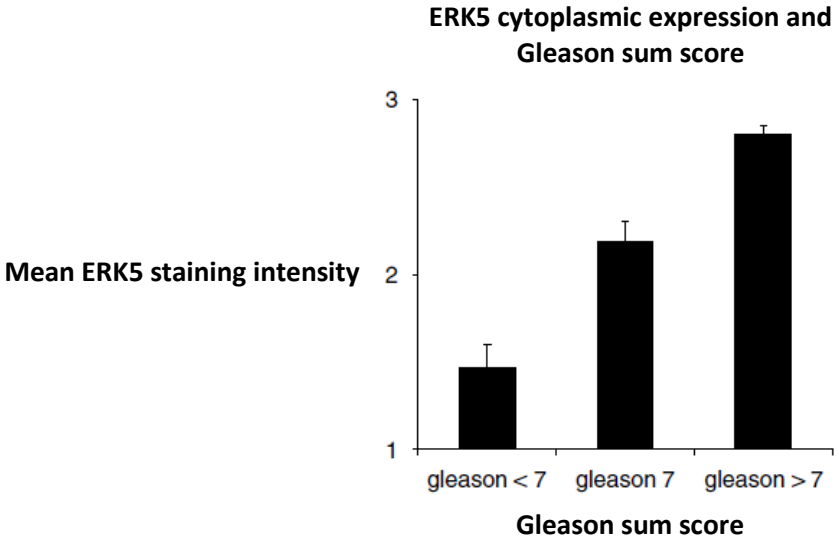


Figure 15. The relationship between ERK5 expression and Gleason sum score. Adapted from ref 42.

Deregulation of the MEK5/ERK5 signalling pathway is also related to increased metastatic potential of prostate cancer cells and poor disease specific (Figure 16). Elevated cytoplasmic and nuclear levels of ERK5 serve as an independent prognostic marker for advanced prostate cancer, with nuclear ERK5 expression present only in malignant cells.[42]

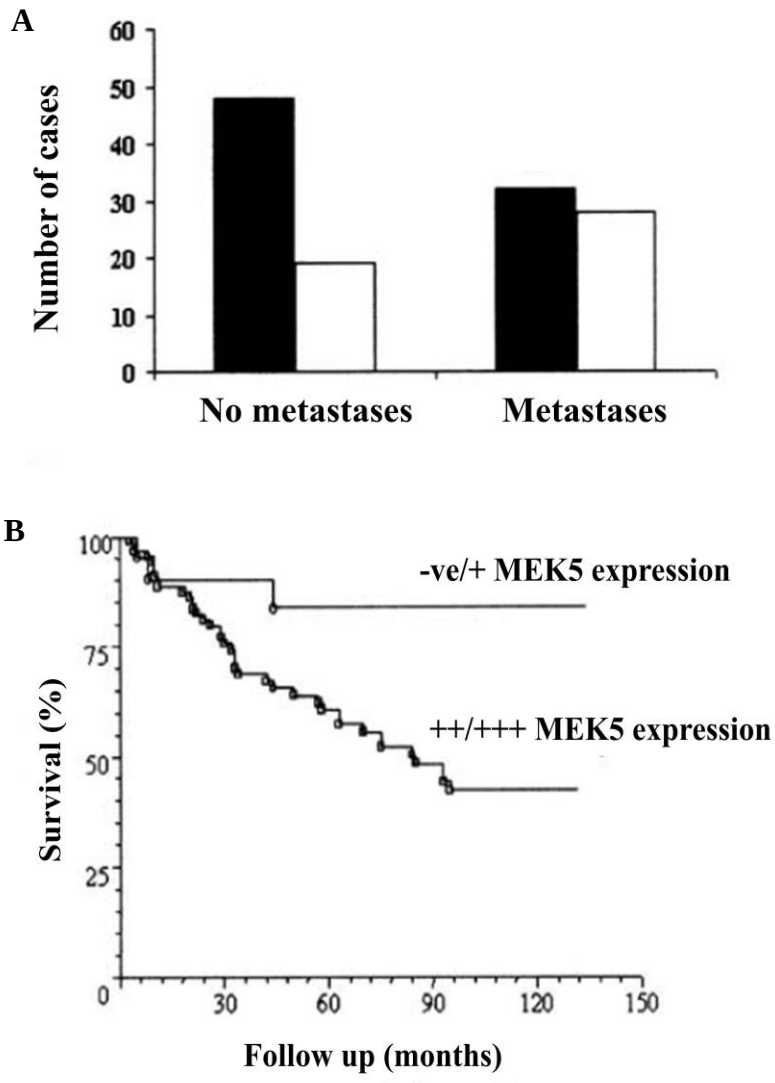


Figure 16. **A**:ERK5 expression in the presence and absence of bone metastases; black = No ERK5 expression detected; white = ERK5 expression detected.
B: Elevated ERK5 expression correlates with poor disease specific survival. Adapted from ref 42.

A prostate cancer cell line (PC3) was used in a xenograft study using mice. Prior to implantation, PC3 cells were either transfected with MAPK7 (the gene encoding ERK5) or empty vector. Cell proliferation was significantly higher in those transfected with MAPK7 with an increase in mean tumour volume at 40 days post implantation measured compared to the control (Figure 17).[42]

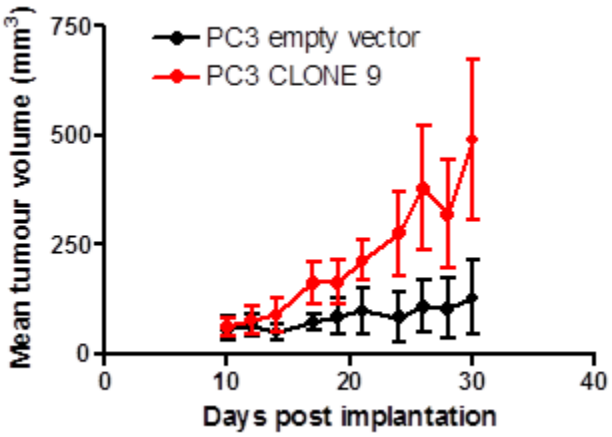
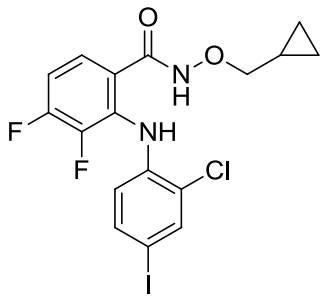


Figure 17. PC3 cells transfected with ERK5 show a more aggressive phenotype. Adapted from ref 42.

Inhibition of ERK5 using small-molecule PD184352 (**7**) was shown to reduce cell growth, migration and invasion in PC3 cells. PD184352 is a known allosteric inhibitor of MEK1/2, but when administered at high concentrations (3 μM in this study) inhibition of ERK5 is observed.[42]These results suggest that clinical inhibition of ERK5 may have a therapeutic application as an anti-proliferative, anti-metastatic agent.



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2.4.3.5.2 ERK5 and breast cancer

Breast cancer is the most commonly diagnosed cancer in women, with approximately 49,000 new cases in the United Kingdom in 2010 (Figure 18). Male breast cancer accounted for around 1% of all UK cancer diagnoses in 2010.[6]

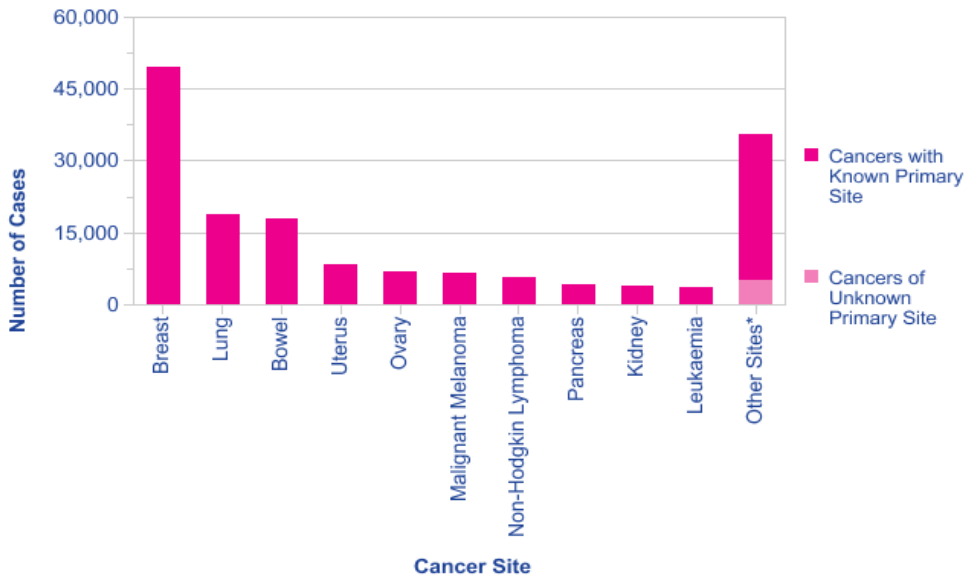


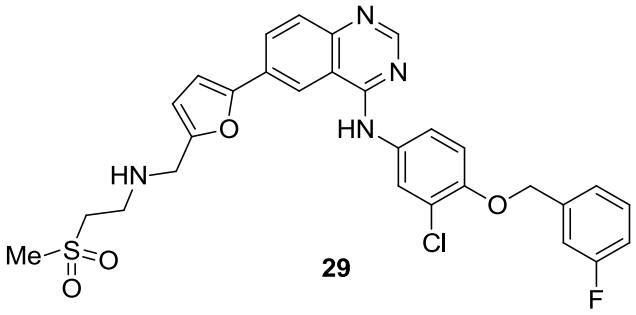
Figure 18. 10 most commonly diagnosed cancer in females in UK in 2010.[6]

The risk of developing breast cancer is strongly associated with production of the female hormone oestrogen and hormonal influences, such as use of the contraceptive pill and reproductive history exert a significant effect on the risk. Genetic predisposition is the most common risk factor. Mutations in the tumour suppressor genes BRCA1 and BRCA2 have been associated with an elevated risk of developing breast, increasing the lifetime risk to ≥80%.[66]

An increase in MEK5 activation was detected in a non-malignant telomerase immortalised breast cancer (TERT) cell line. The increase in levels of activated MEK5 is thought to be related to the oncogene Stat3 (signal transducer and activator of transcription 3), a member of a family of transcription factors responsible for cell growth and survival. Several genes are reported to be up-regulated by Stat3, including the matrix metalloproteinases MMP-1 and MMP-10, vascular endothelial growth factor (VEGF), and connective tissue growth factor (CTGF). In MDA-MB-435 Stat3 expressing breast carcinoma cells, MEK5 expression and activation was inhibited following the use of Stat3 small-interfering RNA (siRNA), suggesting that MEK5 may be directly regulated by Stat3 in some breast cancers.[77]

The HER2 receptor tyrosine kinase oncogene is found over-expressed in 20-30% of breast cancers, and is associated with a more aggressive phenotype, and poor disease specific survival. Monoclonal antibody trastuzamab (Herceptin) and small-molecule

inhibitor lapatanib (**29**) both target the HER2 protein, and are used clinically in patients with advanced or metastatic HER2-positive breast cancers.[78] The extracellular HER2 receptors mediate signalling *via* several pathways, including those involving the MAPKs.[78]



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In certain breast cancer cell lines, ERK5 levels are elevated due to binding of neuregulin to the HER2 receptor. Inhibition of ERK5 in combination with Herceptin has shown chemo-potentiation, sensitising cells to Herceptin treatment. ERK5 may be used as an independent prognostic factor in breast cancers with high levels of ERK5 correlating poor disease specific survival (Figure 19).[79] Therefore, the development of a potent ERK5 inhibitor may provide a more successful approach for the treatment of advanced HER2-positive breast cancer.

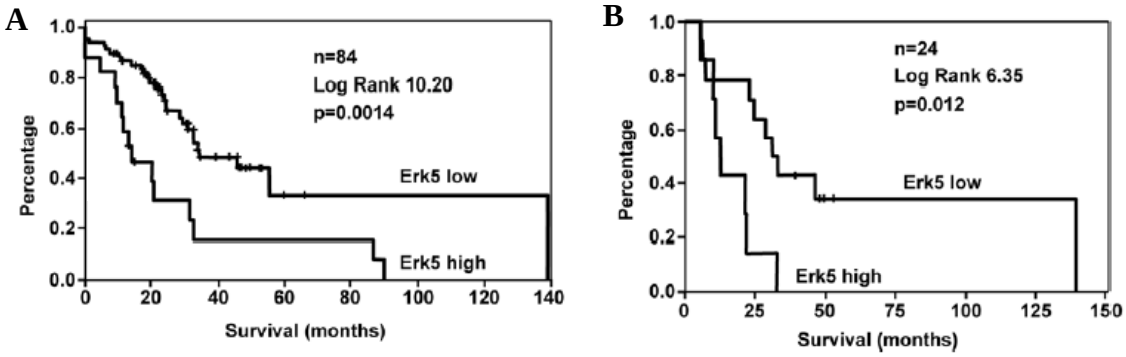


Figure 19. Disease-free survival with respect to ERK5 levels in; (**A**) patients with early stage breast cancer and (**B**) in patients with HER2 positive early stage breast cancer. Adapted from ref 76.

2.4.3.5.3 ERK5 and the development of human lung cancer

Insulin-like growth factor II (IGF-II) is over-expressed in a number of human lung cancer cell lines and correlates with a poor prognosis. ERK5 initiates IGF-II activation

of transcription factor, cAMP response element binding protein (CREB). CREB controls a number of genes including bcl-2, c-fos and erg-1, which are involved in cell-cycle progression. ERK5-mediated activation of IGF-II has been identified as essential in promoting growth of lung cancer cells. Therefore, an ERK5 inhibitor may prove a useful therapy for the treatment of lung cancer.[80-81]

2.4.3.5.4 ERK5 and leukaemia cell survival

ERK5 expression in leukaemia cells containing the Bcr/Abl oncogene promotes cell survival.[82] The Bcr/Abl oncogene is able to stabilise and promote ERK5 expression and activation. Leukemic cell line, MEG-01 were found to more readily enter apoptosis when they expressed lower levels of ERK5 and were treated with imatinib.[82] The level of ERK5 expressed in CML cells had a direct relationship with the effectiveness of treatment with imatinib, suggesting ERK5 may be a factor in development of chemoresistance.

Leukemic T-cells promote cell survival *via* activation of the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells), which is regulated by the MEK5/ERK5 pathway.[83] NF-κB is an anti-apoptotic protein resulting in increased cell survival. ERK5 involvement in the activation of NF-κB has been linked to the activation of RSK1 (a downstream target of ERK5), which liberates NF-κB from the cytoplasm.[83] An ERK5 inhibitor may have potential as a useful therapy for promoting apoptosis in leukemic T-cells or for treatment of phenotypes that have developed chemoresistance to imatinib.

2.4.3.5.5 ERK5 and hepatocellular carcinoma (HCC)

Hepatocellular carcinoma (HCC) is the most commonly diagnosed form of liver cancer.[84] Liver cancer in adults often suffers from poor prognosis at the time of initial diagnosis. The main risk factors associated with the development of liver cancer are exposure to the Hepatitis B and C viruses, and excessive consumption of alcohol.[6]

Investigation of DNA number copy abberations in HCC cell lines (SNU449 amd JHH-7) identified a novel gene amplification at the 17p11, which corresponds to an over-

expression of MAPK7 (the gene encoding for ERK5) (Figure 20). Over-expression of MAPK7 was thought to promote growth of HCC cells *via* regulation of mitosis.[84]

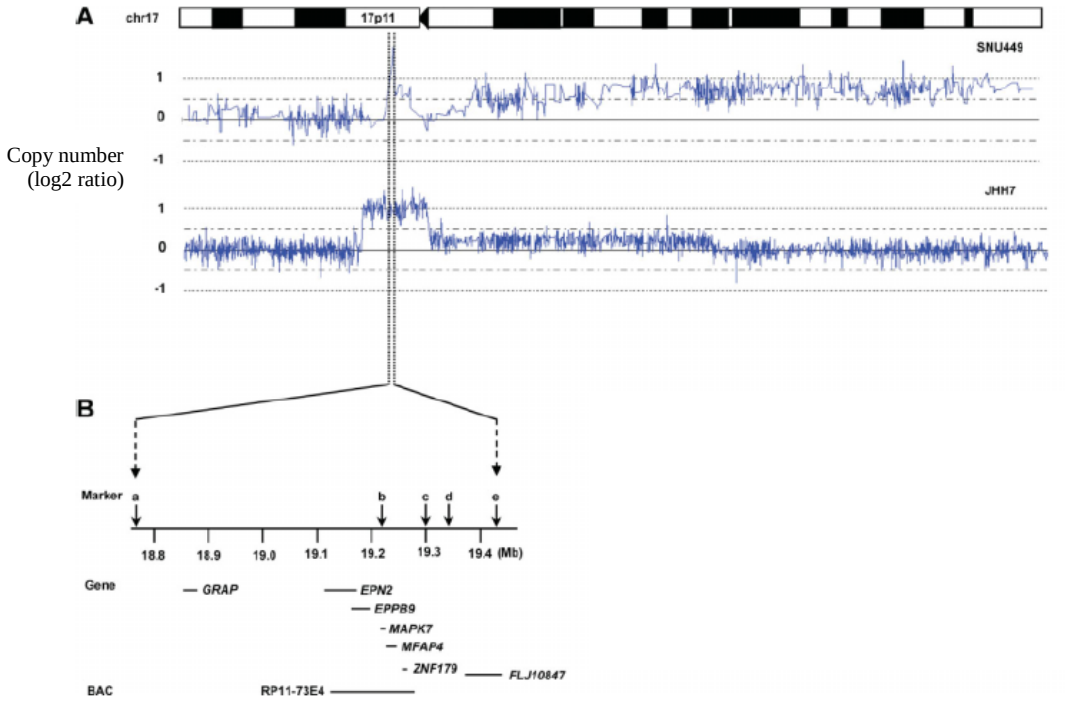


Figure 20. Amplicon map of the 17p11 chromosome in SNU449 and JHH-7 cells corresponding to an over-expression of MAPK7. Adapted from ref 81.

Immunohistochemical studies of 43 primary HCCs revealed ERK5 levels to be raised in aound 25% of cases. RNA interference using siRNA to knockdown MAPK7 in SNU449 cells resulted in suppression of cell growth, an effect attributed to the prevention of ERK5-mediated mitotic entry (Figure 21).[84]

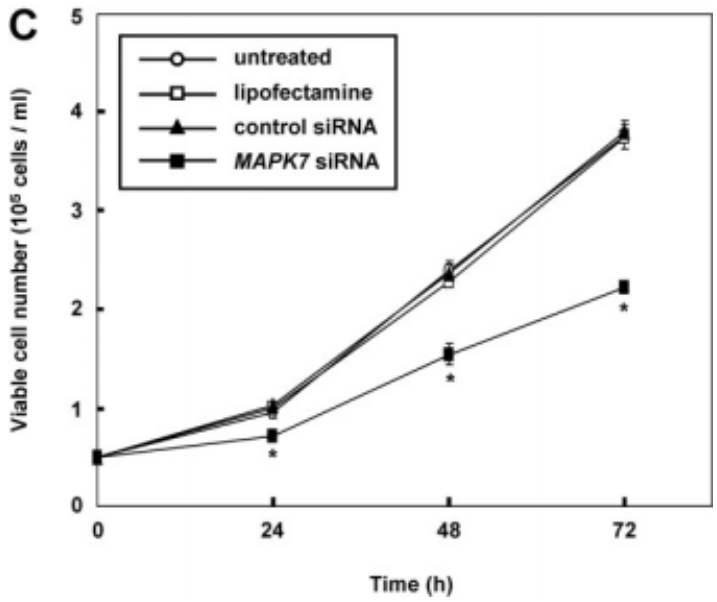


Figure 21. Knockdown of MAPK7 using siRNA in SNU449 cells. Adapted from ref 81.

An ERK5 inhibitor may, therefore, prove a valid therapeutic agent for patients with HCC.

2.4.3.5.6 ERK5 and multiple myeloma

ERK5 has also been implicated in the development of multiple myeloma (MM), which is the accumulation of neoplastic plasma cells of β-lymphocyte origin, associated with the immune system. MM accounts for 10% of all haematological malignancies and leads to the overproduction of antibodies that are no longer able to function normally.[85] The usual treatment is intensive chemotherapy which has little selectivity over normal and neoplastic cells. As for breast cancer, the Stat3 oncongene has been implicated in the progression of MM and is known to stimulate ERK5 activation.[85]

The myeloma growth factor, interleukin 6 (IL-6) has also been shown to activate ERK5 in MM cell lines. Expression of a dominant negative form of ERK5 was shown to reduce IL-6 dependent cell proliferation in MM cell lines.[85] Chemo-potentiation of current therapies is observed following ERK5 knockout in MM cell lines, meaning that development of an ERK5 inhibitor could improve the current treatment of MM by reducing the intensity of the treatment regime.[85]

2.4.3.5.7 ERK5 and malignamt mesotheliomas

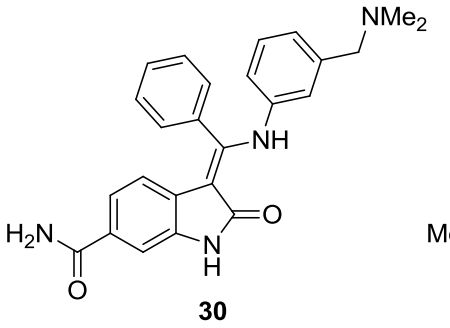
Malignant mesothelioma is a cancer of the pleura, pericardium and peritoneum associated with asbestos exposure.[86] At present there is no successful monotherapy for the treatment of malignant mesothelioma and survival post diagnosis is less than 12 months. Doxorubicin and cisplatin were originally used as a first-line monotherapy for the disease and are still used in combination with other chemotherapeutic agents. The current standard of care is treatment with cisplatin in combination with pemetrexed, a folate antimetabolite drug. This treatment improves patient survival from 9 to 12 months so there is still a need for improved therapeutics.[86]

ERK5 has recently been shown to be constitutively active in human mesothial cell lines (HMESO, H2373, H2595, H2461 and HP-1) and plays a key role in tumoorigenesis.[86] Three of the five cell lines tested showed significant activation of ERK5 after treatment with doxorubicin. Silencing of ERK5 using shRNA in HMESO and H2373 cells showed down-regulation of ERK5 and rendered cells more sensitive to doxorubicin treatment, suggesting the ERK5 may be involved in drug resistance in malignant mesothelioma patients.[86] ERK5 silencing was shown to correlate with a number of genes related to decreased proliferation, invasion and migration. In vivo studies were conducted in SCID mice and reduced tumour growth was observed in ERK5-silenced malignant mesotheliomas treated with doxorubicin compared control experiments.[86] This suggests that an ERK5 inhibitor may be able to attenuate tumour growth by chemo-potentiation of doxorubicin/cisplatin in human malignant mesotheliama. Studies are currently being conducted at Newcastle to see if this is a valid approach.

2.4.4 Inhibitors of the MEK5/ERK5 pathway

There are currently three published inhibitors of the MEK5/ERK5 pathway. BIX02188 (**30**) and BIX02189 (**31**) were developed by Boehringer Ingelheim Pharmaceuticals in 2008 and are indoline-6-carboxamide-based inhibitors. Compounds **30** and **31** inhibit the catalytic function of MEK5, blocking the phosphorylation and subsequent activation of ERK5. This leads to the inhibition of the transcriptional activity of MEF2C, a downstream substrate of the ERK5 pathway, resulting in reduced cell proliferation.[87] The two inhibitors are potent for both MEK5 and ERK5 and show excellent selectivity

for MEK5 and ERK5 against a wide panel of kinases with >100 fold selectivity against 85 of 87 kinases tested. Importantly there is no observed inhibition of the closely related kinases, ERK2 and MEK1/2.[87]



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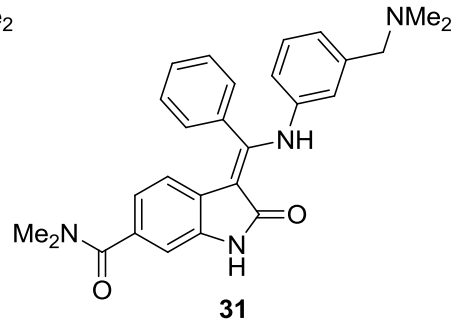
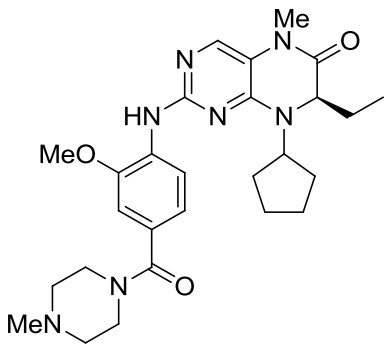
MEK5; IC_{50} = 4.3 nM
ERK5; IC_{50} = 810 nM



31

MEK5; IC_{50} = 1.5 nM
ERK5; IC_{50} = 59 nM

In 2010, an inhibitor of ERK5 was discovered by the Scripps Institute based on a series of isoform selective polo-like kinase (PLK) inhibitors.[53] Analogues of the potent, ATP-competitive PLK inhibitor, BI-2536 (**32**) were made, and after expansion of the aliphatic ring of BI-2536, PLK activity was reduced. The new inhibitor, XMD8-92 (**20**) displayed high selectivity for ERK5 when tested against a panel of 402 kinases in an ATP-site competition binding assay.[53] Notably, MEK5 was not inhibited by XMD8-92 up to a concentration of 50 µM. The IC_{50} was measured in an *in vitro* assay as 300 nM, meaning that XMD8-92 is both a potent and selective ERK5 inhibitor. A cellular assay was conducted also using HeLa cells and the IC_{50} for this was measured as 1.5 µM.[53, 88]



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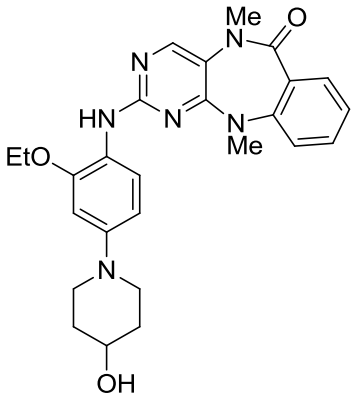


20

The *in vivo* efficacy of XMD8-92 was tested in two mouse tumour xenograft models using HeLa cells and Lewis lung (LL/2) cells. Growth inhibition of the tumours was observed over time, and importantly, inhibition of ERK5 was observed as well as the induction of p21 expression *via* PML activation.[53]

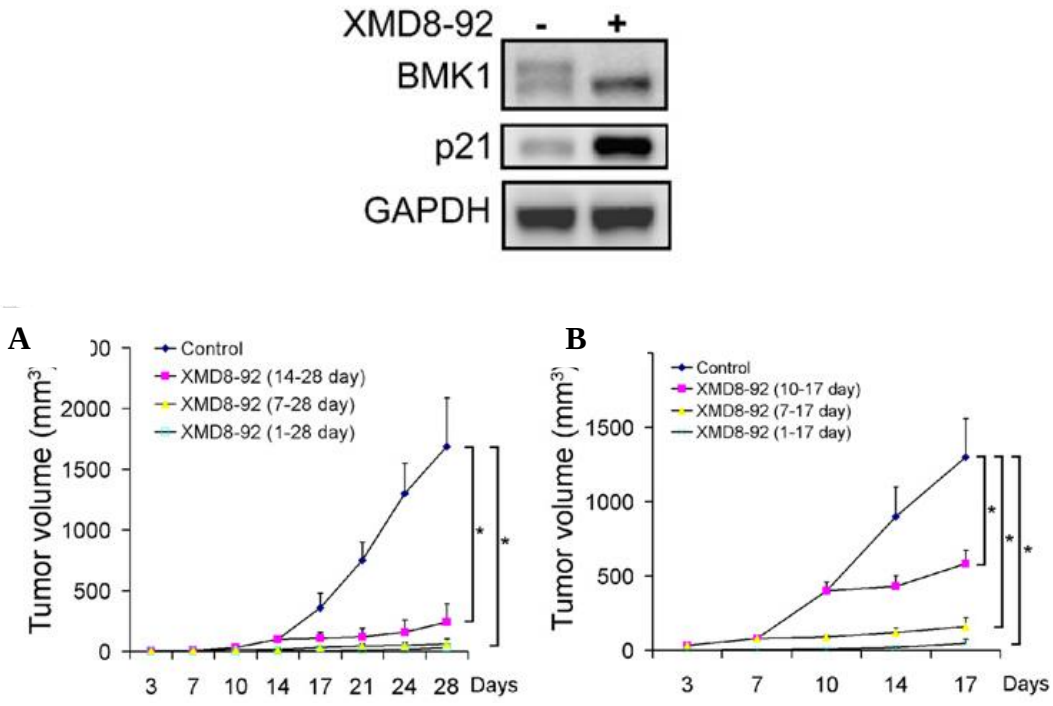


Figure 22. Western blot analysis showing inhibition of ERK5 and increased expression of p21 and (A) mouse xenograft model using HeLa cells and (B) LL/2 cells. Adapted from ref 52.

The discovery of a novel, selective ERK5 inhibitor with proven efficacy has assisted in the validation of our biological assays and guided PK studies on this project. XMD8-92 is now used as a benchmark for our 'in house' development of novel inhibitors of ERK5.

2.4.4.1 X-Ray crystal structure of ERK5 in complex with an analogue of XMD8-92

Until recently, the X-ray crystal structure of ERK5 had not been elucidated, but emerging interest in the MAPK led to the determination of the crystal structure in

complex with an inhibitor.[89] The kinase domain of ERK5 is known to have high structural similarity to a number of MAPKS; ERK1, 51%; ERK2, 51%; p38β, 47%; p38α, 46%; p38δ, 43%; NLK, 43% and p38γ, 38%, but it remains one of only two MAPKs for which the crystal structure remained unsolved.[89] The X-ray crystal structure of the ERK5 kinase domain was published in 2013 in complex with an analogue of XMD8-92 in order to aid the structure-guided design of novel inhibitors. The protein was expressed and purified from baculovirus-infected insect cells and protein crystals were obtained from construct 1-397 of human ERK5. The crystal structure was obtained to 2.8 Å resolution and the protein was in the inactive form.[89]

Inhibitor **33** forms two hydrogen bonds to the hinge region of the ERK5 ATP binding site at Met140 (Figure 23, C) from the pyrimidine nitrogen and the NH. Key hydrogen bonds are also made *via* a water molecule to the DGF motif Asp200 and Glu102 from the carbonyl oxygen (Figure 23, A).[89] The piperidine-piperazine rings point out of the ATP binding site towards solvent. Overall, the shape of inhibitor **33** fits extremely well into the ERK5 ATP binding site, explaining the inhibitory activity of the benzo[*e*]pyrimido[5,4-*b*]diazepine-6(11*H*)-one analogues. Interestingly, the ERK5 ATP binding site was found to have two unique residues; Gly199 before the DFG loop and Leu189 at the bottom of the ATP binding site. Despite structural similarities of the MAPKs, ERK5 is the only member to have these residues in the two specific locations.[89] This partly explains the specificity of inhibitors such as XMD8-92 and **33** for ERK5 and is key information for the development of novel selective inhibitors of ERK5.

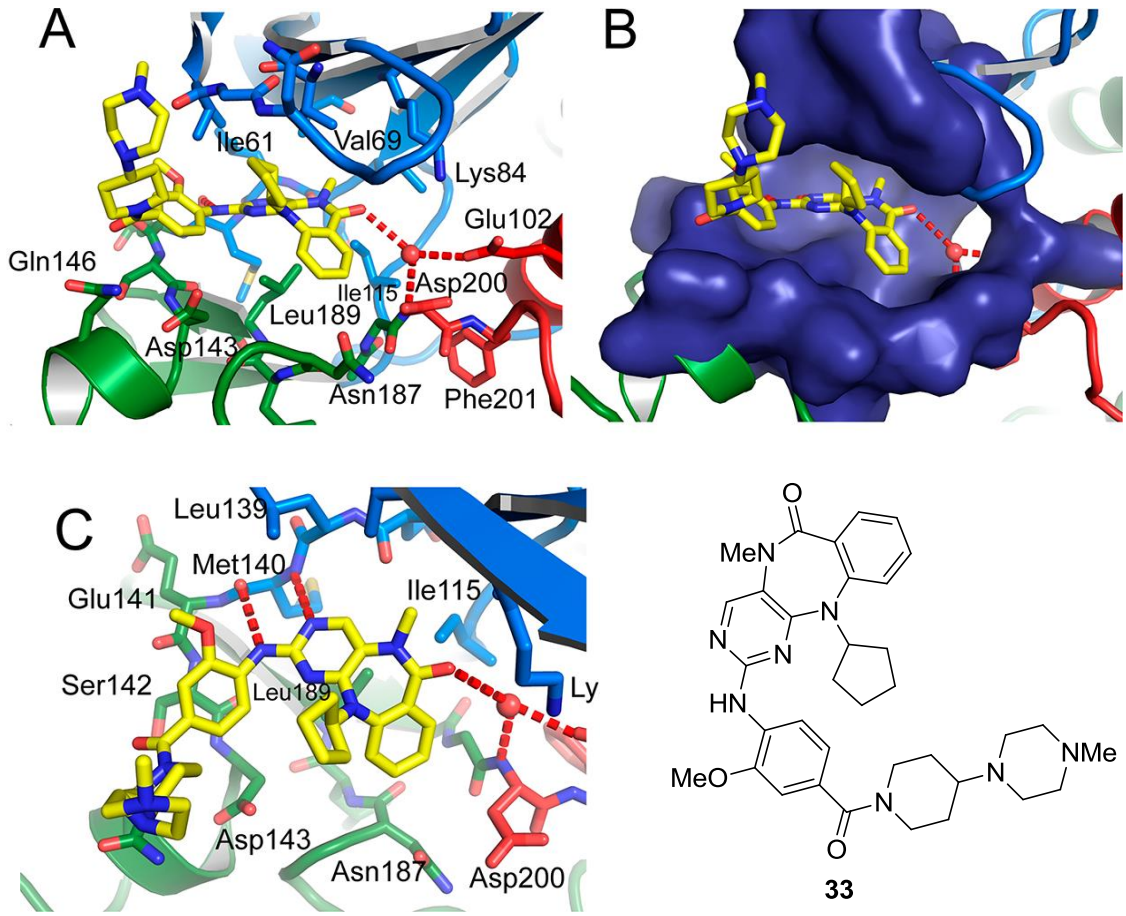


Figure 23. (A) Inhibitor **33** bound in the ERK5 ATP binding site; (B) as in (A) with the surface of the ATP binding site shown around **33**; (C) An alternative angle to A showing the key hinge binding.[86]

Chapter 3. The development of novel inhibitors of ERK5

3.1 HTS for novel inhibitors

In order to identify inhibitors of ERK5, a high throughput screen (HTS) was conducted using an immobilised metal affinity polarisation (IMAP^{TM}) assay at CRT-DL in 2007. The IMAP^{TM} assay assesses the potency of compounds by measuring fluorescence polarisation. As shown in Figure 24, the assay uses a fluorescent labelled peptide, which is specifically selected to bind to the kinase of interest. In this case, the labelled peptide was chosen to be a substrate for ERK5, which is activated *in situ* by the presence of activated MEK5. Out of 60 peptides screened, just one was identified for use in the assay. Once the labelled peptide is bound to the kinase, it is phosphorylated by ATP present in the assay (at 300 µM), enabling it to bind to metal (M(III))-containing nanoparticles *via* a high affinity interaction. This leads to a measurable increase in the fluoresence polarisation readout. In the presence of a potent inhibitor of the kinase, phosphorylation of the labelled substrate is impeded, meaning that fewer phosphorylated substrates are able to interact with the M(III) nanoparticles and the polarisation readout is reduced. The HTS for inhibitors of ERK5 was carried out using a kinase focussed collection of 9000 compounds and a full compound collection comprising 48,500 compounds and confirmed hit compounds as having ERK5 inhibitory activity.

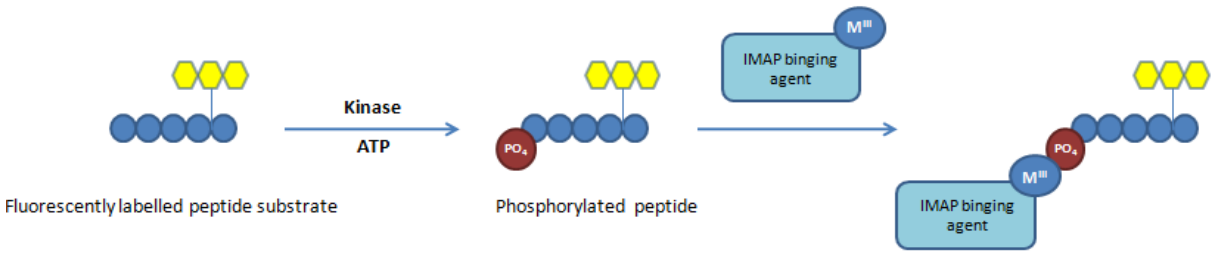
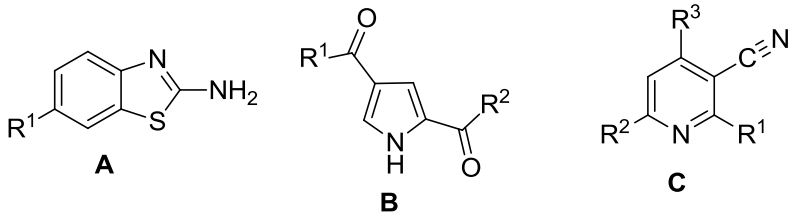


Figure 24. IMAP^{TM} assay format.

Following the high-throughput screening campaign, three distinct chemical series were identified as having good inhibitory activity against ERK5 based on their performance in the assay, the benzothiazoles (**A**), pyrrole carboxamides (**B**) and the cyanopyridines (**C**).



Initially, all three of these chemical series were investigated, with the most potent compounds from each series being re-synthesised and tested in order to validate the series as true 'hits'. In the benzothiazole series, the re-synthesised hits were not validated and so further work into the development of inhibitors based around this chemical series was discontinued. The cyanopyridine series has also been discontinued. In this case, although the original hits were validated, it became apparent that there was a very narrow SAR around this chemical series and as a result, modifications that were made to the core structure did not improve ERK5 inhibitory activity.

The final of these three series, the pyrrole carboxamide series, was validated by re-synthesis of the initial hit compounds, which had activity in the 0.66 – 33 μM range (Table 2). This chemical series has now progressed to the lead optimisation stage of drug discovery with key SARs having been identified.

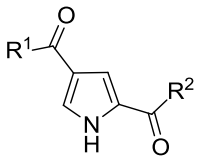
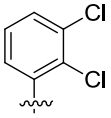
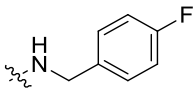
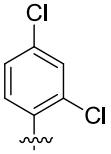
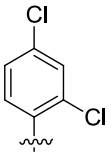
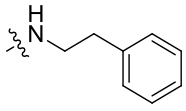
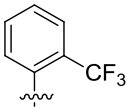
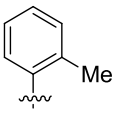
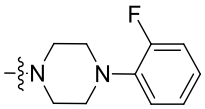
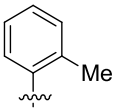
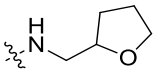
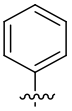
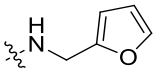
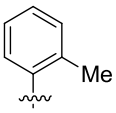
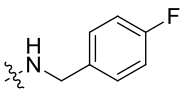
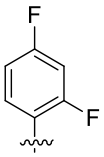
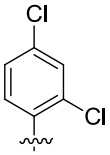
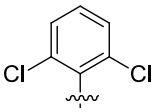
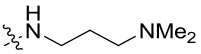
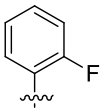
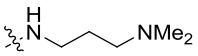
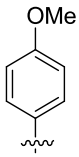
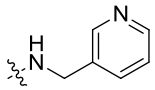
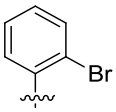
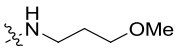
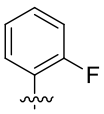
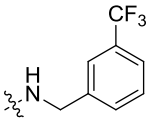
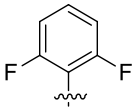
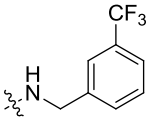
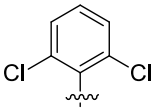


Table 2. Initial pyrrole carboxamide hits identified and corresponding IC_{50} values.

Compound	R^1	R^2	ERK5; IC_{50} (μM)
34			3.7
35			1.9
36			3.5

37			4.3
38			5.5
39			6.0
40			6.3
41			11
42			12
43			14
44			14
45			15
46			16
47			17
48			17
49			23
50			33

3.2 Preliminary structure-activity relationship studies (SARs) around the pyrrole carboxamide scaffold

Initial SARs were conducted by Dr Sandrine Vidot and Dr Ruth Bawn.[90] A 3-fold improvement in potency was considered significant after n = 2. Modifications to the aroyl ring found that substitution in the *para*-position was not tolerated. Dihalogenation in a 2,3- or 2,6-substitution pattern conferred the best potency exemplified by inhibitors with 2,3-dichloro and 2,6-difluorobenzoyl rings. *N*-Methylation of the pyrrole nitrogen abolished activity (ERK5; IC_{50}>120 μM), indicating that the pyrrole NH is important and possibly acts as a hydrogen bond donor in the hinge region of the ATP binding site of ERK5. *N*-Methylation of the carboxamide motif was tolerated, but activity was 5-fold lower than the non-methylated counterpart. Complete removal of the ketone of the benzoyl motif or reduction to the corresponding secondary alcohol also resulted in loss of activity (ERK5; IC_{50}>120 μM), suggesting the possible need for a hydrogen bond acceptor in this position. Finally, 3- and 4-pyridyl substituents were found to be equipotent with the 4-fluorophenyl moiety present in the carboxamide side-chain. The most potent compounds identified in preliminary SARs are shown in Table 3.

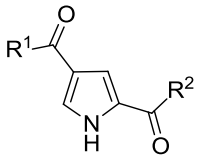
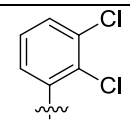
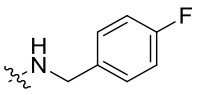
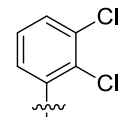
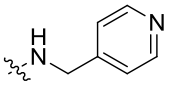
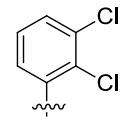
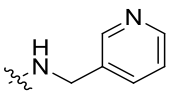
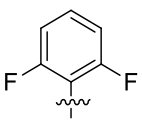
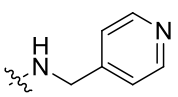
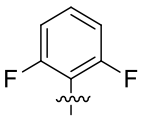
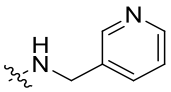
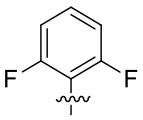
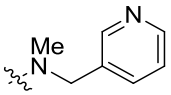


Table 3. Preliminary SAR data around the pyrrole-carboxamide core

Compound	R^1	R^2	ERK5; IC_{50} (μM)
34			3.7 ± 0.8 (n = 2)
51			2.0 ± 2.0 (n = 10)
52			3.8 ± 3.7 (n = 6)
53			2.3 ± 0.1 (n = 2)

54			1.8 ± 0.6 (n = 10)
55			9.0 ± 0.2 (n = 2)

3.3 Selectivity of hit compounds

The MAPK, p38α is a close homologue of ERK5, with 48% sequence identity in the kinase domain and 56% in the active site. ERK2 is the closest homologue with 51% sequence identity in the kinase domain and 78% in the active site. Despite ERK2 having more structural identity with ERK5, it has a larger gatekeeper residue (glutamine) than ERK5 (leucine) meaning that gaining selectivity for ERK5 over ERK2 is easily achieved. The gatekeeper residue in p38α is a threonine residue and, as such, is more similar in size to the leucine gatekeeper residue of ERK5. This means achieving selectivity for ERK5 over p38α is more challenging. It is therefore essential to screen compounds with inhibitory activity for ERK5 against p38α. Compounds are tested for p38α activity using a LANCE assay. The LANCE assay uses time resolved fluorescence energy transfer (TR-FRET) in order to determine IC_{50} values. The assay uses U*Light*, which is an acceptor dye with a red-shifted fluorescent emission, and a europium chelate donor dye. The kinase of interest is labelled with the U*Light* dye and is phosphorylated. A europium labelled antibody is able to bind to the phosphorylated kinase, bringing the donor and acceptor in close proximity. Upon irradiation at 320 nm, FRET occurs between the europium donor and the U*Light* acceptor and light is emitted at 665 nm. The intensity of the light emitted is measured and in turn converted into the IC_{50} value for an inhibitor. If the phosphorylation of the U*Light* labelled kinase has been inhibited, the light emitted after FRET will be less intense (Figure 25).

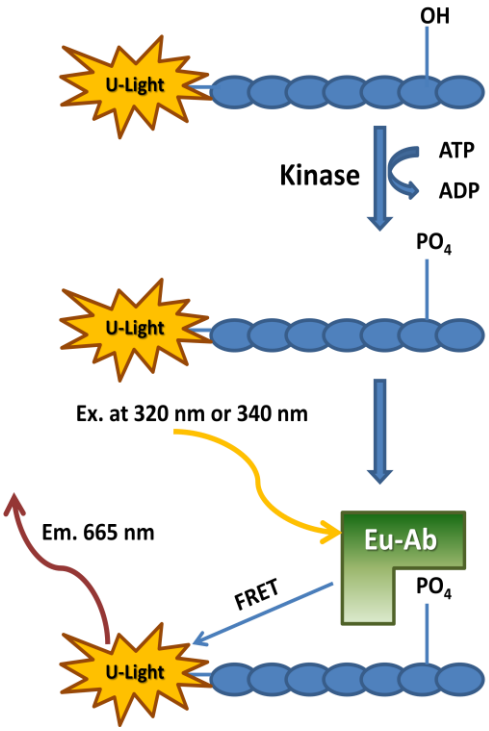
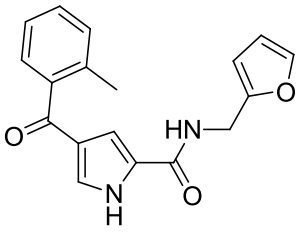


Figure 25. LANCE Assay format.

Pyrrole-carboxamide-based inhibitors of p38α have recently been described in the literature, with compound **56** showing good inhibitory activity (pIC_{50} = 6.6).[91] Compound **56** and the other analogues described are structurally similar to the Newcastle ERK5 pyrrole carboxamide inhibitor series, further highlighting the need for p38α counter-screening on all synthesised compounds. Subtle structural modification of the ERK5 pyrrole-carboxamide series is essential to obtain both potency against ERK5 and selectivity over p38α taking advantage of the key differences in the active sites of the two MAPKs.



56

3.4 Development criteria for pyrrole carboxamide ERK5 inhibitors

Following the HTS, the initial pyrrole carboxamide hit compounds were validated as ERK5 inhibitors *via* resynthesis and testing. Once clear SARs have been established, hit-to-lead studies can be conducted. If an inhibitor meets the criteria *in vitro*, *in vivo* efficacy and DMPK profile will be assessed. The desired criteria identified for a lead compound in the ERK5 project are outlined in Table 4.

Table 4. Desired criteria for a lead compound in the ERK5 pyrrole carboxamide series.

Category	Parameter	Lead criteria
Physicochemical properties	Molecular weight (MW)	<500
	sLogP	<5
	TPSA ($Å^2$)	75-100
	H bond donors	<5
	H bond acceptors	<10
***In Vitro* pharmacology**	ERK5; IC_{50} (µM)	< 1
	LE*	>0.3
	Selectivity	>10-fold
	Cellular ERK5; IC_{50} (µM)	<10
	hERG; IC_{50} (µM)	>25
***In Vitro* ADME**	Solubility L/U (µM)**	>50/50
	PPB (%)***	<99
	Mouse liver microsomal (MLM) clearance (µL/min/mg)	<48
	Human liver microsomal (HLM) clearance (µL/min/mg)	<48
	Caco-2 A2B Papp ($x10^{-6}$ cm/s)	>10
	Efflux Ratio	<2
	CYP1A, CYP2C19, CYP2C9, CYP2D6, CYP3A4; IC_{50} (µM)	All >10

***In Vivo* DMPK and Efficacy**	Clearance (mL/mg/kg)	<30
	Vdss (L/kg)	>1
	Bioavailability (F) (%)	>30
	$t_{1/2}$ Mouse (h)	>1
	$t_{1/2}$ Human (h)	>1

* LE = ΔG/HAC (where ΔG = -1.4 logIC_{50})
**L/U = Lower bound/Upper bound solubility range
*** PPB in human

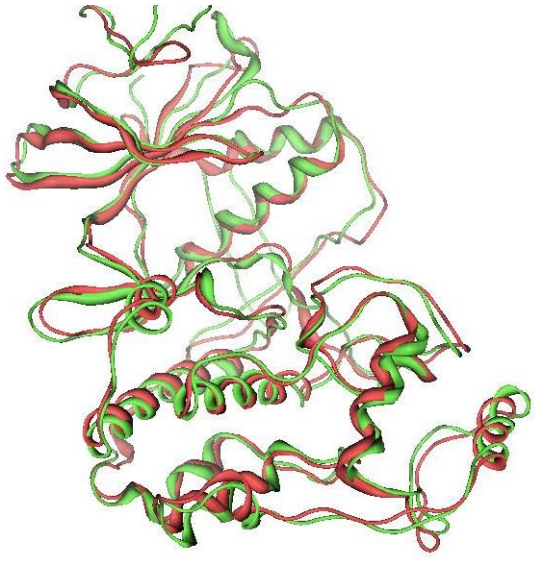
These criteria were utilised throughout the hit-to-lead phase of this drug discovery project. The physicochemical property criteria desired for a lead compound were based upon Lipinski's rule of five (molecular weight, logP and number of H-bond donors and acceptors) since an orally bioavailable compound was desired. TPSA was also evaluated during this stage to give an indication of how cell permeable a compound may be. The desired range of 75-100 $Å^2$ was chosen to give the best chance of having compounds that would be cell membrane permeable but would not cross the blood-brain barrier (compounds with a TPSA <75 may be lipophilic enough to do this) . Solubility data are generated by Cyprotex using a turbidimetric assay and are reported as an estimated precipitation range, L/U (Lower bound/Upper bound). The assay measures solubility of compounds by diluting in DMSO in aqueous buffer. Turbidimetry is used as the endpoint by measuring absorbance at 620 nm. It can be assumed that the compound will precipitate at some point during this range. A solubility value of less than 1µM would represent a highly insoluble compound, between 1 and 100µM partially soluble and >100µM a soluble compound.*In vitro* pharmacology was evaluated based on potency for ERK5 in the cell-free IMAP assay (IC_{50} < 1 µM) and in a cellular assay (IC_{50} < 1 µM), selectivity over other kinases and the compound's ligand efficiency (LE). LE is a measure of overall binding energy per heavy atom, with a value greater than 0.3 for LE indicating efficient binding. Routine counter-screening of inhibitors against p38α was conducted as discussed previously and for interesting compounds a counter-screen for selectivity against a panel of 131 diverse protein kinases was performed at the International Centre for Kinase Profiling in Dundee. Inhibition of hERG may also be measured at this stage.

Assessment of *in vitro* ADME determines if compounds meet the desired criteria for aqueous solubility, plasma protein binding (PPB) and microsomal clearance (tested in both mouse and human liver microsomes). Meeting the outlined criteria for *in vitro* ADME provides the best chance of good oral bioavailability and half-life *in vivo*.

3.5 Structure-guided design

3.5.1 First generation ERK2-derived homology model

In the absence of a crystal structure of ERK5, homology modelling by sequence alignment and superimposition was conducted by Dr Susan Boyd on behalf of CRT-DL. ERK2 was identified as the closest homologue of ERK5 in a basic alignment search tool (BLAST) search, with 51% and 78% sequence homology in the kinase and ATP binding site domains, respectively and so the first generation homology model was based on ERK2. In the development of the homology model, three key differences were identified in the conserved residues of the two kinases; Ile103 (ERK2)/Val134 (ERK5), Gln105 (ERK2)/Leu136 (ERK5) and Cys164 (ERK2)/Gly198 (ERK5). Figure 26 shows the ERK2 template overlayed with the ERK5 homology model.

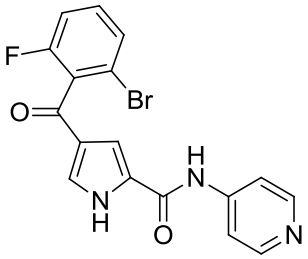


Red – ERK2 Template
Green – ERK5 Template

Figure 26. Overlay of ERK5 homology model with ERK2 template.

3.5.2 Second generation p38α-derived homology model

p38α is a the second closest homologue of ERK5, with 48% sequence identity in the kinase domain and 56% in the active site. A second generation homology model of ERK5 based on p38α was recently developed at Newcastle University. The p38α crystal structure was obtained from the protein data bank (PDB) and computationally mutated using MODELLER software to express the amino acid residues present in ERK5 and generate the homology model. The five key differences were identified as Glu110 (p38α)/Ala107 (ERK5), Leu103 (p38α)/Thr106 (ERK5), Ala111 (p38α)/Ser108 (ERK5) Ala157 (p38α)/Leu155 (ERK5) and Leu167 (p38α)/Gly165 (ERK5) (Figure 27). Pyrrole-carboxamide inhibitors can be built into the ERK5 model using GROMACS (GROningen MAchine for Chemical Simulations) software enabling the structure-based design of new inhibitors exploiting previously unexplored areas of the ERK5 binding site. The docking of pyrrole carboxamide inhibitor **57** will be discussed in more detail in section 5.3.5.2 of chapter 5.



57

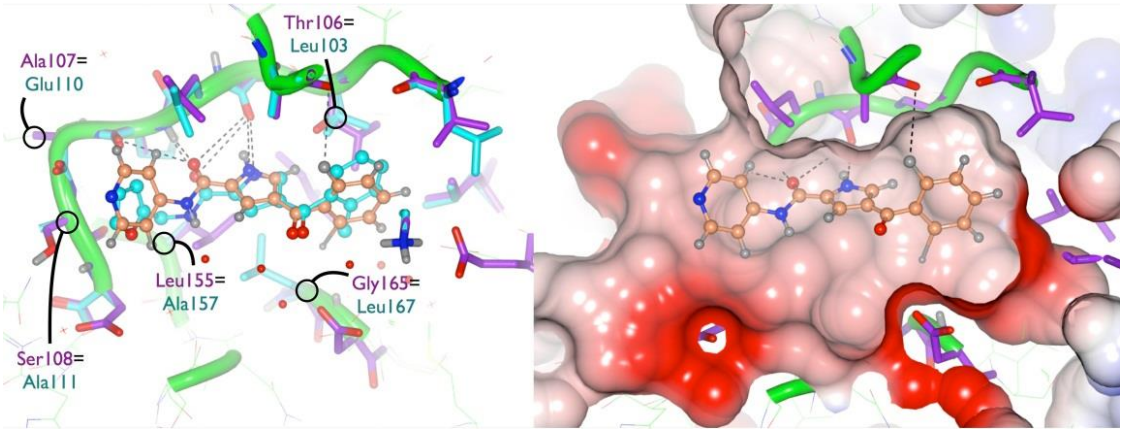


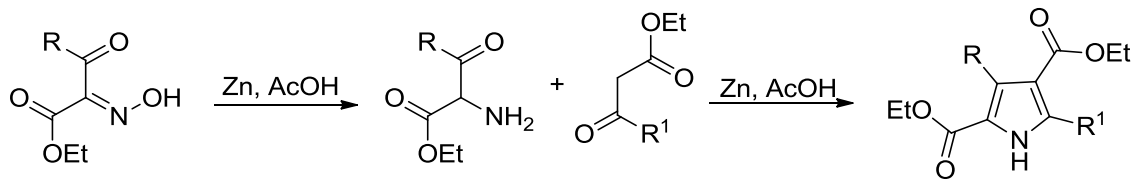
Figure 27. (A) ERK5 model (magenta) compared to p38α crystal structure (cyan), both shown in complex with pyrrole carboxamide inhibitor **57**; (B) **57** docked in the p38α-derived homology model.

3.6 Synthesis of pyrroles

Since we were interested in the synthesis of pyrrole carboxamide inhibitors of ERK5, the synthesis of the core pyrrole scaffold was investigated. There are three classical methods for the synthesis of pyrroles; the Knorr synthesis, Paal-Knorr synthesis, and the Hantzsch synthesis.

3.6.1 The Knorr pyrrole synthesis

The Knorr pyrrole synthesis was first published in 1884 and describes the reaction between an α-aminoketone with a β-ketoester in the presence of zinc and acetic acid to form pyrrole-3-carboxylates.[92] Oximes are used in place of α-aminoketones and are reduced *in situ* by zinc to prevent self-condensation prior to condensation with the β-ketoester (Scheme 1). After condensation with the β-ketoester, an aldol-type intramolecular condensation takes place to afford the substituted pyrrole. In the original reaction the R and R^1 groups were both methyl and this compound is known as Knorr's pyrrole.[92-93]

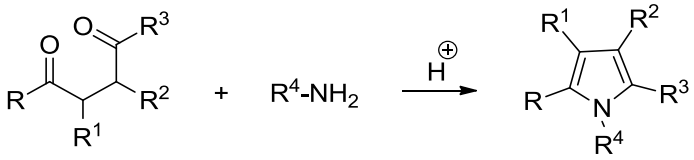


Scheme 1. Knorr pyrrole synthesis

3.6.2 The Paal-Knorr pyrrole synthesis

An updated version of the Knorr pyrrole synthesis was developed by Knorr and Paal independently originally for the synthesis of substituted furans. This was later adapted by the two for the synthesis of substituted pyrroles and thiophenes.[94] For pyrrole synthesis a 1,4-dicarbonyl compound is reacted with an excess of ammonia or a primary amine (Scheme 2). The mechanism for the Paal-Knorr synthesis was not understood until it was investigated in the 1990s by Amarnath *et al*, who discovered that the amine attacks a protonated carbonyl to form a hemiaminal intermediate.[95] The amine is then able to attack the other carbonyl group to form a 2,5-dihydroxytetrahydropyrrole, which

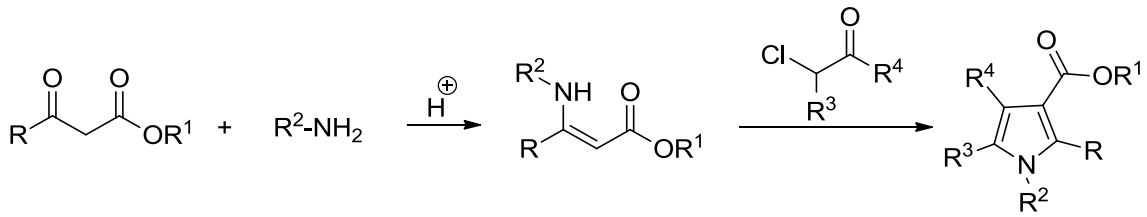
undergoes dehydration to produce the substituted pyrrole. The reaction can be performed in neutral or weakly acidic conditions.[93]



Scheme 2. Paal-Knorr pyrrole synthesis.

3.6.3 The Hantzsch pyrrole synthesis

The Hantzsch pyrrole synthesis was described in 1890 and is a multicomponent reaction.[96] The first step involves the reaction of a β-ketoester with a primary amine to form an enamine (Scheme 3). The enamine then reacts with an α-haloketone and subsequent cyclisation occurs to afford the pyrrole product.[96] The Hantzsch pyrrole synthesis suffers from poor reaction yields due to formation of furan by-products and so a solid-phase version of the reaction has been developed in more recent years to improve reaction yields and reduce by-product formation.[93, 97]



Scheme 3. Hantzsch pyrrole synthesis

Chapter 4. Structure activity relationship studies (SARs) around the amide side-chain

4.1 Synthesis of ERK5 inhibitors based on potent CRT hits 58 and 59

4.1.1 Rationale

A high throughput screening (HTS) campaign was conducted at CRT-DL in 2007 comprising a diverse library of around 48500, and a kinase-focussed library of around 9000 compounds. Following this HTS, two hit triazole compounds (**58** and **59**) were identified as potent ERK5 inhibitors (IC_{50} = 0.31 and 0.20 µM, respectively). To expand the SARs, the synthesis of four compounds derived from primary hits **58** and **59** (Figure 28) was proposed. The new targets contain a core pyrrole ring, a benzoyl substituent, and for compounds **60** and **61**, an amide moiety.Compounds **62** and **63** were more similar to the CRT hits **58** and **59** and have an amine directly attached to the core pyrrole ring at the 2-position.

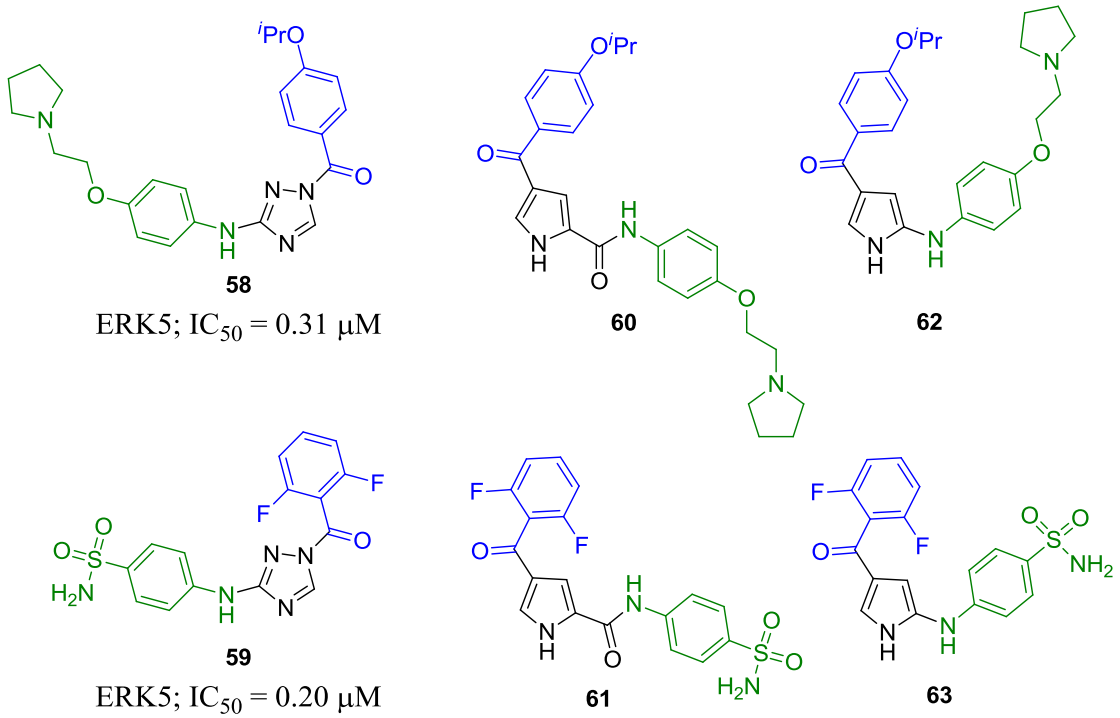
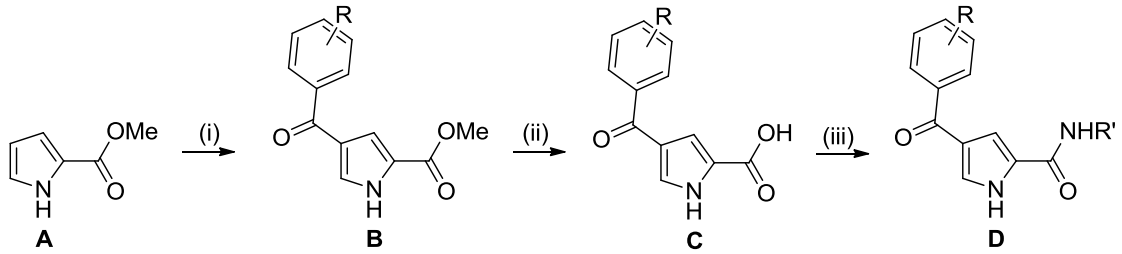


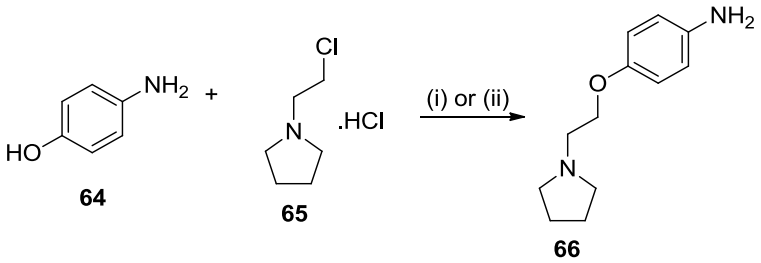
Figure 28. CRT hit compounds and the 4 related compounds proposed for synthesis

4.1.2 Synthesis of 4-(4-isopropoxybenzoyl)-N-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-1H-pyrrole-2-carboxamide (60) based on CRT hit 58

For compounds **60** and **61**, a general synthetic scheme previously optimised within the NICR medicinal chemistry group was utilised (Scheme 4).[90] This 3-step route involved an amide coupling as the final step to make pyrrole carboxamide compounds of general structure **D**. For compound **60**, the amine side chain had to be synthesised, as it was not commercially available. Initially an *O*-alkylation of 4-aminophenol (**64**) using 1-(2-chloroethyl)pyrrolidine hydrochloride (**65**) as shown in Scheme 5 was performed.[98] The reaction was performed under conventional heating (75 ^{o}C, 2h), to give compound **66** in 46% yield. Performing the reaction under microwave irradiation for various times did not improve the yield. The low yield could be attributed to the possible *N*-alkylation of the anilineand the fact that the electron donating amino substituent makes the phenol less acidic.



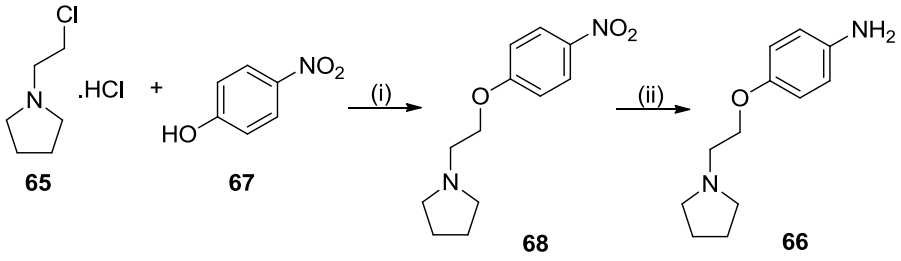
Scheme 4. *Reagents and conditions*: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h; (ii) LiOH (aq), THF, 60 ^{o}C, 18 h; (iii) (a) CDI, THF, 70 ^{o}C, 3 h; (b) appropriate amine, 50 ^{o}C, to RT, 18 h.



Scheme 5. *Reagents and conditions*: (i) DMF, NaOH, 2 h, 75 ^{o}C; (ii) DMF, NaOH, 30 min, MW, 150 ^{o}C.

Due to the low yield of compound **66**, a modified 2-step approach starting with the *O*-alkylation of 4-nitrophenol (**67**) followed by reduction of the nitro group was undertaken (Scheme 6). The electron withdrawing nitro group should increase the

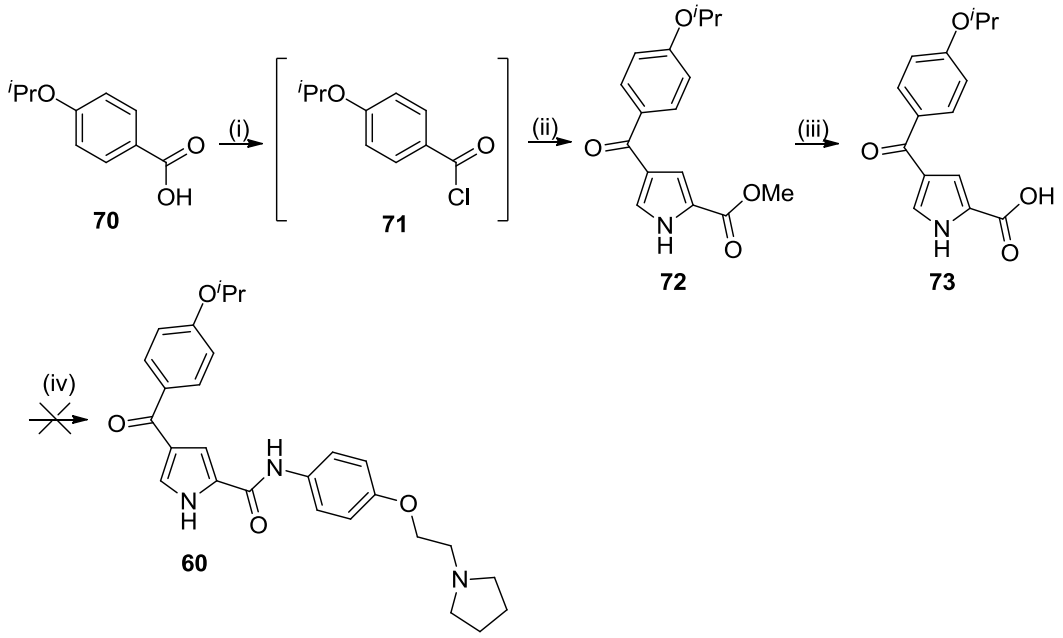
lability of the phenol proton making the alkylation easier, and using the nitro compound rather than the aniline avoided the possibility of amine alkylation. Alkylation of 4-nitrophenol (**67**) with **65** in the presence of potassium carbonate was successful with a good yield of 65%. The subsequent reduction of the nitro group was performed successfully in 92% yield.



Scheme 6. *Reagents and conditions*: (i) K_2CO_3, DMF, 80 ^oC, 2 h; (ii) Zn, AcOH, RT, 18 h.

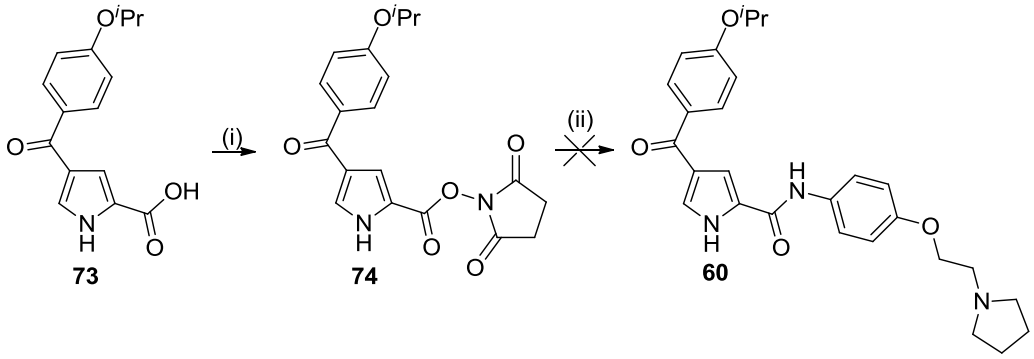
Once the amine side chain was synthesised, the general synthetic route shown in Scheme 4 was applied. The first step was a Friedel-Crafts acylation of the commercially available methyl pyrrole 2-carboxylate (**69**) using 4-isopropoxybenzoyl chloride **71** and aluminium chloride to give methyl ester **72** in 65% yield (Scheme 7). The acylation occurred regioselectively in the 4-position of the pyrrole ring directed by the electron withdrawing substituent in the 2-position

Subsequent ester hydrolysis using lithium hydroxide provided carboxylic acid **73** in 97% yield. In order to couple aniline **66** to carboxylic acid **73**, the optimised conditions using CDI as the coupling agent were employed, but were unsuccessful.



Scheme 7. *Reagents and conditions*: (i) $SOCl_2$, DMF, THF, RT 3 h; (ii) methyl pyrrole 2-carboxylate (**69**), $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (iii) LiOH (aq), THF, 60 ^oC, 18 h; (iv) (a) CDI, THF, 70 ^oC, 3 h; (b) **66**, 50 ^oC to RT, 18 h.

To activate carboxylic acid **73**, *N*-hydroxysuccinimide and DCC were used to form succinimide ester **74** (Scheme 8). Aniline **66** was then added to **74**, but unfortunately the coupling was unsuccessful. The reaction between succiminimide ester **74** and aniline **66** was attempted at RT and under microwave irradiations at various temperatures but with no success (Table 5). A possible explanation for the lack of reaction is the basic nature of the side chain.

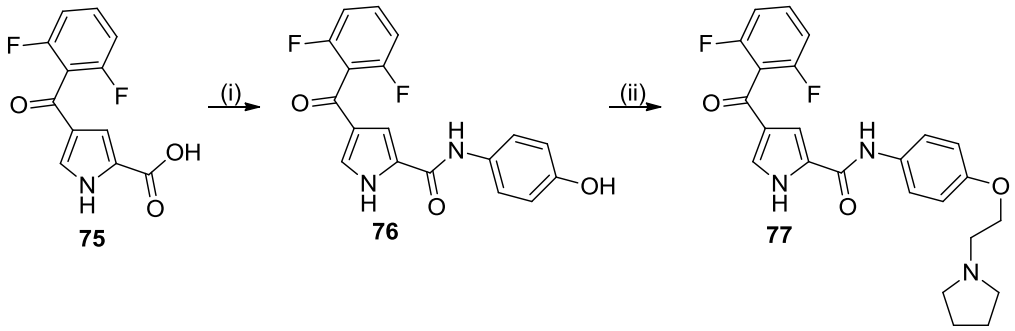


Scheme 8. *Reagents and conditions*: (i) *N*-hydroxysuccinimide, DCC, EtOAc, 0 ^oC, 2 h to RT, 18 h; (ii) See Table 5 for conditions.

Table 5. Attempted reaction conditions for step (ii) Scheme 8.

Entry	Solvent	Temp. (^{o}C)	Heating Method	Time (h)	Result
1	THF	RT	-	18	No reaction
2	THF	100	MW	0.5	No reaction
3	Dioxane	150	MW	0.5	No reaction

A new approach was modelled coupling 4-aminophenol (**64**) to 2,6-difluoro analogue **75** followed by alkylation of phenol **76** (Scheme 9). The first coupling reaction was performed successfully in 54% yield to afford compound **76**. This phenol intermediate was also deemed to be an interesting target for biological testing, but, unfortunately, it was not stable and therefore could not be sent for biological evaluation. The alkylation step to form **77** was attempted under several conditions using potassium carbonate and DMF (Table 6). Entry 4 shows the best conditions (MW, 100 ^{o}C, 45 min) giving **77** in a moderate yield of 35%. In this reaction an extra 0.5 equivalent of potassium carbonate and 1-(2-chloroethyl)pyrrolidine.HCl were used, which did not significantly improve the yield compared to the conditions shown in entry 3.



75 **76** **77**

Scheme 9. *Reagents and conditions*: (i) (a) CDI, THF, 70 ^{o}C, 3 h; (b) 4-aminophenol, 50 ^{o}C to RT, 18 h; (ii) 1-(2-chloroethyl)pyrrolidine.HCl, DMF, NaOH, 80 ^{o}C, 2 h.

Table 6. Conditions attempted for the alkylation reaction in step (ii) Scheme 9.

Entry	Temp. (^{o}C)	Heating Method	Time (h)	% yield
1	80	Conventional	2	5%*
2	80	Conventional	18	17%*
3	100	MW	0.25	31%*
4	100	MW	0.75	35%**

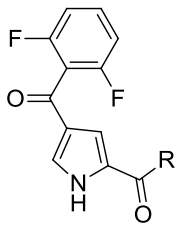
*Conversion by LCMS, **Reaction was performed with an extra 0.5 equivalent of 1-(2-chloroethyl)pyrrolidine.HCl and potassium carbonate.

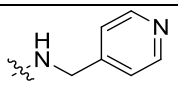
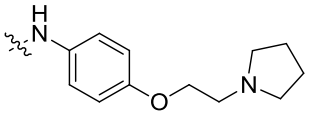
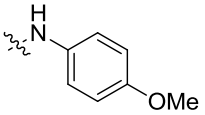
Despite the low yield, compound **77** was successfully synthesised and sent for biological evaluation (section 4.1.3). During the synthesis of compound **77**, a model reaction was conducted whereby carboxylic acid **75** was coupled with *p*-anisidine using CDI, leading to the synthesis of compound **78**, which was also tested for ERK5 inhibitory activity (section 4.1.3).

4.1.3 Biological evaluation of compounds 77 and 78

The ERK5 inhibitory activity for **77** and **78** is shown in Table 7, along with the inhibitory activity of hit pyrrole carboxamide **53** for comparison.

Table 7. Biological evaluation of **77** and **78**.



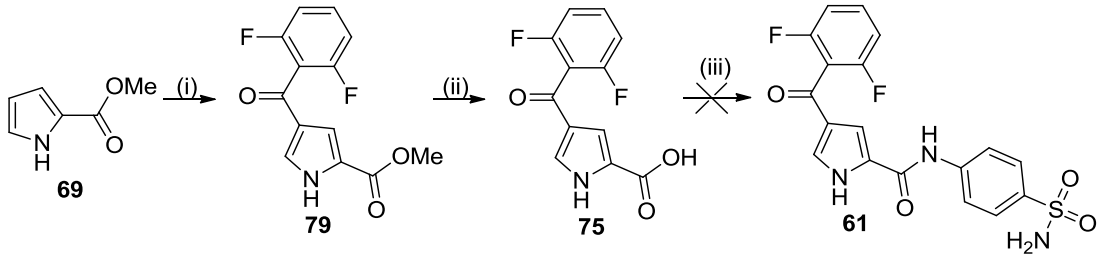
Compound	R	ERK5; IC_{50} (µM)
53		2.4 ± 0.09 (n = 2)
77		>120
78		10 ± 2.1 (n = 2)

Compound **77** showed complete lack of ERK5 inhibition, which led to the decision not to synthesise the original isopropoxy target compound **60**. The ERK5 inhibitory activity

of compound **78** was moderate compared to hit compound **53** (ERK5; IC_{50} = 10 μM). The improvement in inhibitory activity for **78** suggests that a shortened, more rigid amide side chain is more favourable for ERK5 binding.

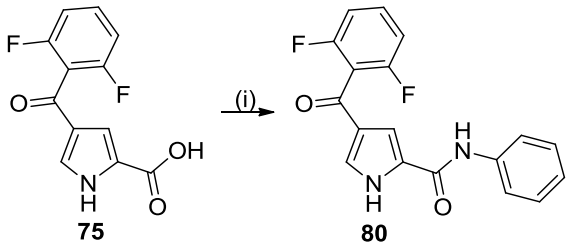
4.1.4 Synthesis of 4-(2,6-difluorobenzoyl)-N-(4-sulfamoylphenyl)-1H-pyrrole-2-carboxamide (61) based on CRT hit 59

For compound **61** the general synthetic scheme was applied again. The Friedel-Crafts acylation of methyl pyrrole 2-carboxylate (**69**) with 2,6-difluorobenzoyl chloride proceeded in 66% yield to give **79**, and the hydrolysis step in 98% yield providing carboxylic acid **75** (Scheme 10). The final CDI coupling with sulphanilamide was unsuccessful, probably due to the reduced nucleophilicity of sulphanilamide.



Scheme 10. *Reagents and conditions*: (i) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) (a) CDI, THF, 70 ^oC, 3 h; (b) sulphanilamide, 50 ^oC to RT, 18 h.

Alongside the attempted CDI coupling of sulphanilamide to carboxylic acid **75**, coupling directly to methyl ester **79** using microwave irradiation was attempted. Unfortunately, this approach was also unsuccessful. A model coupling reaction between **75** and aniline was conducted (Scheme 11). The reaction was successful with a yield of 50%, and the biological evaluation of compound **80** is shown in section 4.1.5. The success of this reaction suggested that amide coupling between an aniline and **75** was possible and that it was most likely the added electron-withdrawing effect of the sulphonamide group of sulphanilamide causing reduced reactivity in the coupling reaction.



75 80

Scheme 11. *Reagents and conditions*: (i) (a) CDI, THF, 70 ^{o}C, 3 h; (b) aniline, 50 ^{o}C to RT, 18 h.

In order to promote coupling between **75** and sulphanilamide, a number of different methods to activate the carboxylic acid were employed (Table 8).

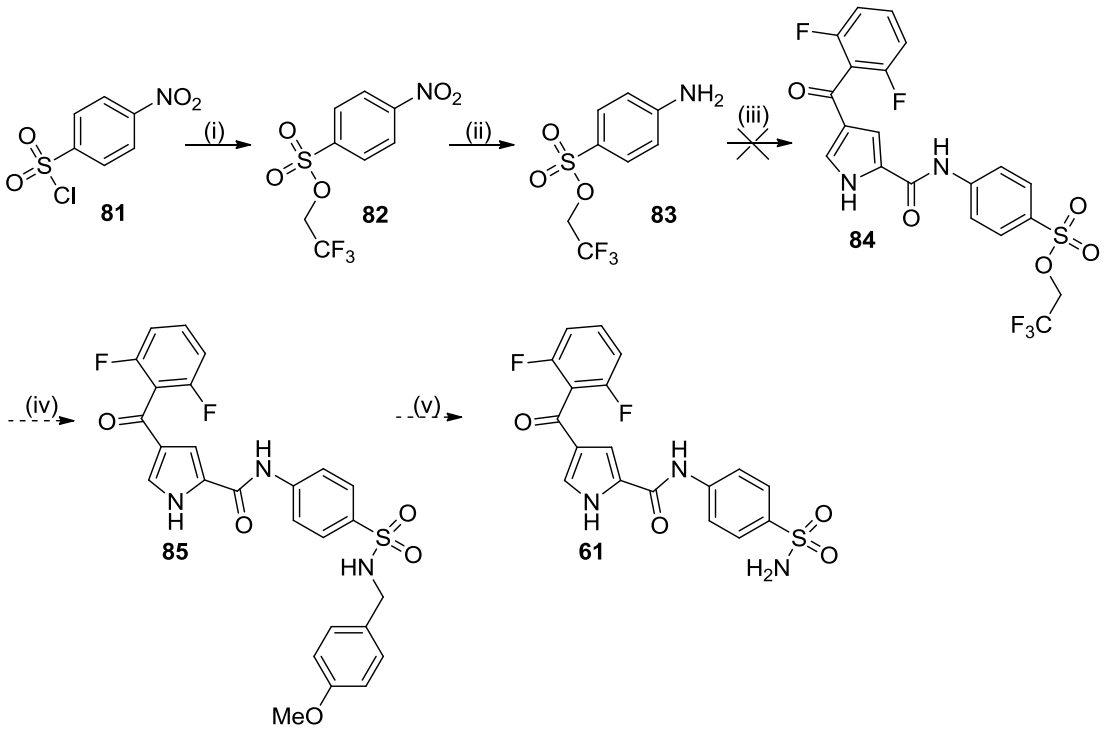
Table 8. Various conditions used to activate **75** prior to reaction with sulphanilamide.

Entry	Activating Agent	Temp. (^{o}C)	Heating Method	Time (h)	Result
1	HBTU, Hunig's base	RT	-	18	No reaction
2	NHS, DCC	RT	-	18	No reaction
3	NHS, DCC	100	Microwave	1	No reaction
4	$SOCl_2$, cat. DMF	RT	-	18	No reaction
5	$SOCl_2$, cat. DMF	70	Conventional	3	No reaction

HBTU was used as an alternative coupling agent to CDI (entry 1, Table 8) but no reaction occurred when sulphanilamide was added. The carboxylic acid was converted to an acid chloride using $SOCl_2$ and catalytic DMF, and *N*-hydroxysuccinimide was employed in order to synthesise the succinimide ester. In each case, subsequent reaction with sulphanilamide failed to yield **61**. Upon formation of the succinimide ester, the reaction with sulphanilamide was attempted at both RT and also under microwave irradiation but was unsuccessful in both cases.

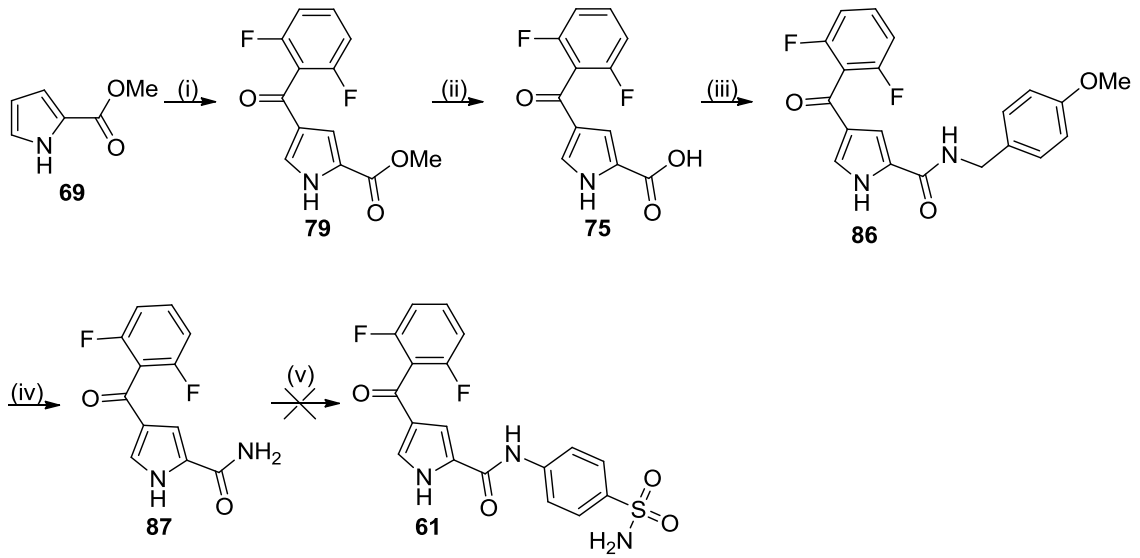
A new approach was attempted starting with the conversion of 4-nitrobenzene-1-sulfonyl chloride (**81**) to 2,2,2-trifluoroethyl 4-nitrobenzenesulfonate (**82**) in 87% yield (Scheme 12). The subsequent reduction of the nitro- substituent gave **83** in 82% yield. The amino- substituent of **83** should be less electron-poor, and potentially reactive enough to couple with carboxylic acid **75**. Once coupled, the 2,2,2-trifluoroethoxysulfonate of compound **84** could be converted to a sulphonamide using a literature procedure *via* reaction with *p*-methoxybenzylamine under microwave irradiation followed by TFA removal of the *p*-methoxybenzyl group.[99] However, the

coupling reaction of **83** with carboxylic acid **75** was unsuccessful using the CDI coupling method. No further coupling conditions were attempted at that stage.



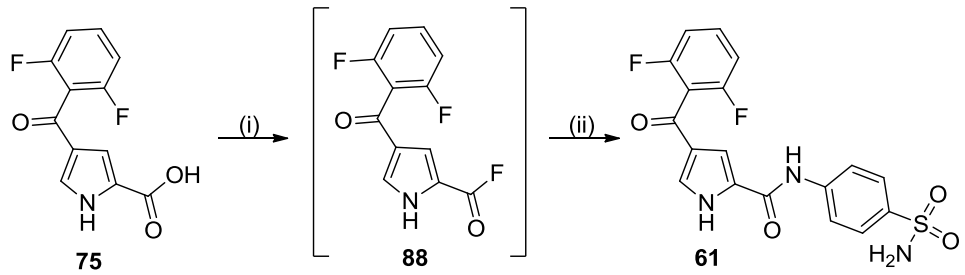
Scheme 12. *Reagents and conditions*: (i) NEt_3, DMAP, TFE, RT, 3 h; (ii) Na_2SO_4, MeOH:H_2O (4:1), MW, 100 ^oC, 15 min; (iii) (a) **75**, CDI, THF, 70 ^oC, 3 h; (b) sulphanilamide, 50 ^oC to RT, 18 h; (iv) *p*-methoxybenzylamine, DBU, THF, MW, 160 ^oC, 15 min; (v) TFA, RT, 1 h.

Another potential route to synthesise **61** utilised compound **86**, which was prepared *via* the general synthetic route (Scheme 13). It was proposed that the *p*-methoxybenzyl group could be cleaved using TFA, to form primary amide **87**. This reaction worked in almost quantitative yield of 97% and following the literature, compound **87** was subjected to a copper-mediated coupling reaction with 4-(bromophenyl)sulfonamide.[100-101] The coupling reaction was conducted in the microwave at 150 ^oC for 15 min, and unfortunately did not afford the desired product **61**. A complex mixture of products was obtained, and the reaction was not attempted again.



Scheme 13. *Reagents and conditions*: (i) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) (a) CDI, THF, 70 ^oC, 3 h; (b) 4-methoxybenzylamine, 50 ^oC to RT, 18 h; (iv) TFA, RT, 3 h; (v) 4-(bromophenyl)sulfonamide, CuI, K_2CO_3, NMP, MW, 150 ^oC, 15 min.

Activation of carboxylic acid **75** by conversion to an acid fluoride using cyanuric trifluoride followed by the reaction with sulphanilamide was conducted. Although previously reported attempts to activate the carboxylic acid were unsuccessful, conversion to the acid fluoride could be sufficiently activating to enable electron-poor sulphanilamide to react (Scheme 14).



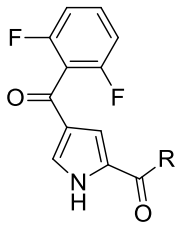
Scheme 14. *Reagents and conditions*: (i) cyanuric trifluoride, pyridine, MeCN, RT, 45 min; (ii) sulphanilamide, RT, 18 h.

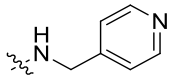
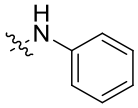
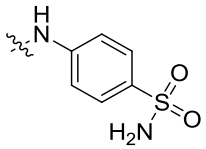
Initially, isolation of the acid fluoride **88** was attempted. However, hydrolysis back to the carboxylic acid was observed and therefore, a one-pot procedure was used with formation of the acid fluoride, followed by addition of sulphanilamide providing **61** in 38% yield.

4.1.5 ERK5 inhibitory activity of 61 and 80

Compound **61** was sent for biological evaluation in the cell-free assay at CRT-DL, along with the phenyl analogue **80** and the results are shown in Table 9. Again, the inhibitory activity of hit pyrrole carboxamide compound **53** is shown for comparison.

Table 9. Biological evaluation of **61** and **80**

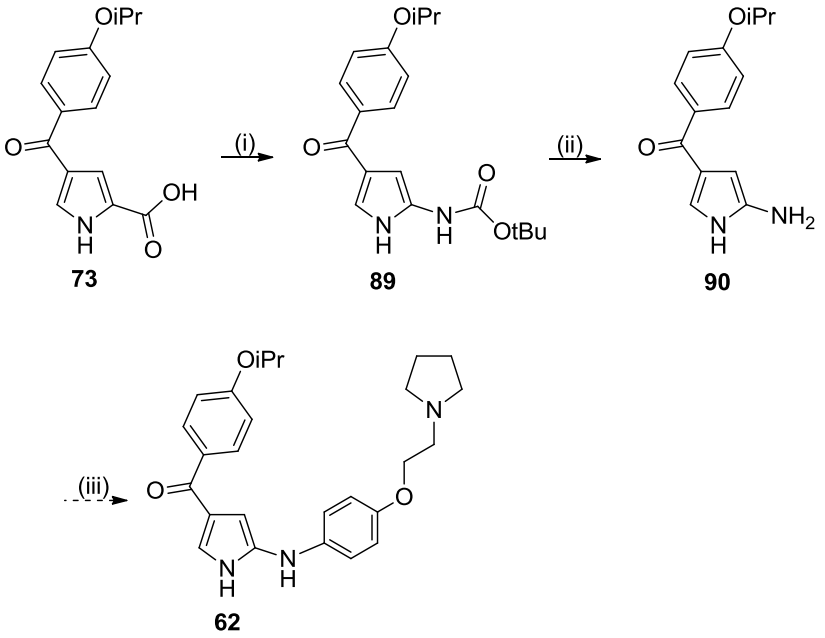


Compound	R	ERK5; IC_{50} (μM)
53		2.4 ± 0.09 (n = 2)
80		9.3 ± 1.4 (n = 2)
61		1.2 ± 0.36 (n = 6)

Phenyl analogue **80** displayed moderate activity against ERK5, but did not show an improvement compared to **61**. Compound **61** displayed a 2-fold improvement in potency (ERK5; IC_{50} = 1.2 μM) compared to original hit **53**, although it was not as potent as the CRT hit **59** (ERK5; IC_{50} = 0.20 μM). The most potent hit pyrrole carboxamide compounds identified from the HTS conducted at CRT-DL incorporated a methylene group in the amide side-chain as in compound **53** and it was thought this was necessary for ERK5 inhibitory activity. Very importantly, compound **61** suggests that the methylene group is not essential and activity can be maintained, or even improved, when it is removed.

4.1.6 Synthesis of inhibitors with 2-aminopyrrole core structures based on CRT hits 58 and 59

For the synthesis of the 2-aminopyrrole compounds **62** and **63**, a Curtius rearrangement was utilised using diphenyl phosphoryl azide (DPPA) (Scheme 15). According to the literature using DPPA with ^{t}BuOH as the solvent, carboxylic acids can undergo a Curtius rearrangement, and the product isocyanate can be trapped as the *tert*-butyl carbamate.[102-103] This route was initially conducted with the isopropoxy carboxylic acid analogue **73** and the rearrangement completed in 71% crude yield. The isolated product was identified as a mixture of the desired compound **89** and some DPPA side products. The crude mixture was taken forward to the TFA deprotection step without purification. The supposedly formed compound **90** was obtained in 28% yield.



Scheme 15. *Reagents and conditions*: (i) DPPA, NEt_3, ^{t}BuOH, 30 ^{o}C, 18 h; (ii) TFA, 1 h, RT; (iii) 1-(2-(4-bromophenoxy)ethyl)pyrrolidine, Pd^0 (cat), $NaO^{t}Bu$.

In order to be sure that the correct 2-amino compound **90** had been synthesised, a sample was sent for LRMS at the EPSRC National Mass Spectrometry service in Swansea. We observed *m/z* 289 $[M+H]^+$, suggesting the formation of carbamic acid **91** *via* hydrolysis of the ^{t}Bu ester (Figure 29). Carboxamic acids are often thought to be unstable, since they can easily decarboxylate. In this case, the carboxamic acid can be stabilised by formation of a 6-membered pseudocycle *via* a hydrogen-bond interaction of the carboxylate with the pyrrole NH (Figure 29). In order to obtain the desired amine

compound **90**, carbamic acid **91** was boiled in ethanol for 30 min and the desired compound obtained in 90% yield.

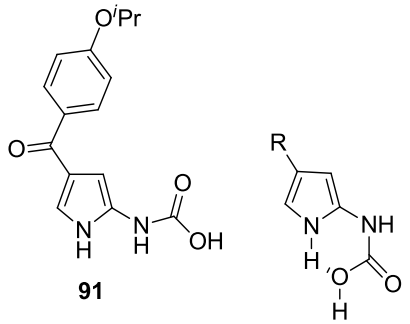
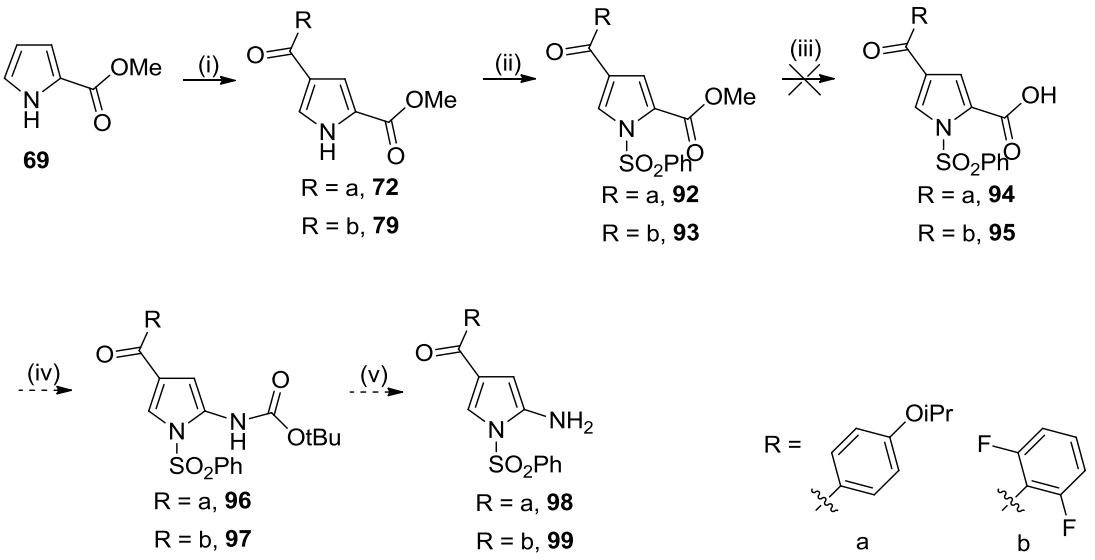


Figure 29. Carbamic acid **91** obtained after TFA deprotection of **89** and postulated 6-membered pseudocycle between the carboxamide and the pyrrole NH.

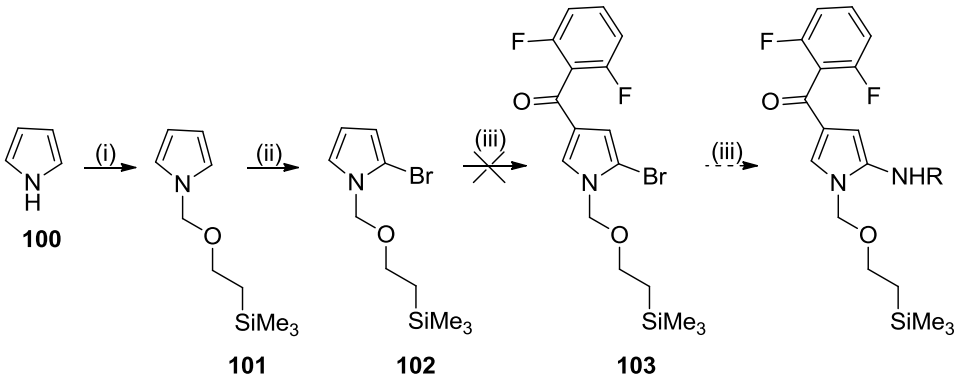
Compound **90** was obtained in very low overall yield, and therefore the subsequent Buchwald coupling could not be attempted. Repetition of the first steps of the scheme in order to gain more material for the final step proved unsuccessful and the method was non-reproducible. At this stage, the same scheme had also been applied to the synthesis of the 2,6-difluoro analogue **63**, and this proved equally unsuccessful, meaning this approach was unreliable. An alternative strategy had to be investigated.

The first approach we attempted was to continue with the same scheme utilising the Curtius rearrangement, but using an *N*-protected pyrrole (Scheme 16). The protecting group of choice was a phenylsulfonyl group, described in the literature for similar reactions on a pyrrole-2-carboxylic acid.[104] This route was attempted using both the isopropoxy and 2,6-difluoro analogues **62** and **63**, with the protecting group being installed using phenylsulfonyl chloride and triethylamine to give protected ester **92** and **93** in 65% yield each. The subsequent hydrolysis of methyl esters **92** and **93** was attempted using lithium chloride in DMF under microwave irradiation at 100 ^{o}C. Unfortunately, the reaction was unsuccessful with removal of the phenylsulfonyl protecting group observed after 10 min.



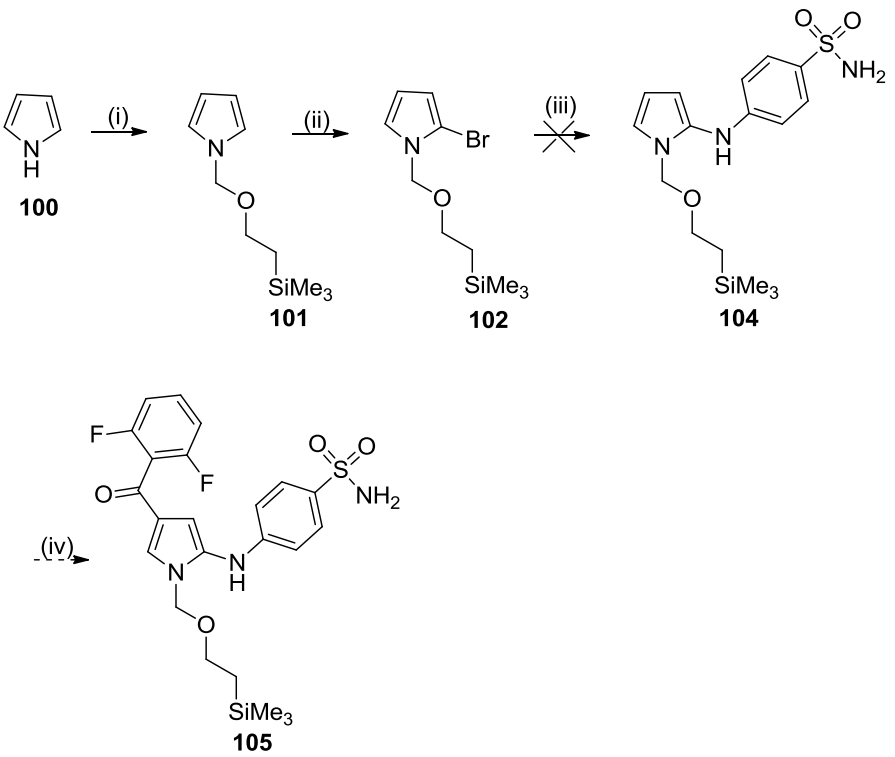
Scheme 16. *Reagents and conditions*: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) NEt_3, SO_2PhCl, DCM, RT, 3 h; (iii) LiCl, DMF, MW, 100 ^oC, 10 min; (iv) DPPA, NEt_3, ^{t}BuOH, 30 ^oC, 18 h (v) TFA, 1 h, RT.

According to the literature, 2-aminopyrroles can be unstable and difficult to prepare.[93, 105-106] An alternative synthesis of the corresponding 2-bromopyrrole was attempted to allow the final Buchwald coupling to be performed with opposite coupling partners. This was achieved following a literature procedure (Scheme 17) starting with SEM protection of the pyrrole to give **101** in 60% yield. [107] Compound **101** was brominated in the 2-position using *N*-bromosuccinimide to give 2-bromopyrrole **102** in 99% yield. The bromination has the benefit of being very fast, taking only 15 min at 0 ^oC. However, a problem was encountered upon attempted scale up of the bromination, with a mixture of mono-, di-, tri- and tetra- brominated pyrrole being observed. The problem was overcome by slow addition of the *N*-bromosuccinimide in small portions. The Friedel-Crafts acylation of **102** was attempted prior to the Buchwald coupling, but was unsuccessful, probably due to the electron donating nature of the SEM protecting group. In order to install a benzoyl group into the 4-position of the pyrrole ring, it is usually required to have an electron withdrawing group in the 2-position.



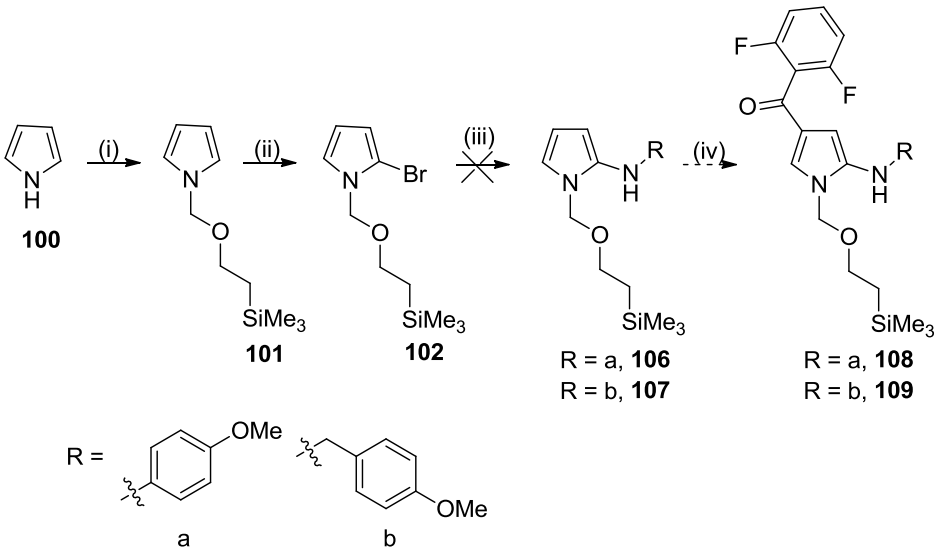
Scheme 17. *Reagents and conditions*: (i) NaH, SEMCl, DMF, 0 ^{o}C to RT, 1 h; (ii) NBS, THF, 0 ^{o}C, 15 min; (iii) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h; (iv) RNH_2, $Pd_2(dba)_3$, $P(^tBu)_3$, ^{t}BuONa, toluene, 80 ^{o}C, 18 h.

As an alternative, the final Buchwald coupling step was attempted prior to the Friedel-Crafts acylation, using sulphanilamide to install the required group at the 2-position. The chosen reaction conditions were $Pd_2(bda)_3$, BINAP, ^{t}BuOK with toluene as the solvent. The reaction was heated to 100 ^{o}C and left to stir for 18 h, which was consistent with methods used for similar Buchwald couplings in the literature.[108-110] The reaction was unsuccessful, possibly due to the electron poor sulphanilamide not being reactive enough to undergo coupling (Scheme 18).



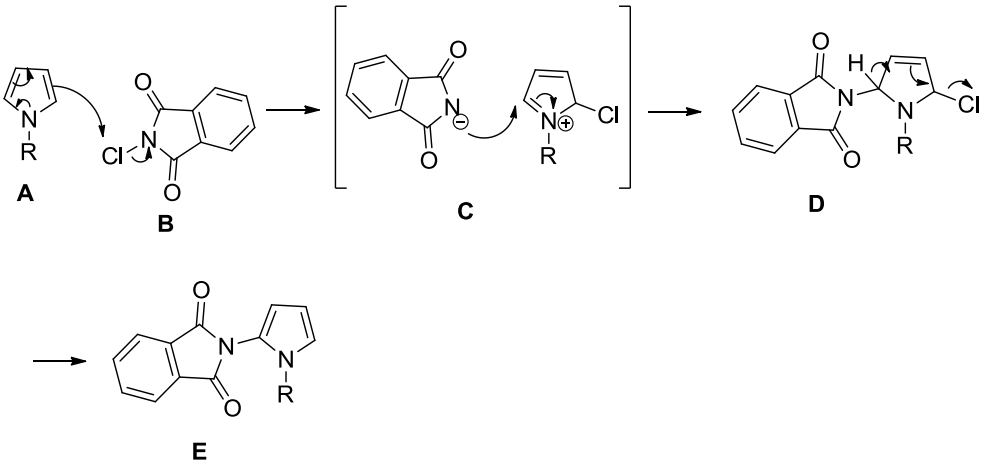
Scheme 18. *Reagents and conditions*: (i) NaH, SEMCl, DMF, 0 ^{o}C to RT, 1 h; (ii) NBS, THF, 0 ^{o}C, 15 min; (iii) sulphanilamide, $Pd_2(dba)_3$, $P(^tBu)_3$, ^{t}BuONa, toluene, 80 ^{o}C, 18 h; (iv) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h.

To determine if the nature of the aniline being coupled was causing the reaction to be unsuccessful, the same Buchwald conditions were attempted using *p*-anisidine and *p*-methoxybenzylamine (Scheme 19). A similar reaction had been carried out in the literature on 1-(5-bromo-1-methyl-1*H*-pyrrol-2-yl)ethanone using *p*-methoxybenzylamine.[109] In both cases, the reaction was unsuccessful and we therefore postulated that the pyrrole ring of **102** may be too electron-rich to undergo coupling. The SEM protecting group is electron donating, and the anilines being used were relatively unreactive meaning that the reaction could possibly be disfavoured. In the literature example the bromo-pyrrole used also incorporated a methyl ketone, which would lower the electron density of the ring making the reaction more favourable.[109]



Scheme 19. *Reagents and conditions*: (i) NaH, SEMCl, DMF, 0 ^{o}C to RT, 1 h; (ii) NBS, THF, 0 ^{o}C, 15 min; (iii) *p*-anisidine or *p*-methoxybenzylamine, $Pd_2(dba)_3$, $P(^tBu)_3$, ^tBuONa, toluene, 80 ^{o}C, 18 h; (iv) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h.

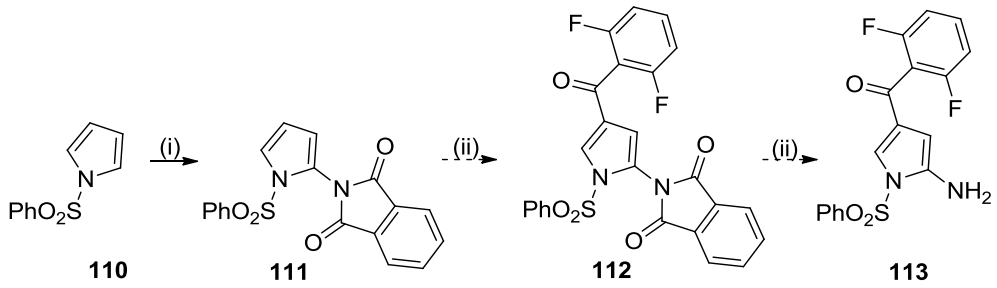
Due to the problems encountered with the Buchwald coupling of 2-bromopyrrole **102** with different anilines, investigation into alternative ways to obtain the 2-aminopyrrole was undertaken. After the failed attempts to install a 2-amino substituent *via* the Curtius rearrangement as discussed previously, a literature procedure using *N*-chlorophthalimide to install a phthalimide group in the 2-position of the pyrrole ring was attempted. This reaction was discovered by De Rosa *et al*, when trying to chlorinate the 2-position of pyrrole using NCP or NCS. Rather than obtaining the expected chlorinated product, they observed the formation of pyrroles substituted in the 2-position with phthalimide or succinimide moieties.[106, 111-113] The reaction is thought to go through the mechanism shown in Scheme 20, whereby the pyrrole ring first acts as a nucleophile and removes the electrophilic chlorine atom from NCP to give ion pair **C**. The phthalimide anion then attacks the pyrrole ring to give **D**. The final step of the proposed mechanism is the loss of HCl to re-aromatise and give product **E**.[106, 111-113] Reaction of the pyrrole with *N*-bromosuccinimide or *N*-iodosuccinimide yields only the halogenated products, and this is thought to be due to the fact that the chloro-substituent affects the aromaticity of the pyrrole ring more than either bromo- or iodo-substituents do, due to its greater electron donating ability.[112]



Scheme 20. Postulated mechanism for the reaction of 1-substituted pyrroles with NCP.[106, 111-113]

In the literature, the reaction was carried out in DCM using $NaHCO_3$. When the reaction was conducted without base or in acidic conditions, the 2-chloropyrrole was obtained. The presence of base prevents the phthalimide anion from being protonated and therefore enables the addition to the pyrrole ring and subsequent loss of HCl to give the 2-phthalimide product. It was postulated that the reaction could be used to install the phthalimide group and this could then be cleaved to leave the required 2-aminopyrrole for use in Buchwald coupling.

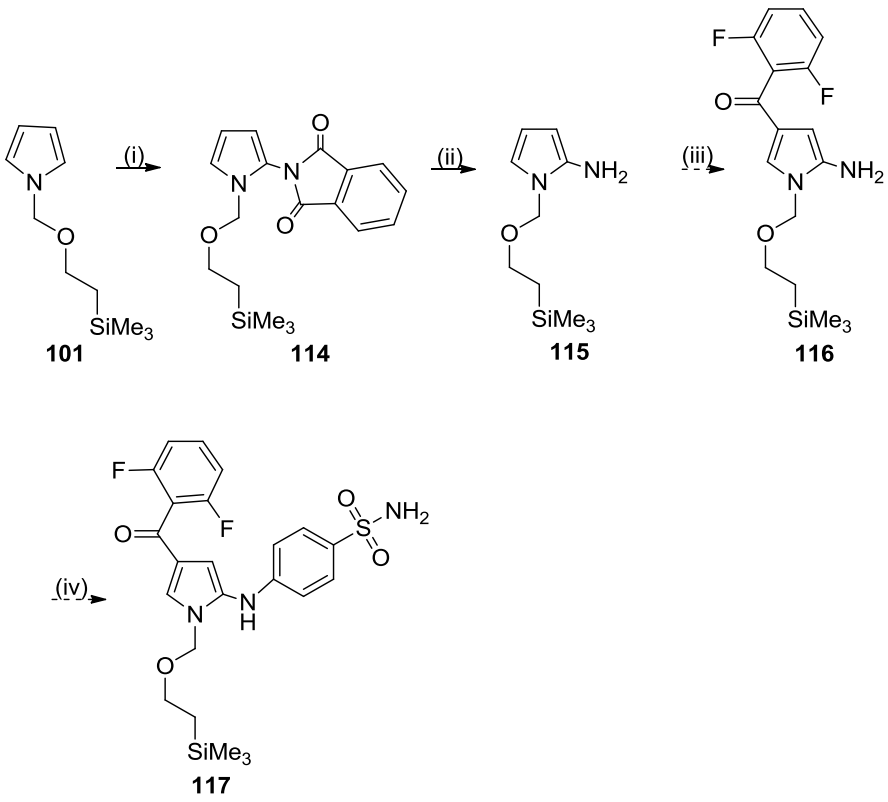
The reaction was first attempted using phenylsulfonyl protected pyrrole. We initially thought that having an electron withdrawing protecting group would help to stabilise the 2-aminopyrrole that would be obtained after phthalimide cleavage.[93] The reaction of NCP and phenylsulfonylpyrrole (**110**) gave the phthalimide product **111**, but in a poor yield of 7% (Scheme 21). After greater understanding of the mechanism of the reaction, it was not surprising that the reaction had not worked in greater yield, since the first step relies on the pyrrole ring to be nucleophilic, and therefore an electron donating protecting group would be more beneficial to aid the reaction. There was not enough material obtained from this initial reaction to progress with the synthesis.



Scheme 21. *Reagents and conditions*: (i) NCP, $NaHCO_3$, DCM, RT, 18 h; (ii) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (iii) hydrazine hydrate, MeCN, RT, 4 h.

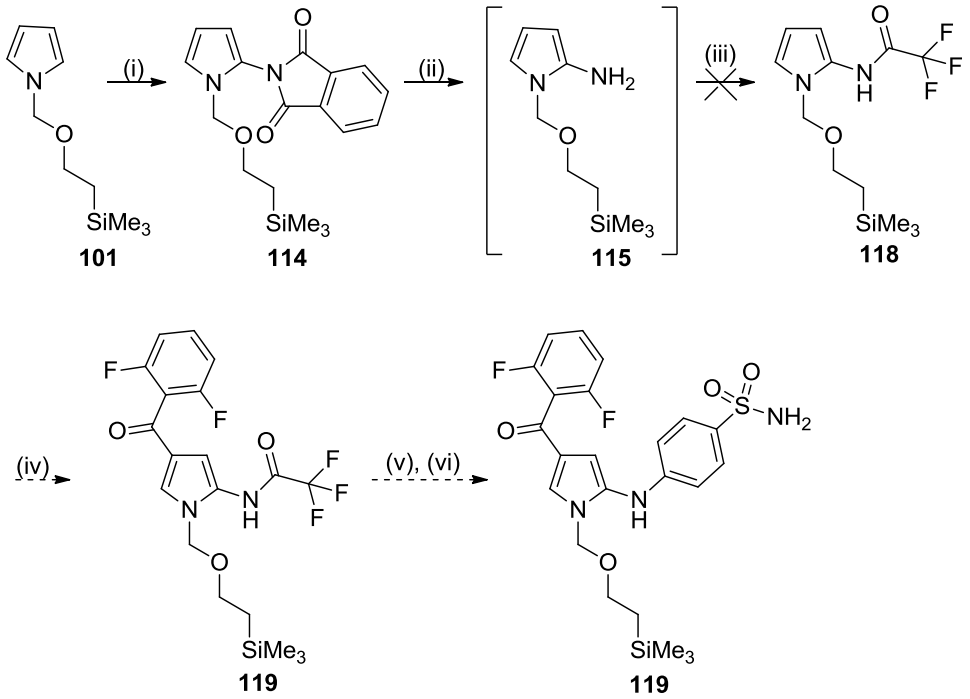
We also tried performing Friedel-Crafts acylation first, followed by phthalimide addition. Unsurpisingly, once the electron withdrawing benzoyl group had been installed in the 4-position, the pyrrole ring was too electron-poor to react as shown in the proposed mechanism shown in Scheme 21.

The phenylsulfonyl protecting group was replaced with a SEM protecting group in order to test whether reaction with NCP would work better with an electron donating protecting group. Using this strategy, the phthalimide product **114** was obtained in 49% yield. However, attempts to perform the Friedel-Crafts acylation on **114**, were not successful. Initially, it was reasoned that Friedel-Crafts reaction would not occur on the benzene ring of the phthalimide as it would be disfavoured due to the electron poor nature of this aromatic ring compared to the pyrrole ring. However, after the reaction it became apparent that the acylation had possibly occurred in several positions, with a complex reaction mixture being obtained. For this reason, the cleavage of the phthalimide prior to Friedel-Crafts reaction (Scheme 22) was conducted using a 40% aqueous solution of methylamine. The use of methylamine had been shown in the literature to be a milder method for cleaving phthalimides compared to the conventional methods using hydrazine hydrate.[114] The reaction worked in an hour, and the correct mass for amine **115** was observed by LC-MS. However, after attempted work up, the product had degraded. As stated previously, the instability of 2-aminopyrroles is well documented, and the original literature which reports this route to their synthesis records that most of the compounds prepared decomposed on attempted isolation.[113] One way to overcome this is to make the salt of the 2-aminopyrrole, which should be stable enough to store.[115]



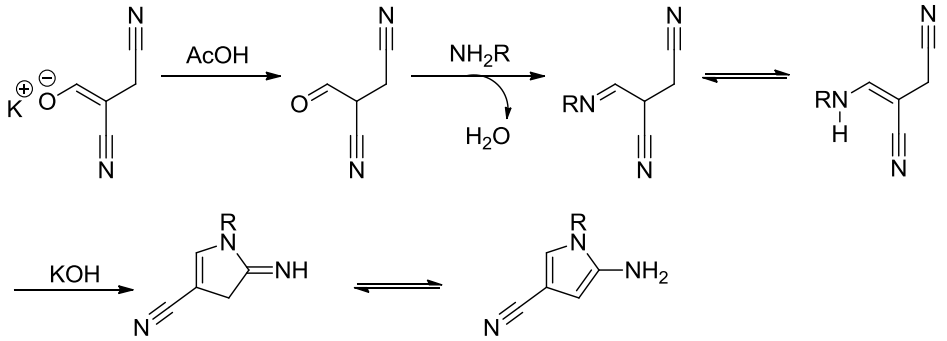
Scheme 22. *Reagents and conditions*: (i) NCP, $NaHCO_3$, DCM, RT, 18 h; (i) $MeNH_2$ (40% in H_2O), EtOH, RT, 1 h; (iii) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (iv) 4-bromobenzenesulfonamide, $Pd_2(dba)_3$, $P(^tBu)_3$, ^tBuONa, toluene, 80 ^oC, 18 h.

Rather than making the salt of the product, we carried out the *in situ* protection of the 2-amino group without isolating the free amine. Using an electron withdrawing protecting group was expected to stabilise the product by pulling electrons from the pyrrole ring and for this reason, we chose a trifluoroacetyl group (Scheme 23). After cleavage with methylamine, the reaction mixture was washed with water to remove any excess methylamine and the N^1,N^2-dimethylphthalamide side product, before trifluoroacetic anhydride was added. It was important to try and remove these as they could both potentially react with trifluoroacetic anhydride. When the protection strategy was conducted, the protected amine **118** was not isolated and the amine starting material had degraded.



Scheme 23. *Reagents and conditions*: (i) NCP, $NaHCO_3$, DCM, RT, 18 h; (i) $MeNH_2$ (40% in H_2O), EtOH, RT, 1 h; (iii) TFAA, pyridine, MeCN, RT, 18 h; (iv) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (iv) 4-bromobenzenesulfonamide, $Pd_2(dba)_3$, $P(^tBu)_3$, ^tBuONa, toluene, 80 ^oC, 18 h.

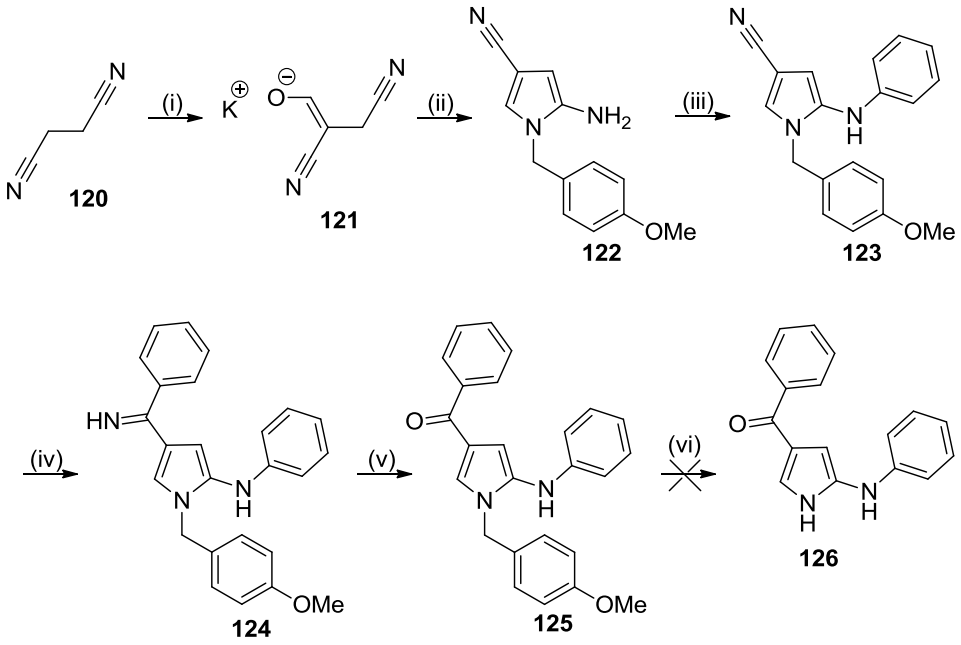
A different synthetic scheme was developed using a literature procedure (Scheme 25). Starting from commercially available succinonitrile (**120**), the formyl potassium salt **121** was produced in 84% yield by reaction with ethyl formate and potassium *tert*-butoxide. Reaction of **121** with *p*-methoxybenzylamine in the presence of acetic acid, followed by addition of potassium hydroxide afforded compound **122** in 70% yield *via* the general route shown in Scheme 24.[116]



Scheme 24. General route for formation of 2-amino-4-cyanopyrroles.[116]

Previous attempts to synthesise 2-aminopyrroles have been unsuccessful due to their instability. Incorporation of a 4-cyano- substituent onto the pyrrole ring not only provides a synthetic handle for subsequent reactions, but also reduces the electron density of the 2-aminopyrrole, hence improving stability.

In order to test whether the route would be viable for the desired targets, a model Buchwald amination of **122** was performed using bromobenzene, $Pd_2(dba)_3$, Xantphos and Cs_2CO_3 in dioxane. The reaction was successful, producing **123** in 75% yield. Another model reaction was conducted, using a Grignard reagent to react with the nitrile group of **123**. Phenylmagnesium bromide was synthesised and used *in situ* to form imine **124**. The initial idea was that the imine would hydrolyse upon acidic work-up to give the ketone **125**. However, this was not the case and the imine **124** was isolated in 90% yield. Refluxing **124** in 1.0 M H_2SO_4 for two hours afforded ketone **125** in 85% yield.[117] Attempted deprotection of **125** by stirring in TFA at both RT and 80 ^oC failed to yield compound **126**.



Scheme 25. *Reagents and conditions*: (i) ethyl formate, ^{t}BuOK, toluene, ^{t}BuOH, RT, 4 h; (ii) (a) PMBamine, EtOH, AcOH, reflux, 45 min; (b) KOH, EtOH, reflux, 2.5 h; (iii) bromobenzene, $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, Dioxane, 110 ^oC, 18 h; (iv) bromobenzene, Mg turnings, THF, 80 ^oC, 5 h; (v) 1M H_2SO_4, reflux, 2 h; (vi) TFA, RT or 80 ^oC, 1 h.

No further conditions to deprotect compound **125** were attempted due to the likelihood that compounds without an amide group would be inactive. Homology modelling suggests that the carbonyl of the amide group makes a H-bond with the hinge region of ERK5 and is therefore important for achieving ERK5 inhibition. Therefore, the synthesis of 2-aminopyrrole inhibitors was ceased.

4.2 Effect of removing a methylene group from the amide side chain on ERK5 inhibitory activity

4.2.1 Rationale

During investigation towards the synthesis of targets based on CRT hit compounds **58** and **59**, three compounds were synthesised without a methylene group in the amide side-chain (**61, 78 and 80**) (Figure 30). The synthesis of these compounds is discussed in section 4.1 of this chapter.

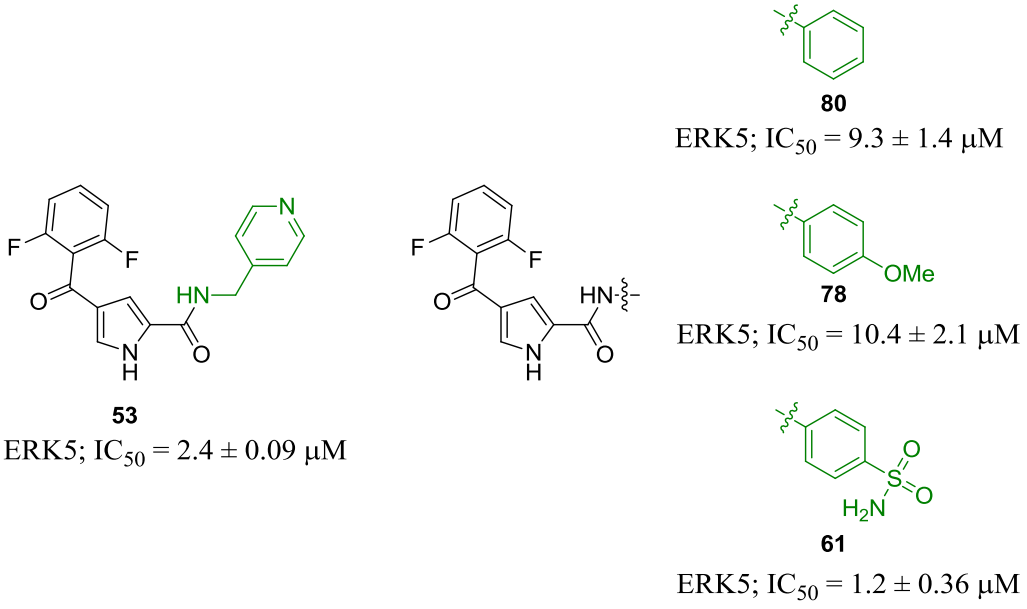
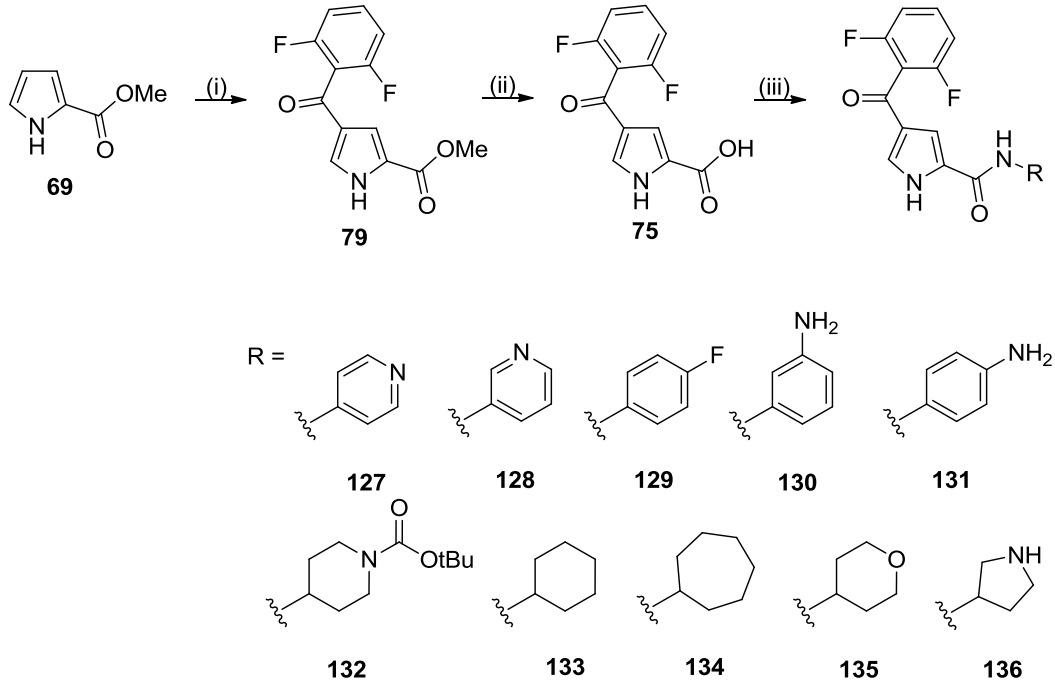


Figure 30. Improvement in ERK5 inhibitory activity for compound **61** compared to hit compound **53**.

In light of the 10-fold improved inhibitory activity observed for compound **61** compared to **78** and **80**, and the 2-fold improvement compared to hit compound **53**, further analogues without a methylene group in the amide side chain were proposed for synthesis. The target compounds contained a range of substituted aromatic and non-aromatic amide side-chains whilst the 2,6-difluorobenzoyl group was maintained.

4.2.2 Synthesis of inhibitors with shorter amide side chains

The compounds were synthesised *via* the 3-step route previously optimised within the group (Scheme 26). The first step was the Friedel-Crafts acylation of the commercially available methyl pyrrole 2-carboxylate **69** using 2,6-difluorobenzoyl chloride, which gave **79** in 66% yield. Lithium hydroxide-mediated hydrolysis of **79** occurred in 99% yield to afford carboxylic acid **75**. Finally, the amide side-chain was introduced using CDI as the coupling agent and occurred in moderate to good yield (35-75%) depending on the amine used.



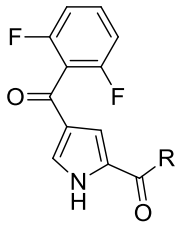
Scheme 26. *Reagents and conditions*: (i) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) (a) CDI, THF, 70 ^oC, 3 h; (b) appropriate amine, 50 ^oC to RT, 18 h.

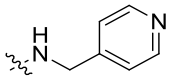
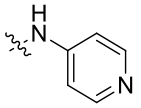
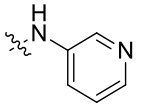
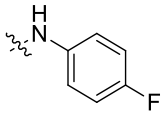
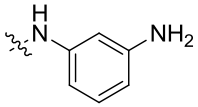
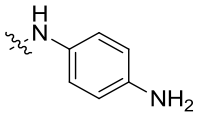
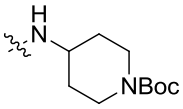
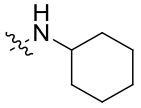
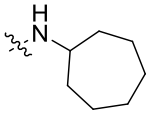
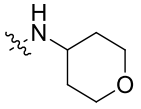
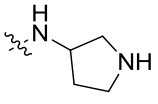
Along with the synthesis of these 10 compounds, Boc-protected analogue **132** was deprotected and the resulting compound sent for biological evaluation. The deprotection was performed using neat TFA to give compound **137** in 87% yield. In addition, the *N*-oxides of pyridyl compounds **127** and **128** were synthesised using *m*-chloroperoxybenzoic acid providing compounds **138** and **139** in 65% and 77% yield, respectively.

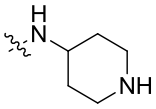
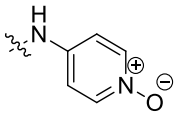
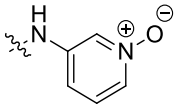
4.2.3 ERK5 inhibitory activity of 127-139

Compounds **127-139** were sent for biological evaluation and the results are shown in Table 10, alongside hit compound **53**.

Table 10. ERK5 inhibitory activity of analogues **125-137**

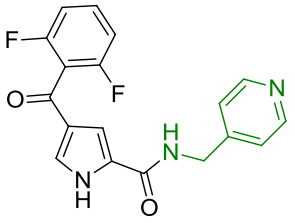


Compound	R	ERK5; IC_{50} (μM)
53		2.4 ± 0.09 (n = 2)
127		0.90 ± 0.15(n = 8)
128		1.1 ± 0.27 (n = 6)
129		7.0 (n = 1)
130		1.2 ± 0.60 (n = 10)
131		1.7 ± 0.60 (n = 4)
132		2.9 ± 0.66 (n = 4)
133		2.7 ± 0.80 (n = 4)
134		13 ± 2.5 (n = 4)
135		2.2 ± 0.35 (n = 8)
136		31 ± 2.0 (n = 4)

137		1.3 ± 0.36 (n = 6)
138		12 ± 0.86 (n = 4)
139		14 (n = 1)

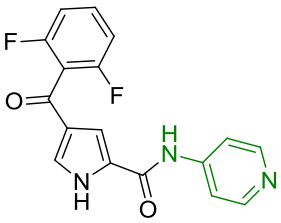
The *N*-oxides **158** and **159** were expected to be major metabolites of pyridyl compounds **127** and **128** *via* CYP450 oxidation. If these expected metabolites retained activity against ERK5, it would mean that metabolism would not inactivate the compound. However, an almost 13-fold loss in potency was observed.

The 4-pyridyl analogue **127** was also tested for ERK5 inhibitory activity in the LANCE assay (IC_{50} = 0.43 ± 0.20 μM). Compound **127** was the first sub-micromolar inhibitor of ERK5, and showed a 3-fold improvement in potency compared to the methylene-containing counterpart **53** (Figure 31). This increase in inhibitory activity provides further evidence that the methylene in the amide side chain is not necessary to achieve good inhibition of ERK5.



53

ERK5; IC_{50} = 2.4 ± 0.09 μM



127

IMAP: ERK5; IC_{50} = 0.90 ± 0.15 μM

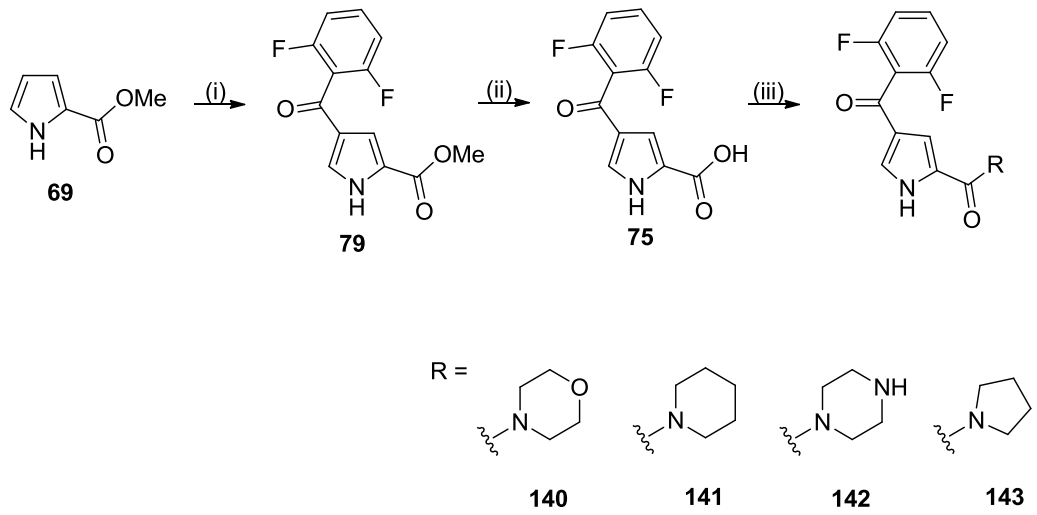
LANCE: ERK5; IC_{50} = 0.43 ± 0.20 μM

Figure 31. Increase in potency observed for **127** compared to **53**.

Interestingly, piperidine analogue **137** was essentially equipotent with the pyridine analogue **127**, suggesting that a non-aromatic group is tolerated in the amide side-chain. *N*-oxide compounds **138** and **139** displayed reduced inhibitory activity, suggesting that the pyridyl nitrogen is required for inhibition of ERK5.

4.2.4 Synthesis of tertiary amide analogues

In order to determine whether the amide side-chain could be shortened further and potency retained, a small library of tertiary amide analogues (**140**-**143**) were synthesised. The general scheme was used and the amide coupling steps were performed in moderate to good yield (47-90%) (Scheme 27).

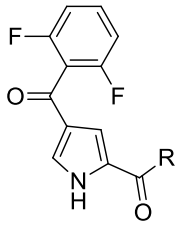


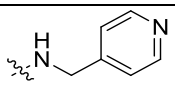
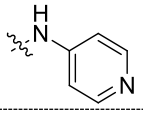
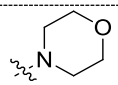
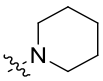
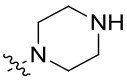
Scheme 27. *Reagents and conditions*: (i) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) (a) CDI, THF, 70 ^oC, 3 h; (b) appropriate amine, 50 ^oC to RT, 18 h.

4.2.4 Biological evaluation of tertiary amide analogues

The inhibitory activity of compounds **140**-**143** is shown in Table 11 alongside hit compound **53** and pyridyl analogue **127**.

Table 11. Biological evaluation of tertiary amide analogues.



Compound	R	ERK5; IC_{50} (μM)
53		2.4 ± 0.09 (n = 2)
127		0.90 ± 0.15 (n = 8)
140		16 ± 2.5 (n = 8)
141		6.1 ± 1.3 (n = 2)
142		17 ± 2.5(n = 4)
143		5.7 ± 0.65 (n = 4)

In each case, the potency was reduced, suggesting that shortening the amide side-chain further is not conducive to potent ERK5 inhibition. It was established in preliminary SARs that the amide NH is not essential for activity (chapter 3), therefore compounds **140**-**143** were expected to retain potency for ERK5. Figure 32 shows the energy minimised structures for analogues **137** and **142**. It is evident that replacing the secondary amide of potent compound **137** (ERK5; IC_{50} = 1.3 ± 0.36 μM) with a tertiary amide in **142** (ERK5; IC_{50} = 17 ± 2.5 μM) effects the conformation of both the amide side-chain and the benzoyl group, potentially affecting binding in the ERK5 ATP binding site. This change in conformation could explain the 13-fold loss in potency observed for **142** compared to **137**. As a result of the lack of ERK5 inhibitory activity, the library of tertiary amide analogues was not expanded further.

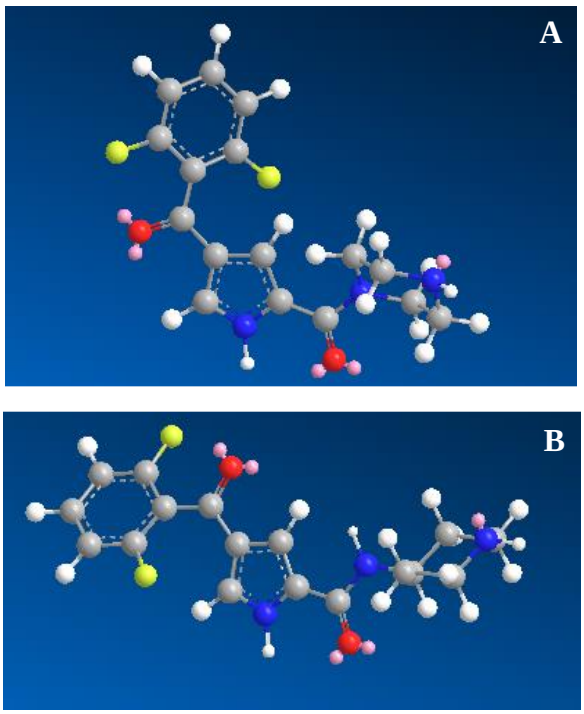
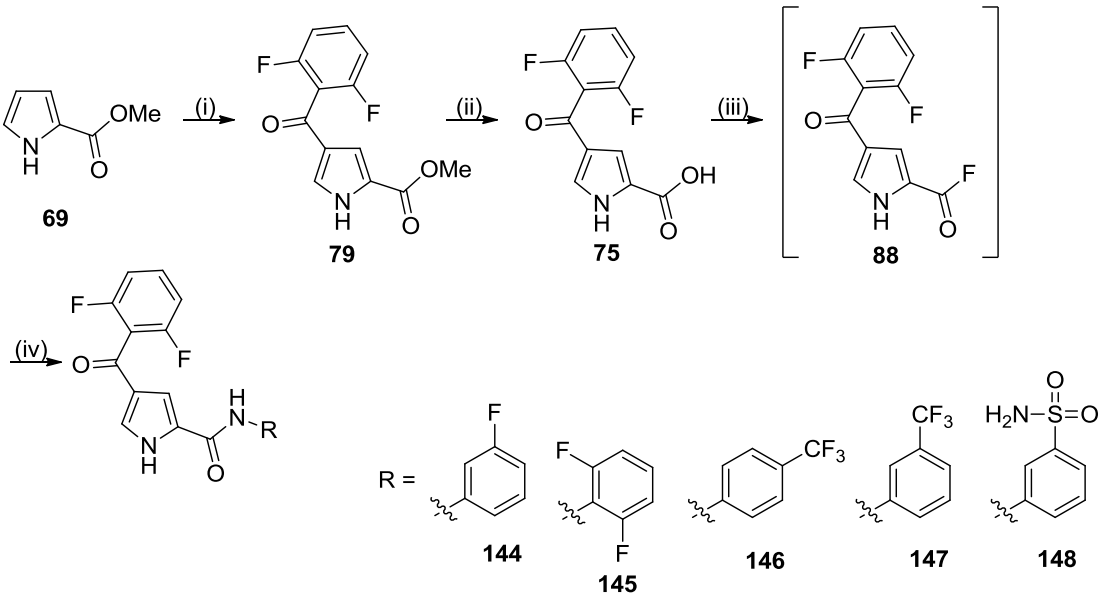


Figure 32. Energy minimised conformations of (A) tertiary amide analogue **142** and (B) secondary amide analogue **137**. Energy minimised using Chem3D.

4.2.5 Development of an alternative amide coupling strategy for coupling of electron-poor anilines

Following the success of removing the methylene group in the amide side-chain, further analogues were synthesised (**144**-**148**) using the general scheme. In these cases, the final CDI coupling was found to be unsuccessful possibly due to the reduced nucleophilicity of electron poor anilines to be coupled.

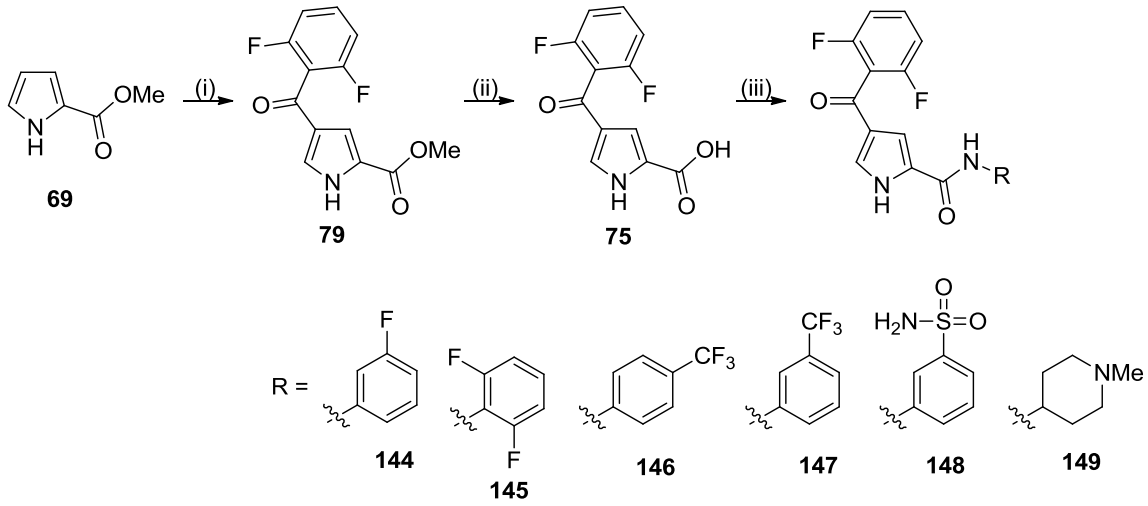
An alternative strategy was to convert carboxylic acid **75** to the more activated acid fluoride **88** using cyanuric fluoride (Scheme 28). Conversion to the desired products was observed, but each reaction mixture was complex meaning the target compounds **144** to **148** were difficult to purify.



Scheme 28. *Reagents and conditions*: (i) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) cyanuric trifluoride, pyridine, MeCN, RT, 45 min; (iv) appropriate amine, RT, 18 h.

A review of the literature revealed phosphorus trichloride as a useful amide coupling agent for coupling of electron-poor anilines to carboxylic acids.[118] Phosphorus trichloride-mediated coupling reactions are well reported, but the method has not been favoured due to the requirement of high reaction temperatures and long reaction times.[119-120] Recently it has been shown that phosphorus trichloride-mediated coupling reaction rates can be enhanced by microwave irradiation at high temperature, exemplified by the coupling of a number of electron-poor anilines to benzoic acid.[118] The optimised reaction conditions were one equivalent of phosphorus trichloride and one equivalent of aniline in acetonitrile with heating under microwave irradiation at 150 ^oC for 5 min. Applying these coupling conditions to the synthesis of 3-fluoro analogue **144** was successful in 30% yield. To improve the yield, the number of equivalents of aniline was increased to 2.5, leading to **144** in 65% yield. Compounds **145**-**148** were also synthesised using the improved coupling method in good yield (**145**, 83%, **146**, 64%, **147**, 57% and **148**, 75%). The phosphorus trichloride coupling procedure was attractive for the reduced reaction time in comparison with both, the CDI and acid fluoride methods. The reaction mixtures were also generally much less complex meaning the products were easier to purify. The coupling procedure was also successfully applied to the synthesis of *N*-methyl piperidyl analogue **149** (72% yield),

showing that the reaction can be equally applied to coupling of aromatic and non-aromatic amines.



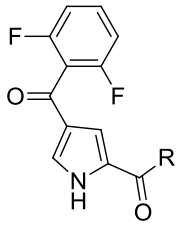
Scheme 29. *Reagents and conditions*: (i) 2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) PCl_3, appropriate amine, MeCN, MW, 150 ^oC, 5 min.

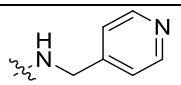
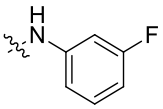
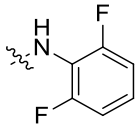
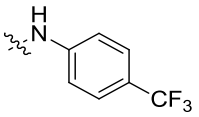
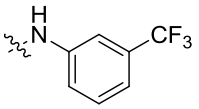
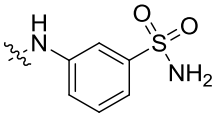
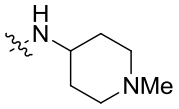
The *N*-methyl piperidyl analogue **149** was synthesised in order to investigate what effect changing the basicity of the piperidine nitrogen would have on ERK5 inhibitory activity.

4.2.6 ERK5 inhibitory activity compounds 144-149

The biological results for compounds **144**-**149** are shown in Table 12 and are compared to hit compound **53**.

Table 12. Biological evaluation of **144**-**149**



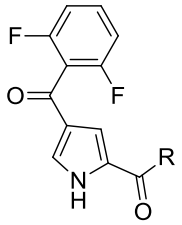
Compound	R	ERK5; IC_{50} (μM)
53		2.4 ± 0.09 (n = 2)
144		47 ± 27 (n = 4)
145		3.1 ± 0.64 (n = 4)
146		>120
147		>120
148		2.0 ± 0.05 (n = 4)
149		1.1 ± 0.1 (n = 8)

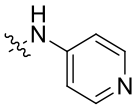
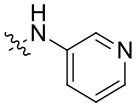
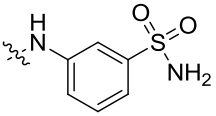
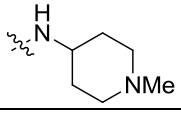
Interestingly, *N*-methylpiperidyl analogue **149** was shown to be equipotent with piperidyl analogue **137** (ERK5; IC_{50} = 1.3 μM), suggesting that modifying the basicity of the piperidyl nitrogen is tolerated. Incorporation of a trifluoromethyl substituent into the 3- or 4-position of the aryl ring was not tolerated. 2,6-Difluoro and 3-sulphonamide analogues **145** and **148** displayed moderate ERK5 inhibitory activity and were essentially equipotent with hit compound **53**.

4.2.7 Physicochemical properties and in vitro ADME profiling for selected analogues containing shortened amide side-chains

A number of the more potent shorter amide side-chain analogues were sent to ADME and toxicology profiling company Cyprotex for assessment of *in vitro* drug metabolism and pharmacokinetic (PK) properties. Prior to this, the physicochemical properties of the four compounds were reviewed (Table 13) to determine if they met the criteria for a lead compound (as outlined in chapter 3). The physicochemical properties were calculated using Optibrium StarDrop software.

Table 13. Physicochemical properties of compounds **127**, **128**, **148** and **149**.

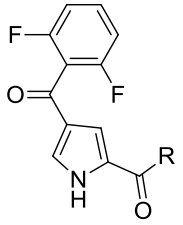


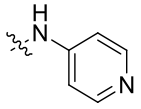
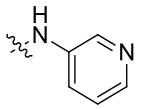
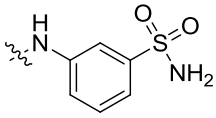
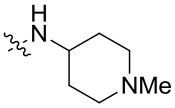
Compound	R	MW	H-bond donors	H-bond acceptors	sLogP	TPSA
Lead criteria	-	<500	<5	<10	<5	75-100
127		327	2	5	2.4	75
128		327	2	5	2.4	75
148		405	3	7	1.9	122
149		347	2	5	2.0	65

All of the compounds meet the lead criteria for MW (<500), H-bond donors/acceptors (<5/<10) and sLogP (<5). Compounds **127** and **128** also have TPSA within the acceptable range (75-100), whereas the TPSA of compounds **148** and **149** fall outside the optimum range. Despite this, the physicochemical properties of each compound were deemed acceptable enough to proceed to *in vitro* PK studies.

Aqueous solubility, plasma protein binding (PPB) and stability in mouse and human liver microsomes were investigated for each compound and the results are shown in Table 14 and compared with the desired properties for a lead candidate. The ligand efficiency (LE) is also shown in Table 14 for each compound (calculated using the formula LE = ΔG/HAC (where ΔG = -1.4 logIC_{50})).

Table 14. *In vitro* ADME profiling for compounds **127**, **128**, **148** and **149**.



Compound	R	LE	Solubility L/U (μM)	PPB (%)	Mouse CLint (μL/min/mg)	Human CLint (μL/min/mg)
Lead criteria	-	>0.30	>50/50	<99	<48	<48
127		0.35	10/65	ND	61.2	16.1
128		0.34	10/30	84	15.5	0
148		0.28	30/100	ND	ND	ND
149		0.33	100/100	ND	0	10.1

ND – Not determined

All compounds except **148** have a ligand efficiency above the desired value for a lead candidate. Pyridyl analogues **127** and **128** both displayed lower aqueous solubility compared to the optimal value, which could be an indicator of poor cell permeability *in vivo*. Human microsomal clearance was good for **127** (16.1 μM/min/mg), but mouse microsomal clearance was high (61.2 μM/min/mg). Microsomal stability was improved for **128** (0 and 15.5 μM/min/mg, in human and mouse, respectively). Compound **128** was tested for plasma protein binding and displayed an optimum value of <99%. Solubility for **148** and **149** was improved (**148**; S (L/U) = 30/100 μM and **149**;

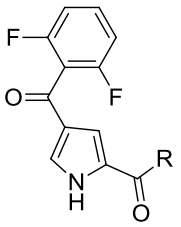
S (L/U) = 100/100 μM). Clearance from both human and mouse microsomes was also good for **149**, meaning this compound had a good balance of *in vitro* ADME properties.

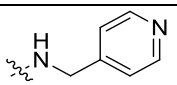
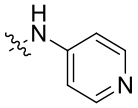
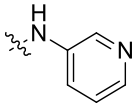
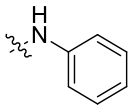
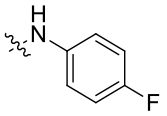
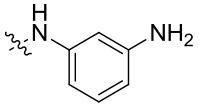
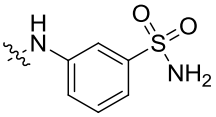
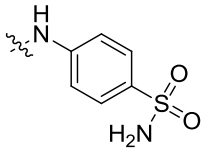
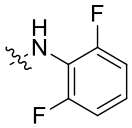
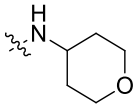
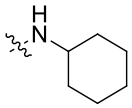
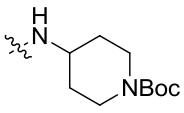
4.2.8 Effect of shortening the amide side chain on selectivity for ERK5 over p38α

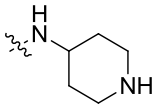
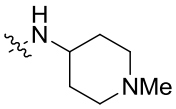
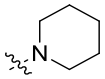
The MAPK, p38α is a close homologue of ERK5, with 48% sequence identity in the kinase domain and 56% in the active site. ERK2 is the closest homologue with 51% sequence identity in the kinase domain and 78% in the active site. Despite ERK2 having more structural identity with ERK5, it has a larger and electronically different gatekeeper residue (glutamine) compared to ERK5 (leucine) meaning that gaining selectivity for ERK5 over ERK2 is easily achieved. The gatekeeper residue in p38α is a threonine residue and, as such, is more similar in size to the leucine gatekeeper residue of ERK5. This means achieving selectivity for ERK5 over p38α is more challenging. It is therefore essential to screen compounds with inhibitory activity for ERK5 against p38α to ensure selectivity is achieved. Compounds are tested for p38α activity using a LANCE assay. The LANCE assay uses time resolved fluorescence energy transfer (TR-FRET) in order to determine IC_{50} values.

The ERK5 inhibitory activity was improved for all inhibitors lacking the methylene group in the amide side-chain. In order to determine the effect this would have on selectivity over p38α, a selection of the most potent of the shortened amide side analogues (ERK5; IC_{50} <10 μM) were counter-screened and the results are shown in Table 15. All of the compounds with aromatic side chains retained selectivity for ERK5 over p38α, particularly **61** and **148**, which showed no inhibition of p38α. The compounds with non-aromatic side chains were generally less selective for ERK5 over p38α. Compound **141** was included to determine if a tertiary amide side chain would have any effect on selectivity for ERK5 over p38α and it was shown to have reversed selectivity. These results highlight the fact that selectivity for ERK5 over p38α can be achieved by removing the methylene in the amide side chain without modification of the phenyl ring of the benzoyl group. Two of the best amide side-chains identified were the 3- and 4-pyridyl rings as they were both potent and selective for ERK5 in comparison to methylene-containing hit compound **53**.

Table 15. p38α counter-screening data for most potent shortened amide side-chain analogues.



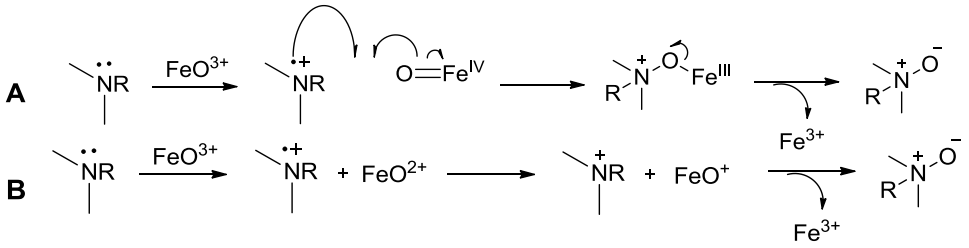
Compound	R	ERK5; IC_{50} (μM)	p38α; IC_{50} (μM)
53		2.4 ± 0.09 (n = 2)	0.58 ± 0.27 (n = 2)
127		0.90 ± 0.15 (n = 8)	100 ± 35(n = 2)
128		1.1 ± 0.27 (n = 6)	35 ± 0.90(n = 2)
80		9.4 ± 1.4 (n = 2)	60 (n = 1)
129		7.0 (n = 1)	60 (n = 1)
130		1.2 ± 0.60 (n = 10)	20 ± 1.3 (n = 2)
148		2.0 ± 0.05 (n = 4)	>120
61		1.3 ± 0.40 (n = 2)	>120
145		3.1 ± 0.64 (n = 4)	43 (n = 1)
135		2.3 ± 0.35 (n = 8)	4.8 ± 2.2 (n = 2)
133		2.7 ± 0.80 (n = 4)	23 ± 26 (n = 2)
132		2.9 ± 0.66 (n = 4)	24 ± 21 (n = 2)

137		1.3 ± 0.36 (n = 6)	9.8 ± 0.52 (n = 2)
149		1.1 ± 0.14 (n = 8)	6.8 ± 0.10 (n = 2)
141		6.1 ± 1.3 (n = 2)	2.1 ± 0.11 (n = 2)

4.3 Modification of the amide side-chain to reduce CYP450 inhibition

4.3.1 Rationale

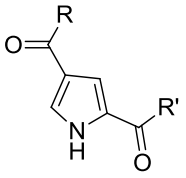
One of the anticipated problems with some of the more potent inhibitors in the shortened amide side-chain series was that they may also be potent inhibitors of CYP450 enzymes. CYP450 enzymes are involved in the metabolism of chemicals *in vivo.* Their main function is to increase the ability of compounds to be excreted *via* conjugation of polar functionality, which is usually achieved *via* an oxidation reaction. Oxidation of potential drugs can lead to poor bioavailability and potentially toxic metabolites, meaning it is important to investigate whether any potential drug candidates will bind to, and be metabolised by, CYP450 enzymes.[121] It is also important to ascertain whether compounds will inhibit CYP450 enzymes and prevent the metabolism of other substances in the body. All compounds with pyridyl side chains were expected to bind to CYP450 enzymes and be substrates for *N*-oxidation. Until recently, it was thought that the main enzyme involved in metabolism *via N*-oxidation was flavin-containing monooxygenases (FMO). However, mechanisms for *N*-oxidation by CYP450 enzymes have now been proposed. The active site of CYP450 enzymes contains a haem centre, which is tethered to the P450 enzyme *via* a thiolate ligand from a cysteine residue. The catalytic cycle of CYP450 enzymes begins with the binding of a substrate at a site near the haem centre. There are two postulated mechanisms for *N*-oxidation by CYP450 enzymes. The first proposes an electron transfer from the nitrogen to the iron centre of FeO^{3+}. The nitrogen radical can then attack the oxygen before Fe^{3+} is regenerated and the *N*-oxide is formed (mechanism A, Scheme 30). The alternative mechanism involves a second electron transfer from the nitrogen to the iron centre before the *N*-oxide is formed (mechanism B, Scheme 30).[121]

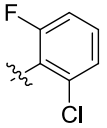
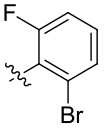
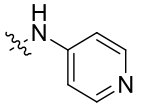
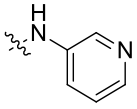


Scheme 30. Two proposed mechanisms for *N*-oxidation by CYP450 enzymes. Modified from reference 26.

With this in mind, two of the most potent compounds containing a pyridyl side chain were tested against a panel of CYP450 enzymes, and these results are shown in Table 16. Compounds **150** and **151** have either a 2-chloro-6-fluorobenzoyl group or a 2-chloro-6-fluorobenzoyl group as these groups were identified by another member of the groups as optimal for good selectivity for ERK5 over p38α. The 4-pyridyl compound **150** was a potent inhibitor of all of the enzymes in the panel, particularly CYP2C19 and CYP2C9. The 3-pyridyl compound **151** showed reduced inhibition against the same panel of CYP450 enzymes, but was still shown to inhibit the enzymes with an IC_{50} <10 μM, which could still mean a more rapid rate of metabolism than is desirable. The reason for the reduced CYP inhibition of the 3-pyridyl compounds could be due to the fact that the nitrogen atom in the ring is no longer in the optimal position to interact with the haem centre of the enzyme.

Table 16. CYP450 Inhibition profile of **150** and **151**



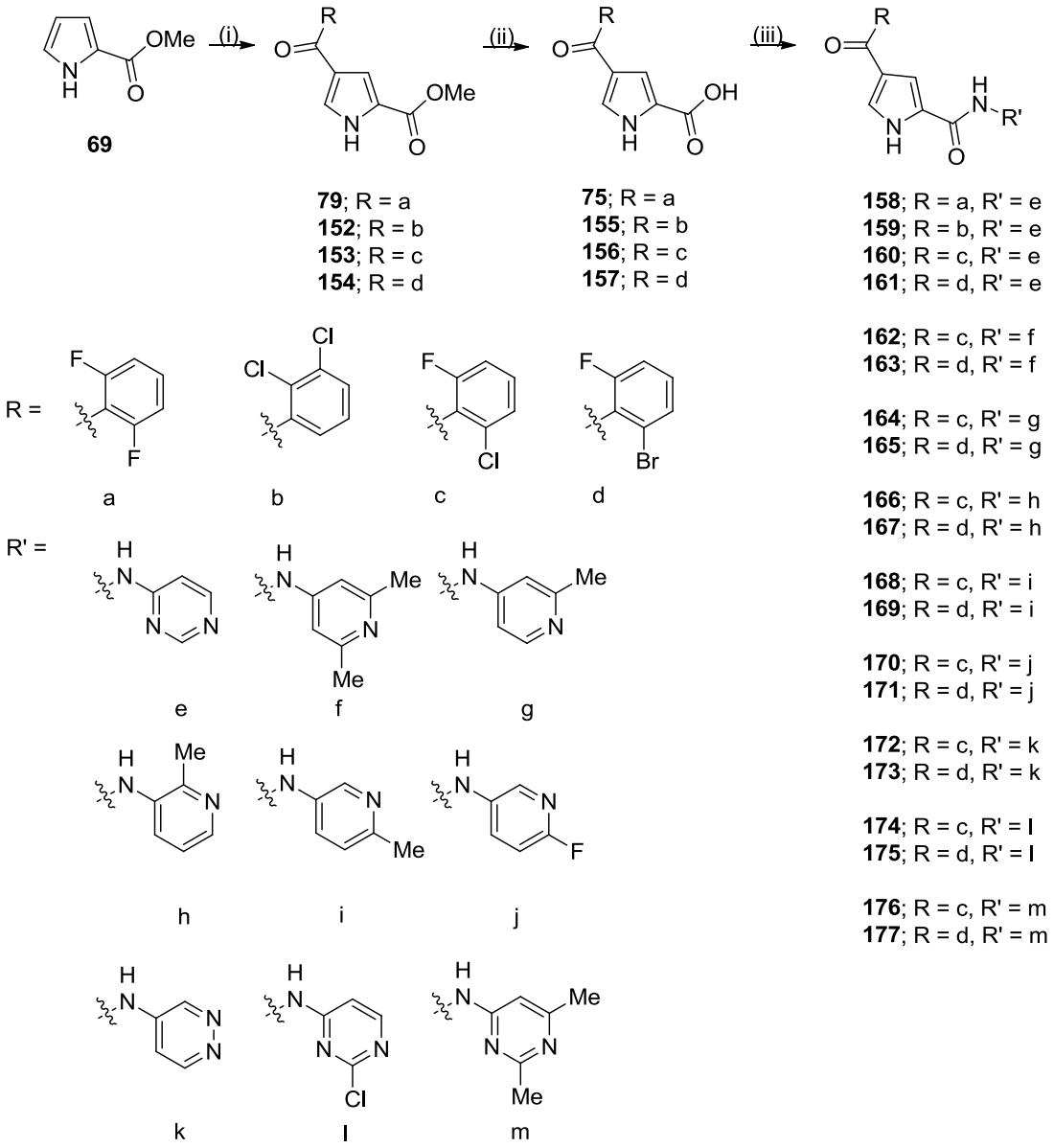
Compound	150	151
R		
R'		
CYP1A; IC_{50} (μM)	-*	6.0 ± 0.60
CYP2C9; IC_{50} (μM)	0.35 ± 0.01	14 ± 1.7
CYP3A4; IC_{50} (μM)	1.2 ± 0.18	8.4 ± 1.6
CYP2C19; IC_{50} (μM)	0.07 ± 0.02	8.5 ± 2.5
CYP2D6; IC_{50} (μM)	1.1 ± 0.16	9.2 ± 1.3

*unable to calculate an IC_{50} value over concentration range tested. 32.8% inhibition observed at 5 μM

In order to try and reduce the CYP450 inhibition of these inhibitors, alternative side chains were investigated. A review of the literature found that replacement of a pyridyl group for substituted pyridyl moieties, various non-substituted and substituted pyrimidine groups or a pyridazine ring had been successful in reducing CYP450 inhibition.[122-124]

4.3.2 Synthesis of inhibitors to reduce CYP450 inhibition

A new series of inhibitors were synthesised and tested against ERK5 and p38α to ensure potency and selectivity were retained when the amide side-chain was modified (Scheme 31). For the first set of inhibitors with a 4-aminopyrimidine side chain, compounds were synthesised with 2,6-difluorobenzoyl, 2,3-dichlorobenzoyl, 2-chloro-6-fluorobenzoyl and 2-bromo-6-fluorobenzoyl groups in the 4-position of the pyrrole ring. All subsequent compounds were synthesised with the 2-chloro-6-fluorobenzoyl and 2-bromo-6-fluorobenzoyl solely as these groups had been found to be optimal in this position. Methyl pyrrole-2-carboxylate (**69**) was acylated regioselectively in the 4-position using the Friedel-Crafts procedure with the appropriate benzoyl chloride in good yield (63-80%) (Scheme 31). Each methyl ester was then treated with aqueous LiOH to give the corresponding carboxylic acids **75** and **155-157** in excellent yield (99%). The appropriate aniline was then coupled using PCl_3 under microwave irradiation to give the 20 desired compounds **158-177** with yields ranging from 45-76%. Table 17 shows the relevant yields for each compound.



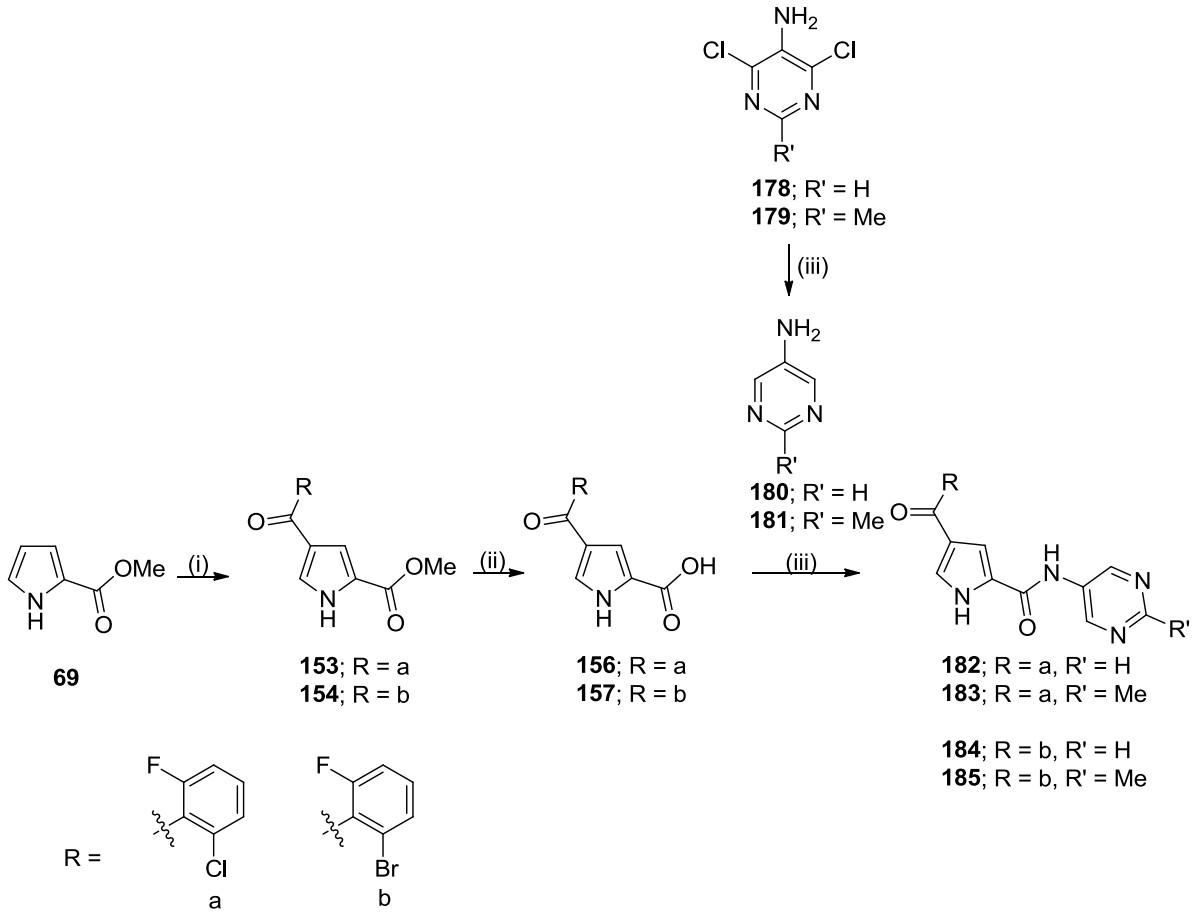
Scheme 31. *Reagents and conditions*: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) PCl_3, appropriate aniline, MeCN, MW, 150 ^oC, 5 min.

Table 17. Yields achieved for Steps (i), (ii) and (iii) in Scheme 31.

	Yield (%)		
Compound	**Step (i)**	**Step (ii)**	**Step (iii)**
158	66	99	76
159	71	99	72
160	63	99	60
161	80	99	62
162	63	99	50
163	80	99	62

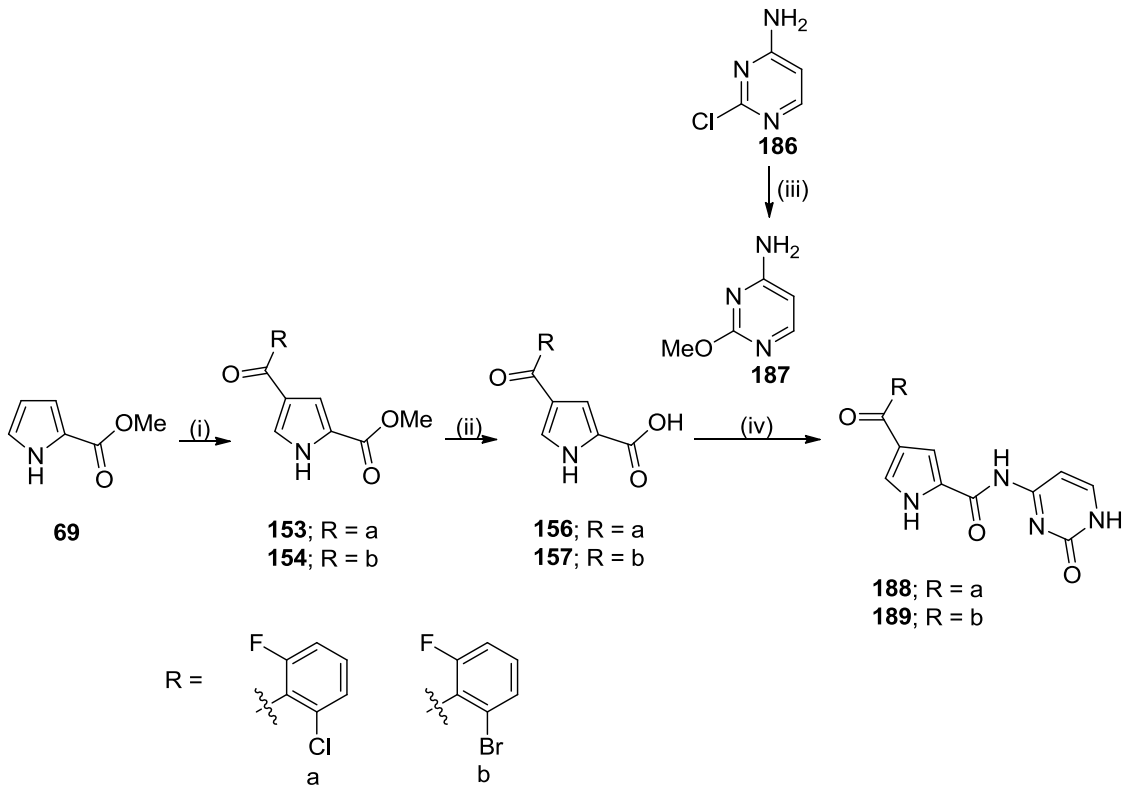
164	63	99	60
165	80	99	69
166	63	99	60
167	80	99	52
168	63	99	52
169	80	99	63
170	63	99	50
171	80	99	45
172	63	99	65
173	80	99	61
174	63	99	55
175	80	99	58
176	63	99	48
177	80	99	55

Three of the amine side chains to be incorporated were not commercially available and had to be synthesised prior to the amide coupling. The syntheses of 5-aminopyrimidine and 5-amino-2-methylpyrimidine are shown in Scheme 32. A literature procedure was followed for the dehalogenation reaction between commercially available 5-amino-4,6-dichloropyrimidine (**178**) and 5-amino-4,6-dichloro-2-methylpyrimidine (**179**) with ammonium formate and palladium on carbon.[125] The reaction proceeded smoothly at reflux for 1 h, providing 5-aminopyrimidine **180** and 5-amino-2-methylpyrimidine **181** in 80% yield respectively. Amide coupling of **180** and **181** with carboxylic acid intermediates **156** and **157** provided **185-185** in 40-79% yield. Using the PCl_3-mediated coupling method, the reactions required further heating time (30 min) due to the electron-poor pyrimidine coupling partners.



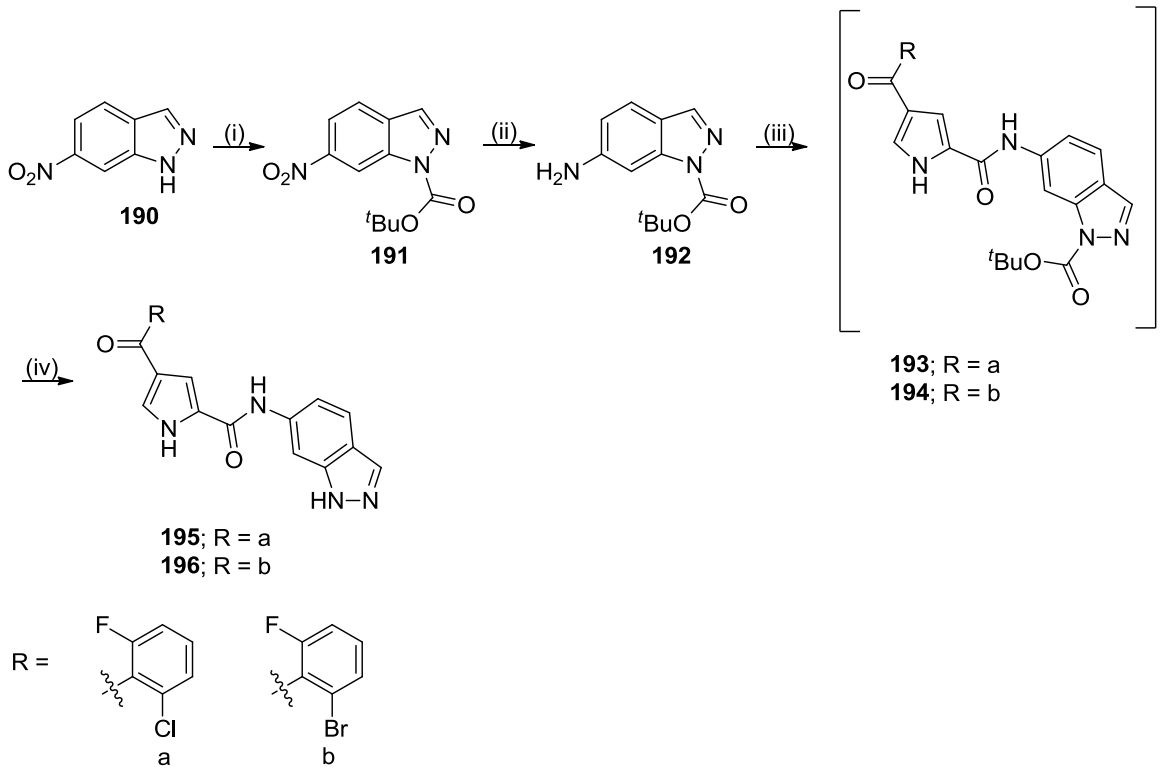
Scheme 32. *Reagents and conditions*: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) NH_4HCOO, Pd/C, MeOH, reflux, 1 h; (iv) PCl_3, appropriate amine, MeCN, MW, 150 ^oC, 30 min.

4-Amino-2-methoxypyrimidine was synthesised following Scheme 33. Substitution of the chloro- substituent of 4-amino-2-chloropyrimidine (**186**) using sodium methoxide produced 4-amino-2-methoxypyrimidine (**187**) in 90% yield. Reaction of 4-amino-2-methoxypyrimidine (**187**) with carboxylic acid intermediates **156** and **157** using the PCl_3 coupling method produced pyrimidones **188** and **189**, with *in situ* demethylation of the methoxy substituent, in 30% and 45%, respectively. The low yields in this case could be attributed to the fact that there was a mixture obtained of the pyrimidone compounds as the major product and also the methoxypyrimidine compounds. There was too little of the methoxypyrimidine compounds to purify.



Scheme 33. *Reagents and conditions*: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) Na, MeOH, 50 ^oC, 4 h; (iv) PCl_3, 4-amino-2-methoxypyrimidine, MeCN, MW, 150 ^oC, 5 min.

Compounds incorporating an indazole amide side-chain were also synthesised according to Scheme 34. 6-Nitroindazole (**190**) was protected with a Boc group using Boc_2O to produce **191** in 92% yield. Reduction of the nitro group of **191** *via* continuous flow hydrogenation gave **192** in 90% yield. Amide coupling of **192** to carboxylic acid intermediates **156** and **157** using CDI followed by *in situ* deprotection using TFA gave compounds **195** and **196** in 16 and 20% yield, respectively.

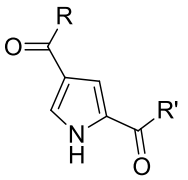


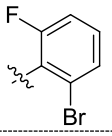
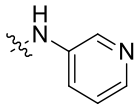
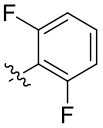
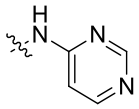
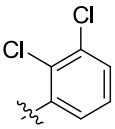
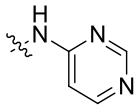
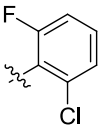
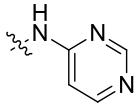
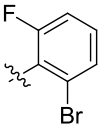
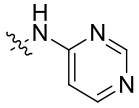
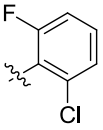
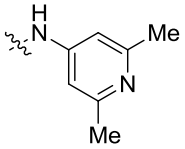
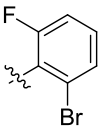
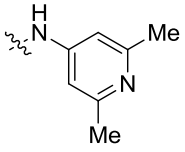
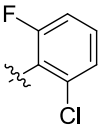
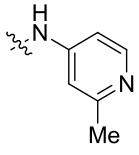
Scheme 34. *Reagents and conditions*: (i) Boc_2O, NEt_3, DCM, RT, 18 h; (ii) THF, H-cube, full H_2 mode, 1 mL/min, 2.5 h; (iii) (a) **156** or **157**, CDI, THF, 70 ^oC, 3 h; (b) appropriate amine, 50 ^oC to RT, 18 h; (iv) TFA, RT, 1.5 h.

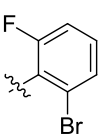
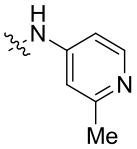
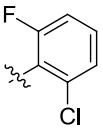
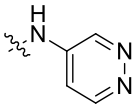
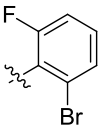
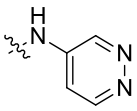
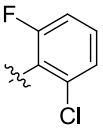
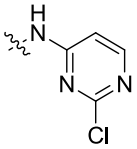
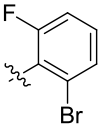
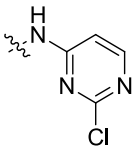
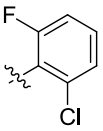
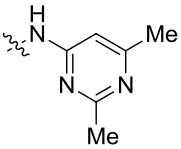
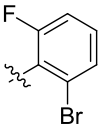
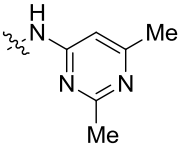
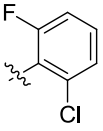
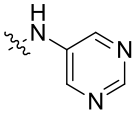
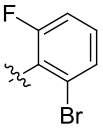
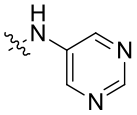
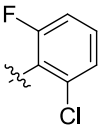
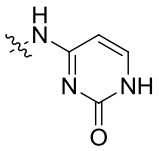
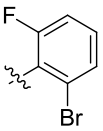
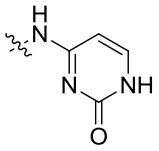
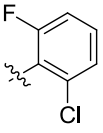
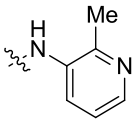
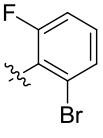
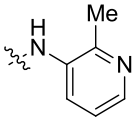
4.3.3 Biological evaluation of compounds 158-196

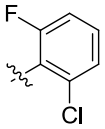
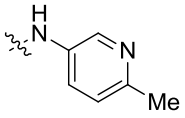
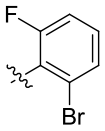
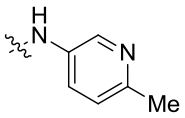
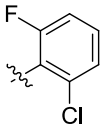
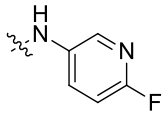
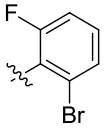
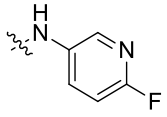
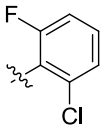
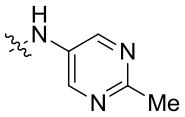
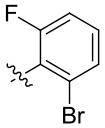
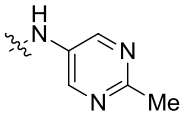
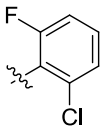
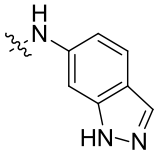
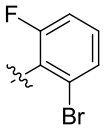
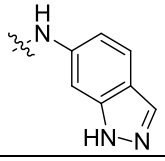
The ERK5 inhibitory activity and p38α counterscreening data for compounds **158-196** are shown in Table 18 with each compound compared to 3-pyridyl compound **151**.

Table 18. Biological results for compounds **158-196**.



Compound	R	R'	ERK5; IC_{50} (μM)	P38α; IC_{50} (μM)
151			0.82 ± 0.07 (n = 4)	>120
158			2.1 ± 0.11 (n = 4)	>120
159			2.7 ± 1.6 (n = 6)	>120
160			1.2 ± 0.84 (n = 6)	>120
161			1.8 ± 1.1 (n = 6)	>120
162			0.34 ± 0.14 (n = 6)	>120
163			0.52 ± 0.25 (n = 6)	>120
164			0.35 ± 0.17 (n = 6)	>120

165			1.1 ± 0.48 (n = 6)	>120
172			0.44 ± 0.26 (n = 6)	>120
173			0.68 ± 0.37 (n = 6)	>120
174			4.4 ± 0.92 (n = 2)	>120
175			7.8 ± 2.3 (n = 2)	>120
176			4.1 ± 0.55 (n = 2)	>120
177			5.0 ± 0.64 (n = 2)	>120
182			0.36 ± 0.10 (n = 4)	>120
183			0.79 ± 0.16 (n = 4)	>120
188			2.9 ± 0.24 (n = 2)	>120
189			34 ± 3.2 (n = 2)	>120
166			0.96 ± 0.16 (n = 2)	84 ± 5.0
167			2.4 ± 0.60 (n = 2)	>120

168			1.0 ± 0.07 (n = 2)	56 ± 4.4
169			1.5 ± 0.40 (n = 4)	93 ± 5.9
170			1.5 ± 0.80 (n = 2)	>120
171			1.7 ± 0.02 (n = 2)	>120
183			1.2 ± 0.60 (n = 6)	>120
185			1.2 ± 0.20 (n = 2)	>120
195			1.5 ± 0.40 (n = 2)	>120
196			7.2 ± 0.60 (n = 2)	>120

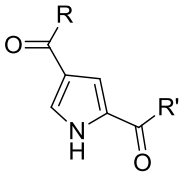
All of the inhibitors in Table 18 are selective for ERK5 over p38α. Introduction of one or two methyl groups flanking the pyridyl nitrogen greatly improved ERK5 inhibitory activity compared to compound **151** (i.e. **162**, ERK5; IC_{50} = 0.34 μM and **164**, ERK5; IC_{50} = 0.35 μM). Replacement of the pyridyl ring in **151** with a pyridazine or a pyrimidine moiety also provided more potent ERK5 inhibitors (i.e. **172**, ERK5; IC_{50} = 0.44 μM and **182**, ERK5; IC_{50} = 0.36 μM).

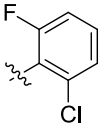
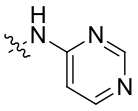
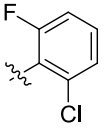
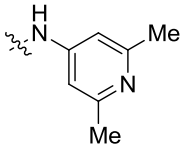
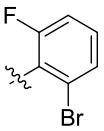
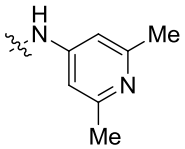
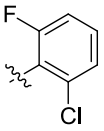
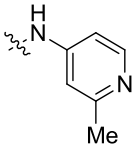
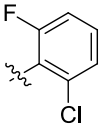
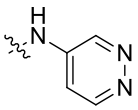
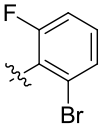
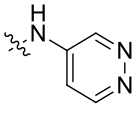
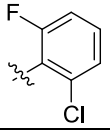
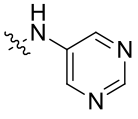
Introduction of a chloro substituent on the pyrimidine ring was detrimental to ERK5 inhibitory activity (**175**, ERK5; IC_{50} = 7.8 μM) as was the introduction of an indazole ring (**196**, ERK5; IC_{50} = 7.2 μM), suggesting that increasing the size of the amide side-chain was not tolerated in the ERK5 ATP binding site.

4.3.4 Physicochemical properties and in vitro ADME profiling of selected potent compounds

The most potent compounds determined by the initial cell-free ERK5 inhibition assay were taken forward for *in vitro* ADME profiling and initial drug metabolism studies at Cyprotex. The physicochemical properties (calculated by StarDrop) of the seven compounds were reviewed initially in order to ensure they met the lead criteria (Table 19).

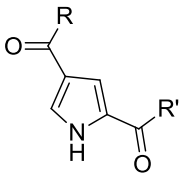
Table 19. Physicochemical properties of compounds **160-182**.

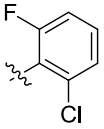
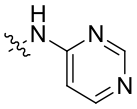
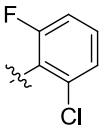
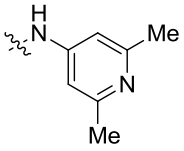
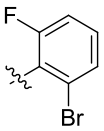
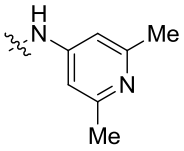
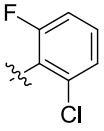
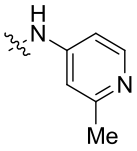
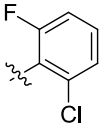
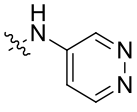
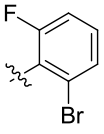
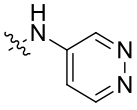
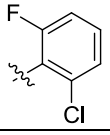
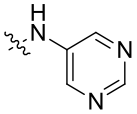


Compound	R	R'	MW	H-bond donors	H-bond acceptors	sLogP	TPSA
Lead criteria	-	-	<500	<5	<10	<5	75-100
160			345	2	6	3.3	88
162			372	2	5	3.8	75
163			416	2	5	3.9	75
164			358	2	5	3.4	75
172			345	2	6	3.3	88
173			389	2	6	3.3	88
182			345	2	6	3.3	88

All of the seven compounds fulfilled the lead candidate criteria in terms of physicochemical properties and were selected for *in vitro* ADME profiling and drug metabolism studies at Cyprotex (Table 20).

Table 20. *In vitro* PK and drug metabolism properties of compounds **160**-**182**.



Compound	R	R'	LE	Solubility L/U (µM)	PPB (%)	Human CLint (µL/min/mg)	Mouse CLint (µL/min/mg)
Lead criteria	-	-	>0.30	>50/50	<99	<48	<48
160			0.37	100/100	94	7.7	49
162			0.34	10/30	91	24	13
163			0.33	3/20	93	33	26
164			0.35	10/30	94	27	25
172			0.36	100/100	87	7.9	39
173			0.35	100/100	85	6.4	64
182			0.37	100/100	88	4.6	5.0

The most potent compounds from this series were also sent for testing against a panel of CYP450 enzymes in order to see if replacement of the pyridyl side chain does reduce CYP450 inhibition. These results are shown in Table 21 and are compared to those for compound **151**. The optimal IC_{50} value against each CYP450 isoform would be >10 μM.

Table 21. CYP450 Inhibition profile of most potent compounds in Table 18.

Compound	CYP1A; IC_{50} (μM)	CYP2C9; IC_{50} (μM)	CYP3A4; IC_{50} (μM)	CYP2C19; IC_{50} (μM)	CYP2D6; IC_{50} (μM)
151	6.0 ± 0.6	14 ± 1.7	8.4 ± 1.6	8.5 ± 2.5	9.2 ± 1.3
160	23 ± 3.6	0.58 ± 0.06	4.5 ± 1.1	0.29 ± 0.05	6.0 ± 1.8
162	>25	>25	>25	>25	>25
163	>25	>25	>25	>25	>25
164	>25	>25	>25	9.8*	>25
172	>25	1.3*	13*	0.50*	10*
173	>25	1.1*	16*	0.90*	6.8*
182	22 ± 3.2	24 ± 3.3	12 ± 2.8	22 ± 3.7	>25

*n = 1

Compound **160** was the most potent of the four compounds containing a 4-aminopyrimidine side chain (IC_{50} = 1.2 μM) and also has excellent aqueous solubility (100 μM), plasma protein binding (94%) and human microsomal clearance (CLint = 7.68 μL/min/mg). Clearance in mice is higher (CLint = 48.7 μL/min/mg), but the overall profile is good. However, **160** inhibits the CYP450 isoforms tested, with particularly potent inhibition observed in both CYP2C9 (IC_{50} = 0.58 μM) and CYP2C19 (IC_{50} = 0.29 μM), meaning the replacement of the 4-pyridyl ring of **151** with a 4-pyrimidyl group does not reduce CYP450 inhibition. Both **162** and **163** showed potent ERK5 activity (IC_{50} = 0.34 μM and IC_{50} = 0.52 μM, respectively). Although plasma protein binding and clearance were good for both inhibitors, aqueous solubility was reduced (S (L/U = 10/30 μM and 3/20 μM). This is due to the introduction of lipophilicity through the introduction of the two methyl groups flanking the pyridyl nitrogen. For both of these inhibitors the CYP450 inhibition profile was greatly improved with IC_{50} >25 μM against all CYP450 isoforms tested. This validates the theory that blocking the pyridyl nitrogen from possible oxidation by CYP450 enzymes reduces CYP450 inhibition. Introduction of just one flanking methyl group onto the pyridyl moiety was also interesting. Compound **164** was shown to be a potent inhibitor of ERK5 (IC_{50} = 0.35 μM) and also had reduced activity against all of the CYP450 enzymes in the panel.

Again the aqueous solubility was less than optimal (S (L/U) = 10/30 µM), but plasma protein binding and clearance were good (PPB = 94%; human CLint = 27 µL/min/mg and mouse CLint = 25 µL/min/mg). The two compounds containing pyridazine side chains, **172** and **173** were both potent inhibitors of ERK5 (IC_{50} = 0.44 and 0.68 µM respectively). They both had excellent aqueous solubility (S (L/U) = 100/100 µM), plasma protein binding (87% and 84%) and no clearance, with the exception of **173** in mouse microsomes (**172**; human Clint = 7.9 µL/min/mg, mouse CLint = 39 µL/min/mg; **173**; human CLint = 6.4 µL/min/mg, mouse CLint = 64 µL/min/mg). However, in both cases the CYP450 inhibition is not reduced, with both compounds showing potent inhibition of CYP2C19 (**172**; IC_{50} = 0.50 µM; **173**; IC_{50} = 0.90 µM). Finally, **182** has excellent ERK5 inhibitory activity (IC_{50} = 0.36 µM), aqueous solubility (S (L/U) = 100/100 µM), plasma protein binding (88%) and clearance (human CLint = 4.6 µL/min/mg; mouse CLint = 5.0 µL/min/mg). Compound **182** has also been shown to have an excellent CYP450 inhibition profile with an IC_{50} value of >20 µM for four out of the five CYP450 isoforms tested and >10 µM for the final isoform. Overall compound **182** has an excellent drug-like profile with the right balance of *in vitro* ADME properties.

Chapter 5. Structure-activity relationship studies (SARs) around the aroyl ring

5.1 Effect of *para*-substitution of the aroyl ring on ERK5 inhibitory activity

5.1.1 Rationale

In the absence of a crystal structure of ERK5, homology modelling by sequence alignment and superimposition was conducted by Dr Susan Boyd on behalf of CRT-DL. As stated in chapter 3, ERK2 was identified as the closest homologue of ERK5 with 51% and 78% sequence homology in the kinase and ATP binding site domains, respectively. The first generation homology model was based on ERK2 and was generated using MOE software from Chemical Computing Group, using the Amber99 force field with Born solvation.

Using the homology model, two pyrrole carboxamide inhibitors were docked into the ERK5 binding site, and two potential binding modes were proposed. Modelling of **197** led to the development of binding mode A (Figure 33). This binding mode suggests that the halogenated aryl ring points out towards solvent whilst the pyridyl nitrogen interacts with a salt bridge lysine.

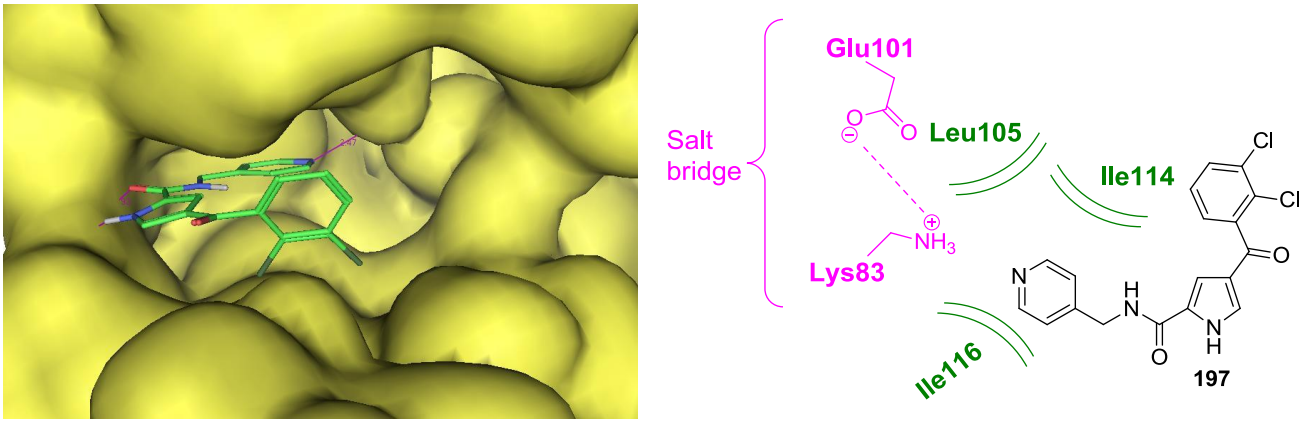


Figure 33. Proposed binding mode A for the pyrrole carboxamide series, modelled using **197**.

Binding mode B was obtained using a compound with a penta-substituted aroyl ring (**198**) (Figure 34). In this binding mode, the pyrrole ring was thought to bind in the opposite orientation, with the pyridylmethylamine ring pointing out towards solvent.

The aroyl ring is directed into a hydrophobic pocket in the binding site, and is therefore constrained. The chloro substituents are shown in space-filling mode to illustrate the limited size of the binding pocket. This is consistent with previous SARs, which suggest the aroyl ring may be sensitive to substitution pattern.

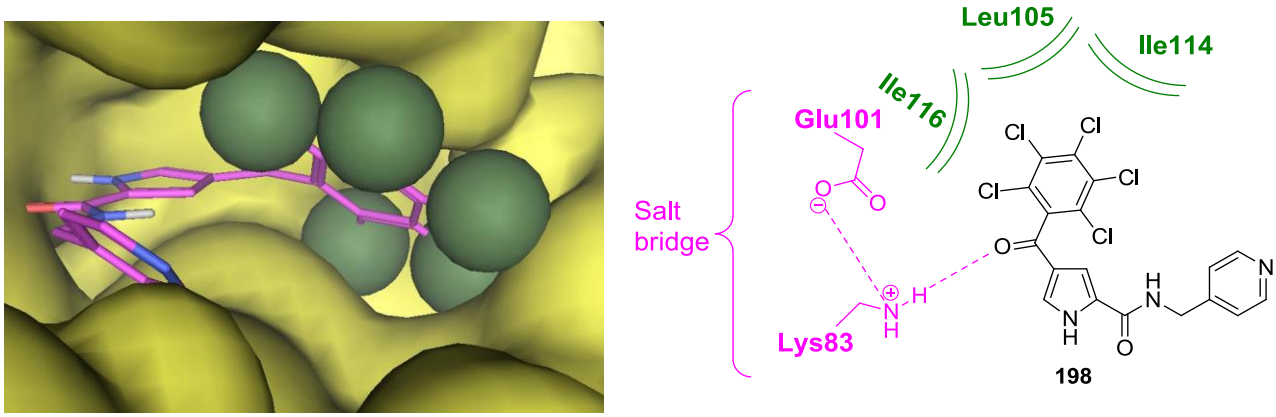
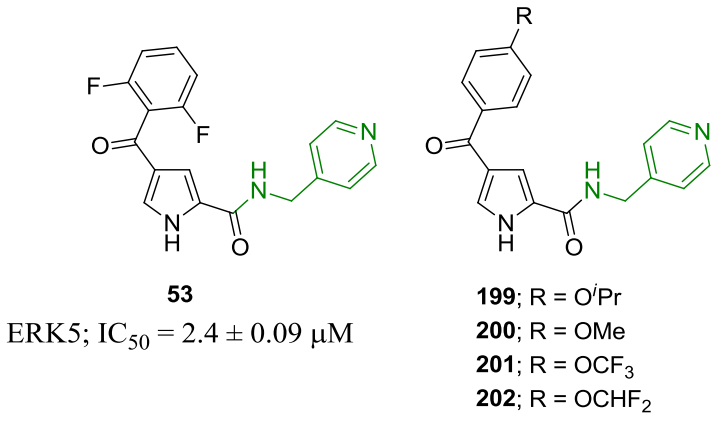


Figure 34. Alternative binding mode B for the pyrrole carboxamide series.

The rationale behind the synthesis of compounds with a *para*-substituent on the aroyl ring was to determine which of two proposed binding modes was preferred. If the *para*-substituted compounds were potent inhibitors of ERK5, it would suggest that binding mode A was adopted as the benzoyl ring would not be constrained in this binding mode. Conversely, if inactivity was observed, it would appear binding mode B is preferred since this proposed mode suggests the benzoyl ring is tightly bound within a hydrophobic pocket.

The inhibitors to be synthesised are shown in Figure 35. The amine substituent is fixed as the 4-pyridylmethylamine group based on the original hit compounds (i.e. **53**, Figure 35). The shortened amide side-chains had not been discovered at the time of synthesis. Varying the size and electronic properties of the groups to be incorporated at the *para*-position, helps to further probe which groups will be tolerated in this position and gives information on the surrounding ATP binding site of ERK5.



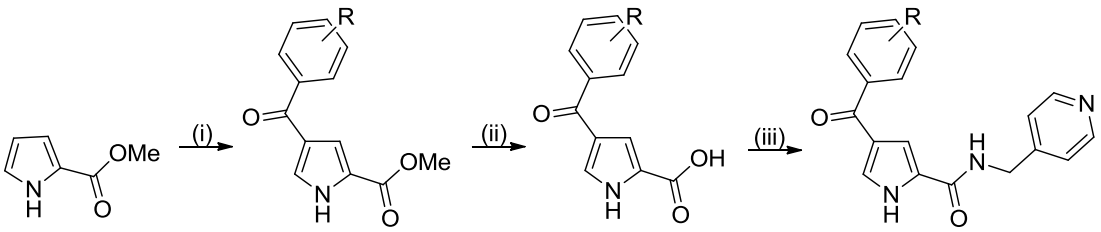
53
ERK5; IC_{50} = 2.4 ± 0.09 μM

199; R = O^{i}Pr
200; R = OMe
201; R = OCF_3
202; R = $OCHF_2$

Figure 35. Proposed targets to probe tolerance of *para*-substitution.

5.1.2 Synthesis of inhibitors 199-201 with a para-substituted aroyl ring

The target compounds **199**, **200** and **201** were synthesised following the previously optimised 3-step route (Scheme 34). The first step Friedel-Crafts acylation gave esters **72**, **203** and **204** in good yield (67%, 77% and 79%, respectively). Hydrolysis of the methyl ester using lithium hydroxide gave the corresponding carboxylic acids, **73**, **205** and **206** in 94%, 84% and 84%, respectively. CDI-mediated coupling of picolylamine with the previously prepared carboxylic acids, provided **199**, **200** and **201** in excellent yields (90%, 85% and 85%, respectively).



69

72; R = OiPr
203; R = OMe
204; R = OCF_3

73; R = OiPr
205; R = OMe
206; R = OCF_3

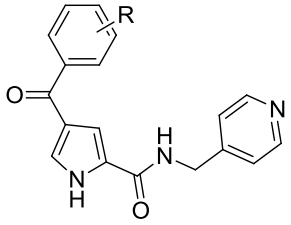
199; R = OiPr
200; R = OMe
201; R = OCF_3

Scheme 34. *Reagents and conditions*: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH(aq), THF, 60 ^oC, 18 h; (iii) (a) CDI, THF, 70 ^oC, 3 h; (b) 4-picolylamine, 50 ^oC to RT, 18 h.

5.1.3 ERK5 inhibitory activity of 199-201

Compounds **199**, **200** and **201** were tested for ERK5 inhibitory activity and the results are shown in Table 20 with compounds being compared to hit compound **53**. All three compounds proved totally inactive against ERK5, with all IC_{50} values >120 μM.

Table 20. Biological results for **199**, **200** and **201**.



Compound	R	ERK5; IC_{50} (μM)
53	2,6-diF	2.4 ± 0.09 (n = 2)
199	4-O^{i}Pr	>120
200	4-OMe	>120
201	4-OCF_3	>120

The observed lack of activity provides further evidence that *para*-sustitution is not tolerated on the aroyl ring, as suggested by previous SARs. It also shows that these compounds are more likely to bind within the ERK5 active site according to proposed binding mode B illustrated in Figure 34. This binding mode suggests that the aroyl ring is directed into a small hydrophobic pocket imposing limited tolerance around this ring.

5.1.4 Synthesis of para-difluoromethoxybenzoyl analogue 202

The general synthetic scheme outlined previously for the synthesis of the other *para*-substituted analogues could not be employed for the synthesis of **200**. Problems were encountered with the Friedel-Crafts acylation with none of the desired product isolated despite several attempts. Conversion of the commercially available 4-$OCHF_2$ benzoic acid to the corresponding benzoyl chloride was conducted using thionyl chloride and the product used immediately in order to rule out potential instability as a reason for the lack of reaction. A review of the literature suggested that the -$OCHF_2$ group could react with the $AlCl_3$ Lewis acid catalyst used in this step. It has been observed that C-F bonds can be activated by aluminium reagents. The C-F bond can be cleaved upon coordination with a strong activating agent such as aluminium or boron, both of which have been shown to bind strongly to fluorine (159 and 181 kcal/mol respectively),

meaning Friedel-Crafts type arylations can possibly occur on the CHF_2 group with the loss of fluorine gas (Figure 36).[126]

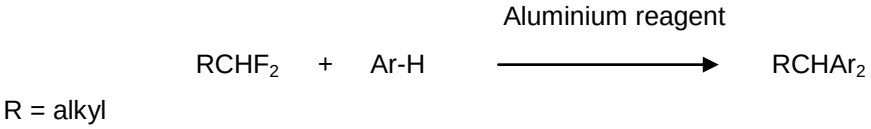
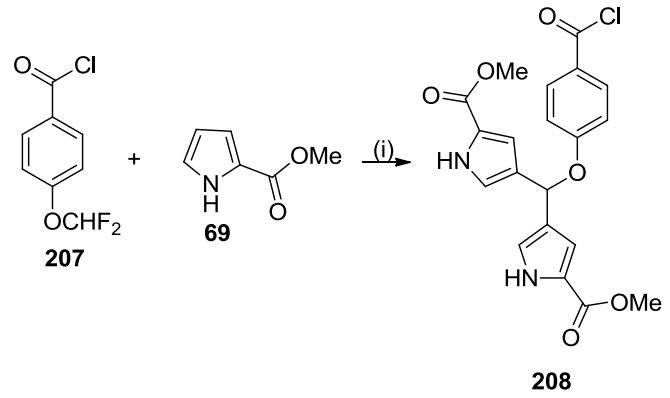


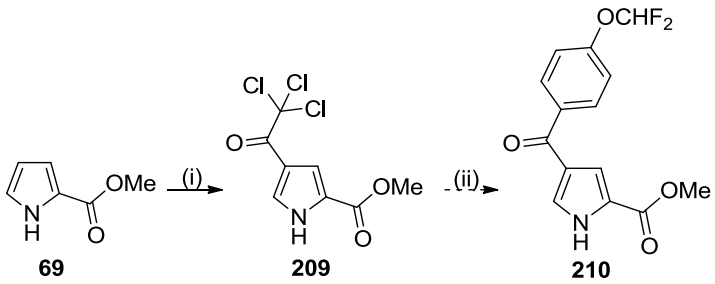
Figure 36. Possible Friedel-Crafts arylation of the CHF_2 group in the presence of an aluminium reagent.

According to the literature, this reaction has been investigated using a variety of hydrofluorocarbons (HFCs), and the expected arylated products according to the above proposed reaction were isolated when the reaction was completed at 0 ^{o}C.[126] It was also observed that there were differences in reactivity between CH_2F, CHF_2, and CF_3 groups, with the following identified as the reactivity pattern, $CH_2F>CHF_2>CF_3$, with CHF_2 fluorine atoms being substituted selectively in the presence of CF_3 groups in the same molecule. This could explain why there were no problems encountered during the Friedel-Crafts acylation reaction involving the 4-OCF_3 substituted benzoyl chloride, due to the fact that additional fluorine atoms in a molecule can strengthen neighbouring C-F bonds.[126] According to these observations, the potential product from the originally attempted Friedel-Crafts reaction could be as shown in Scheme 35. Our hypothesis was also strengthened by ^{19}F NMR of the crude isolated material where a lack of fluorine was observed.



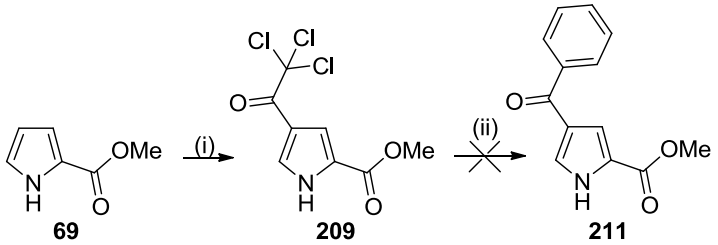
Scheme 35. *Reagents and Conditions*: (i) $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h.

An alternative synthetic approach was attempted (Scheme 36), using a Friedel-Crafts reaction to insert a trichloroacetyl substituent into the 4-position of the pyrrole ring. We used the optimised reaction conditions with trichloroacetyl chloride, and the desired product **209** was obtained in 89% yield. (4-(Difluoromethoxy)phenyl)magnesium bromide could then be used in order to displace the trichloromethyl anion and give **210**.



Scheme 36. *Reagents and conditions*: (i) trichloroacetyl chloride, $AlCl_3$, 0 ^oC to RT, 18 h; (ii) (4-(difluoromethoxy)phenyl)magnesium bromide, Et_2O, RT, 18 h.

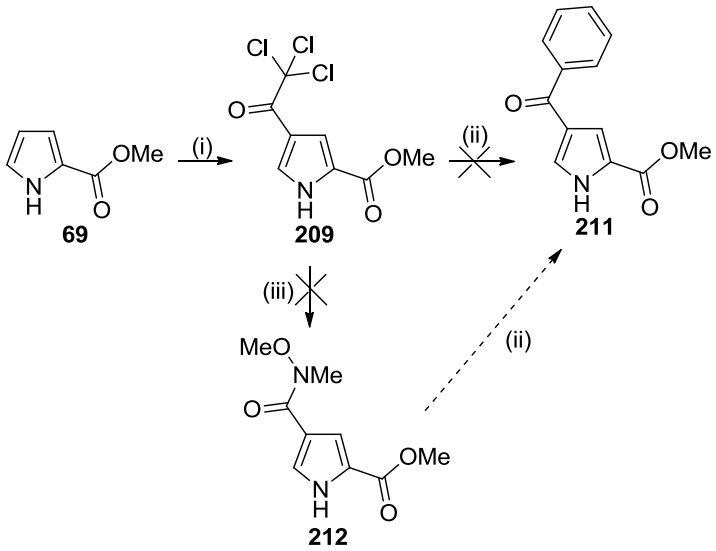
The Grignard reaction was tested in a model reaction using phenylmagnesium bromide (Scheme 37). This reaction was unsuccessful when attempted either with commercial Grignard reagent, or when the reagent was formed *in situ*. The lack of success could be explained by a possible halogen metal exchange between the Grignard reagent and the trichoroacetyl group. As described in the literature, the trichloroacetyl group can act as a Cl^+ donor.[127] It was also suggested that the Grignard reagent may be sufficiently basic to deprotonate the pyrrole NH, and chelation could occur between the pyrrole nitrogen and the carbonyl group, therefore preventing reaction in the desired position.



Scheme 37. *Reagents and conditions*: (i) trichloroacetyl chloride, $AlCl_3$, 0 ^oC to RT, 18 h; (ii) phenylmagnesium bromide, Et_2O, RT, 18 h.

Conversion of the trichloroacetyl group to a Weinreb amide to give **212** was attempted using *N,O*-dimethylhydroxylamine (Scheme 38) followed by reaction with the Grignard reagent.[128] The trichloroacetyl group should react readily with an amine as this had been

performed successfully by other members of the group. However, using *N,O*-dimethylhydroxylamine this reaction was unsuccessful, and so the subsequent Grignard reaction could not be attempted. Conversion to the Weinreb amide **212** was attempted using a variety of conditions in which the number of equivalents of the amine and base were modified, along with the temperature (Table 21). Since none of these modifications were successful, an alternative strategy towards the target compound was sought.



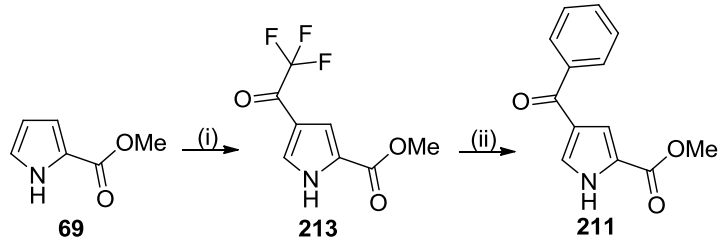
Scheme 38. *Reagents and conditions*: (i) trichloroacetyl chloride, $AlCl_3$, 0 ^oC to RT, 18 h; (ii) phenylmagnesium bromide, Et_2O, RT, 18 h; (iii) *N,O*-dimethylhydroxylamine hydrochloride, NEt_3, DMF, see Table 21 for attempted conditions.

Table 21. Conditions attempted for conversion of **207** to **210**.

Entry	Temp. (^oC)	Time (h)	Equiv. *N,O*-dimethylhydroxylamine . HCl	Equiv. NEt_3	Result
1	RT	18	1.2	1.0	No reaction
2	RT	18	3.2	3.0	Degradation
3	50	18	1.2	1.0	Degradation
4	RT	18	1.0	3.6	No reaction

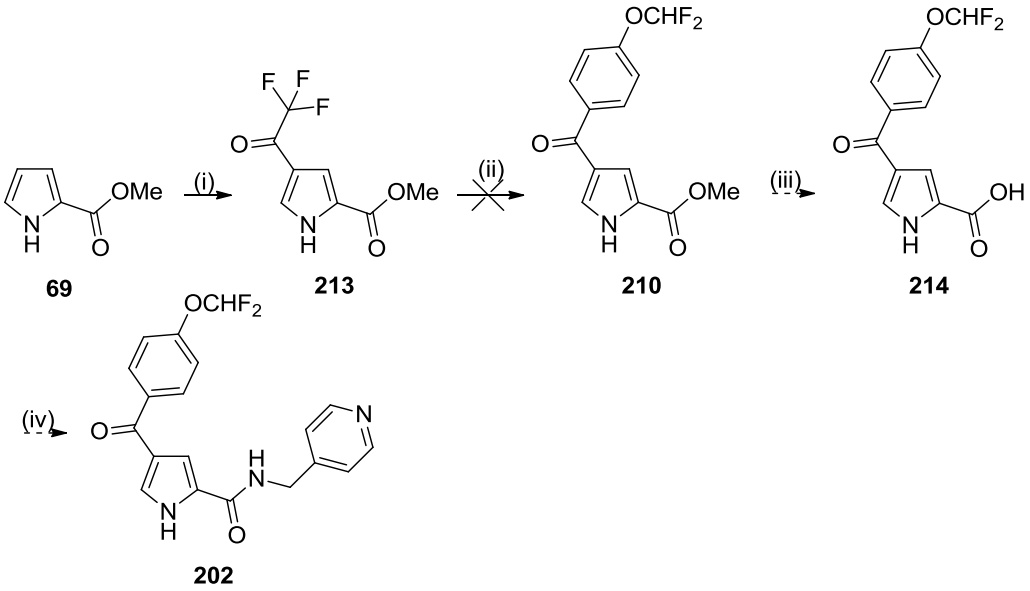
The next approach we explored consisted of using an alternative leaving group to the trichloro- group, the trifluoro- group. The Friedel-Crafts reaction was utilised to introduce a trifluoroacetyl substituent onto the 4-position of the pyrrole ring, using trifluoroacetic anhydride. Compound **213** was obtained in a good yield of 75%. The

subsequent model Grignard reaction was performed using phenylmagnesium bromide, and the desired product **211** was isolated in 40% yield (Scheme 39).



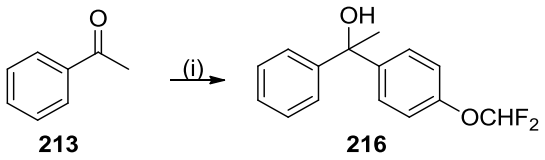
Scheme 39. *Reagents and conditions*: (i) TFAA, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) phenylmagnesium bromide, THF, RT, 18 h.

We applied this methodology to the synthesis of the **210** (Scheme 40). The Grignard reagent for this transformation was generated *in situ* using 1-(bromo-4-difluoromethoxy)benzene and magnesium turnings before being reacted with **213** in a 1:1 ratio of equivalents. After overnight reaction, a 6% conversion to the desired product **210** could be observed by LC-MS, and no further reaction occurred after further stirring overnight. Attempts to isolate **210** were unsuccessful due to the small scale of the reaction.



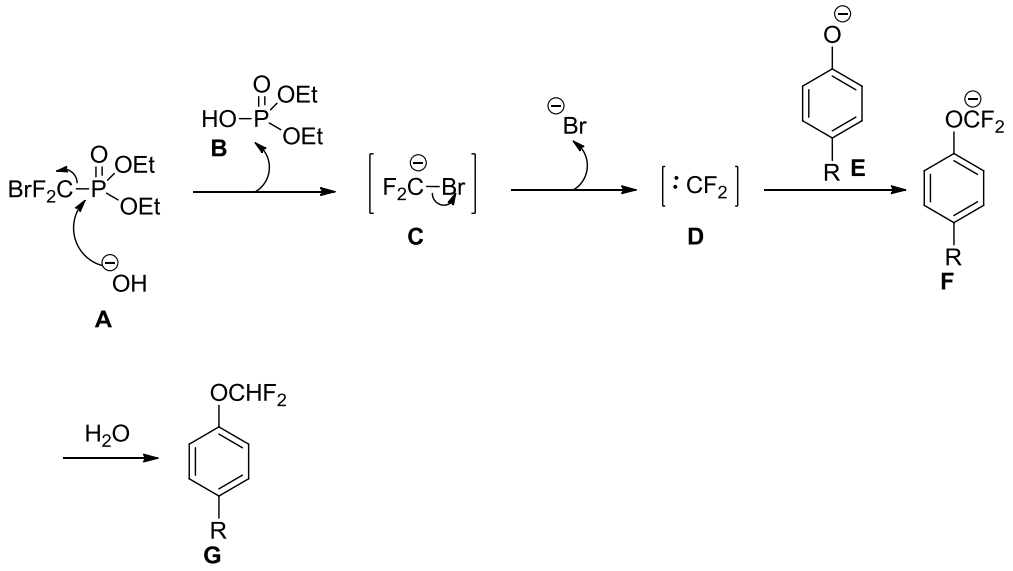
Scheme 40. *Reagents and conditions*: (i) TFAA, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) (4-(difluoromethoxy)phenyl)magnesium bromide, THF, RT, 18 h; (iii) LiOH(aq), THF, 60 ^oC, 18 h; (iv) (a) CDI, THF, 70 ^oC, 3 h; (b) 4-picolylamine, 50 ^oC to RT, 18 h.

In order to determine whether the *in situ* Grignard reagent had formed successfully, a model reaction was performed with acetophenone using the same conditions. This reaction was successful in 5 h, and the correct product **216** was obtained in 80% yield (Scheme 41). This suggested that (4-(difluoromethoxy)phenyl)magnesium bromide was being formed and could react successfully. The Grignard reagent may be sufficiently basic to deprotonate the pyrrole nitrogen and this could prevent the desired reaction occurring. In order to try and overcome this, the reaction was attempted using 2 equivalents of the Grignard reagent. However, this only improved the conversion to 15% (detected by LC-MS). An alternative approach could be to protect the pyrrole nitrogen prior to reaction with the Grignard reagent; however, this was never attempted due to an alternative approach to the desired compound being identified in the literature.



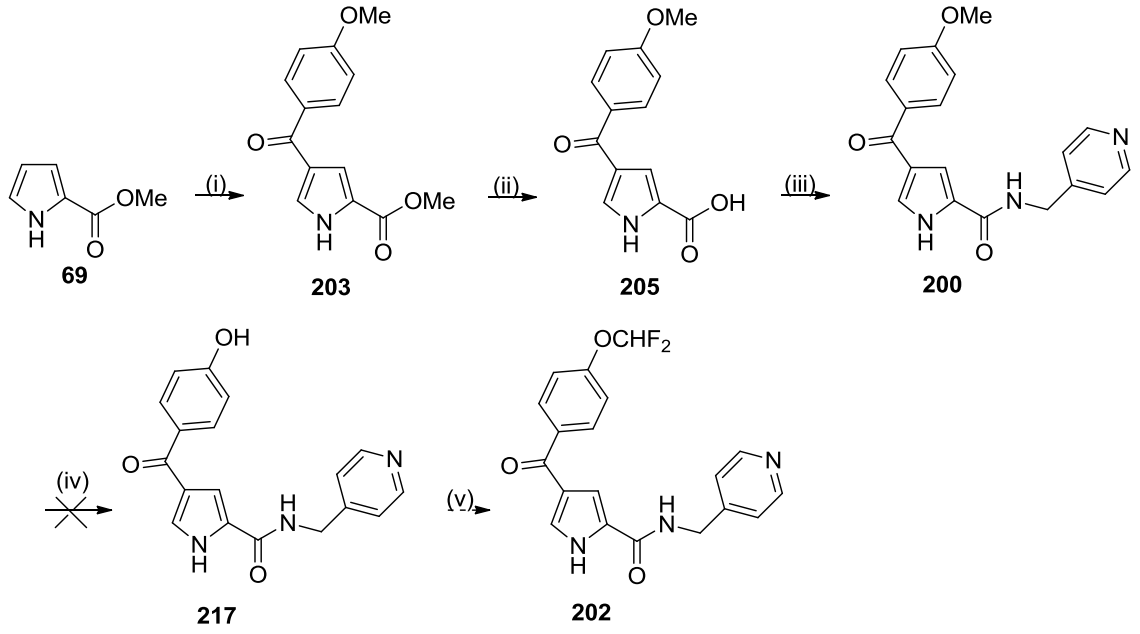
Scheme 41. *Reagents and conditions*: (i) Mg turnings, 1-(bromo-difluoromethoxy)benzene, Et_2O, RT, 5 h.

Alkylation of a 4-phenol on the aryl ring of the aroyl substituent using diethyl bromodifluoromethylphosphonate was attempted. Use of diethyl bromodifluoromethylphosphonate as an efficient difluorocarbene precursor has been shown in the literature to be useful for the difluoromethylation of phenols. The reaction is performed in the presence of potassium hydroxide, and is thought to proceed *via* the hydroxide-mediated cleavage of the P-C bond to give the bromodifluoromethyl anion **C** and diethyl phosphate **B**. The bromodifluoromethyl anion is unstable and, as such, eliminates bromide in order to generate the difluorocarbene **D**, which can react with the phenol to generate difluoromethoxy compound **G** (Scheme 42).[129]



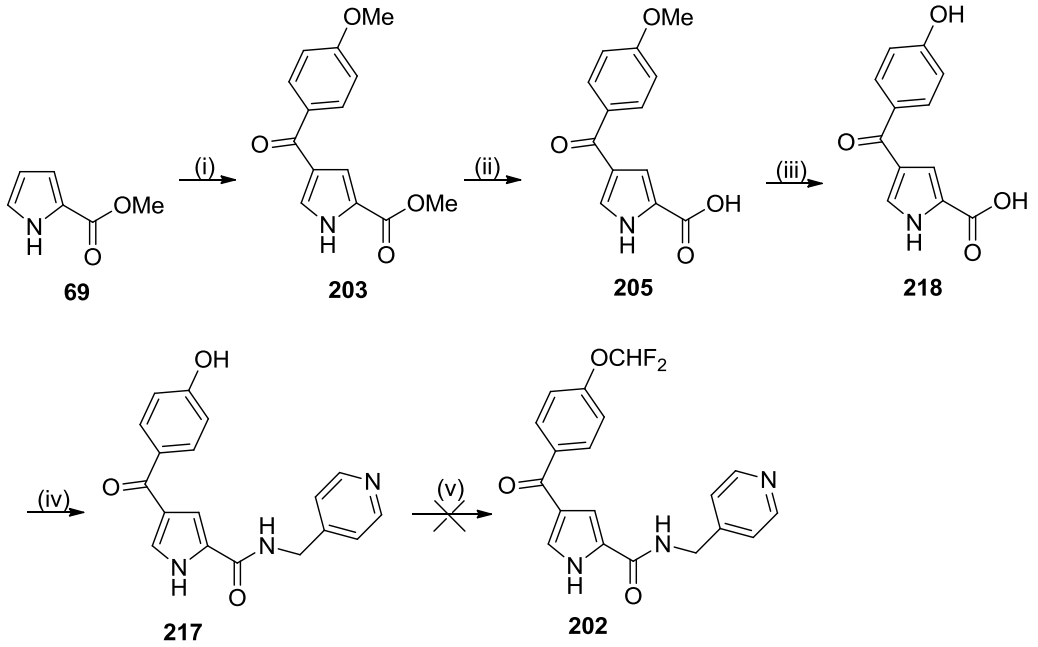
Scheme 42. Proposed mechanism of phenol alkylation using diethylbromodifluoromethylphosphonate[129]

Demethylation of previously prepared methoxy compound **200** was conducted using boron tribromide with the aim of performing the discussed alkylation to give difluoromethoxy compound **202** (Scheme 43).



Scheme 43. *Reagents and conditions*: (i) 4-methoxybenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH(aq), THF, 60 ^oC, 18 h; (iii) (a) CDI, THF, 70 ^oC, 3 h; (b) 4-picolylamine, 50 ^oC to RT, 18 h; (iv) BBr_3 (1.0 M in DCM), 0 ^oC to RT, 18 h ;(v) diethyl bromodifluoromethylphosphonate, KOH, MeCN-H_2O, -78 ^oC to RT, 2 h.

The synthesis of **200** was discussed previously. The boron tribromide demethylation of **200** was successful affording phenol **217** in 80% yield indicated by LC-MS. Isolation of phenol **217** was difficult due to remaining starting material **200**, so removal of the methyl group earlier in the synthesis was attempted by reacting **205** with boron tribromide (Scheme 44).



Scheme 44. *Reagents and conditions*: (i) 4-methoxybenzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH(aq), THF, 60 ^oC, 18 h; (iii) BBr_3 (1.0 M in DCM), 0 ^oC to RT, 18 h (iv) (a) CDI, THF, 70 ^oC, 3 h; (b) 4-picolylamine, 50 ^oC to RT, 18 h; (v) diethyl bromodifluoromethylphosphonate, KOH, MeCN-H_2O, -78 ^oC to RT, 2 h.

In this case the purification of **218** was achieved using MPLC producing the pure product in 79% yield. Subsequent amide coupling using CDI was performed to give phenol compound **217** in 32% yield. The low yield was possibly due to the side reaction of the phenol moiety with CDI. After successful isolation of **217**, the alkylation of the phenol using diethyl bromodifluoromethylphosphonate was attempted, but without any success.

Since the synthesis of compound **202** had proven difficult, and in light of the poor ERK5 inhibitory activity of the other *p*-substituted aroyl compounds (**199**, **200** and **201**)**,** we decided to cease the synthesis of difluoromethoxy compound **202**. The intermediate phenol **217** was sent for biological evaluation, and showed poor activity against ERK5 with an IC_{50} of 44 μM.

5.2 *meta*-Substitution of the aroyl ring

5.2.1 Rationale

In addition, two *meta*-substituted aroyl analogues were synthesised and tested for ERK5 inhibitory activity. The 3-OMe substituted compound **219** was synthesised previously by another member of the group, and showed moderate activity against ERK5 (Figure 37).[90] The large increase in potency for this compound compared with the 4-OMe counterpart (**198**, IC_{50} > 120 μM) was encouraging, and showed that the synthesis of 3-substitued analogues may provide interesting SARs.

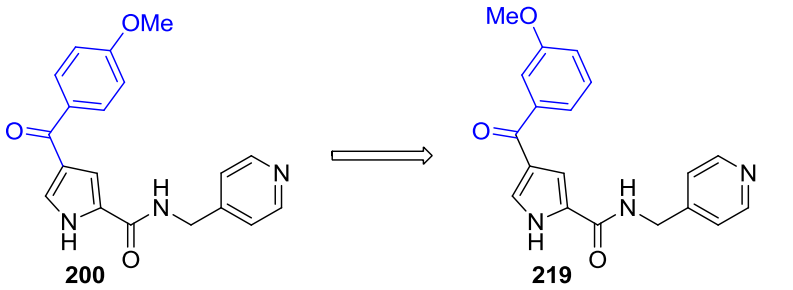
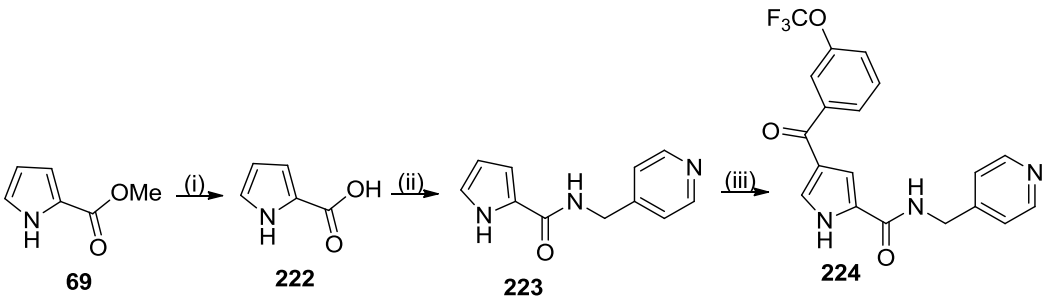


Figure 37. Improved potency for 3-substituted methoxy compound **219** compared to 4-methoxy compound **200**.

5.2.2 Synthesis of inhibitors with a meta-substituted aroyl ring

The 3-OCF_3 analogue was synthesised following the general scheme outlined previously, with the Friedel-Crafts acylation being performed in 79% yield (**220**) and the subsequent hydrolysis in 89% yield (**221**). The amide coupling step proved to be very low yielding, with only 6% of the final compound obtained. In order to remedy this problem the synthesis was performed in a different order, with the hydrolysis and subsequent amide coupling being performed first followed by the Friedel-Crafts acylation (Scheme 45). Performing the reactions in this order improved the yields for each step (hydrolysis, compound **222**; 98%, amide coupling, compound **223**; 89% and acylation, compound **224**; 84%).

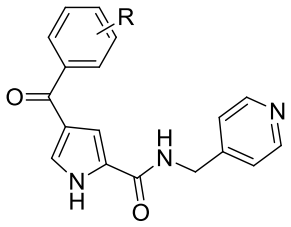


Scheme 45. *Reagents and conditions*: (i) LiOH (aq), THF, 60 ^{o}C, 18 h; (ii) (a) CDI, THF, 70 ^{o}C, 3 h; (b) 4-picolylamine, 50 ^{o}C to RT, 18 h; (iii) (3-trifluoromethoxy) benzoyl chloride, $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h.

5.2.3 Biological evaluation of meta-substituted inhibitors

The biological evaluation of compound **224** is shown in Table 22. Unsurprisingly, the 3-OCF_3 substituted compound showed improved inhibitory activity compared to the 4-OCF_3 substituted analogue (**201**, ERK5; IC_{50} > 120 μM).

Table 22. ERK5 inhibitory activity of **219** and **224**.



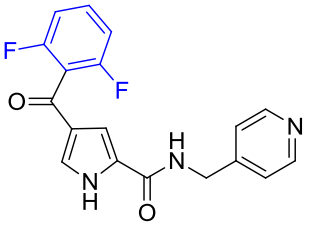
Compound number	R	ERK; IC_{50} (μM)
200	4-OMe	>120
201	4-OCF_3	>120
219	3-OMe	27 ± 1.2 (n = 2)
224	3-OCF_3	12 ± 1.2 (n = 2)

Despite the improved ERK5 inhibitory activity observed for compounds **219** and **224**, compared to 4-substituted analogues **200** and **201**, the ERK5 inhibition remained quite poor compared to hit compound **53** (ERK5; IC_{50} = 2.4 μM) and no further analogues were synthesised.

5.3 Modification of the aroyl substituent to gain selectivity over p38α

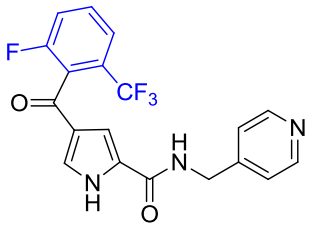
5.3.1 Rationale

As stated previously, the MAPK p38α is a close homologue of ERK5, and as such, it is essential to screen compounds with inhibitory activity for ERK5 against p38α to ensure selectivity is achieved. It had been observed that original hit compound, **53** showed greater inhibitory activity against p38α compared to ERK5. Modification of the substituents on the aroyl ring was shown to improve selectivity for ERK5 over p38α (i.e. **225**, **226** and **227**) (Figure 38).



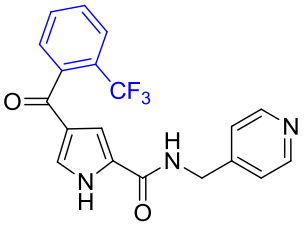
53

ERK5; IC_{50} = 2.4 ± 0.09 μM
p38α; IC_{50} = 0.58 ± 0.27 μM
ERK5:p38α = 4:1



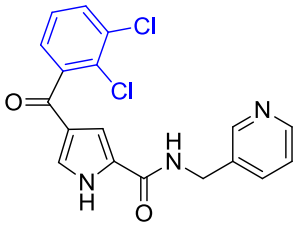
225

ERK5; IC_{50} = 4.9 ± 1.2 μM
p38α; IC_{50} = 66 ± 35 μM
ERK5:p38α = 1:13



226

ERK5; IC_{50} = 6.3 ± 2.8 μM
p38α; IC_{50} = 35 ± 30 μM
ERK5:p38α = 1:6



227

ERK5; IC_{50} = 2.4 ± 0.19 μM
p38α; IC_{50} = 10 ± 2.5 μM
ERK5:p38α = 1:4

Figure 38. Improvement in selectivity for ERK5 over p38α by modification of the benzoyl group.

In light of this and the success of the shorter amide side chain analogues (as discussed in Chapter 4), a small library of 15 compounds was proposed for synthesis, incorporating the three benzoyl groups that had shown improved selectivity over p38α and the five amide side chains that had conferred best potency against ERK5 (Figure 39).

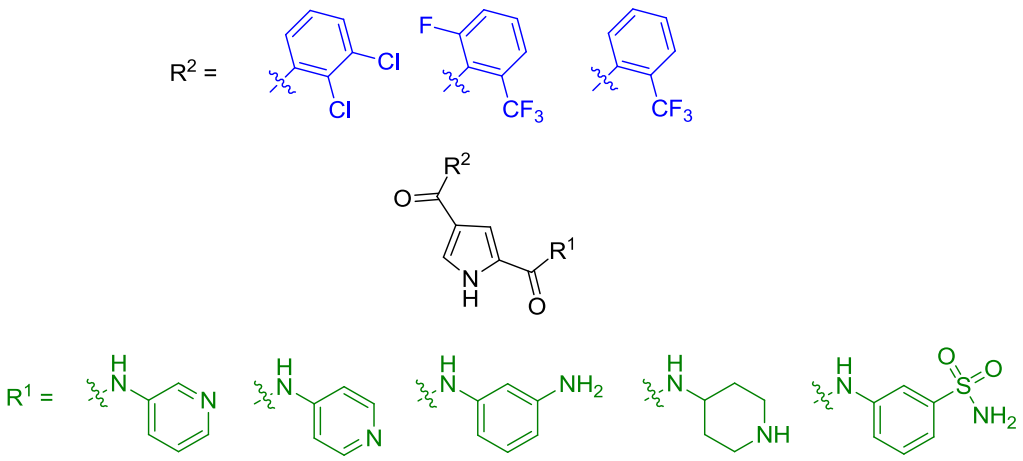
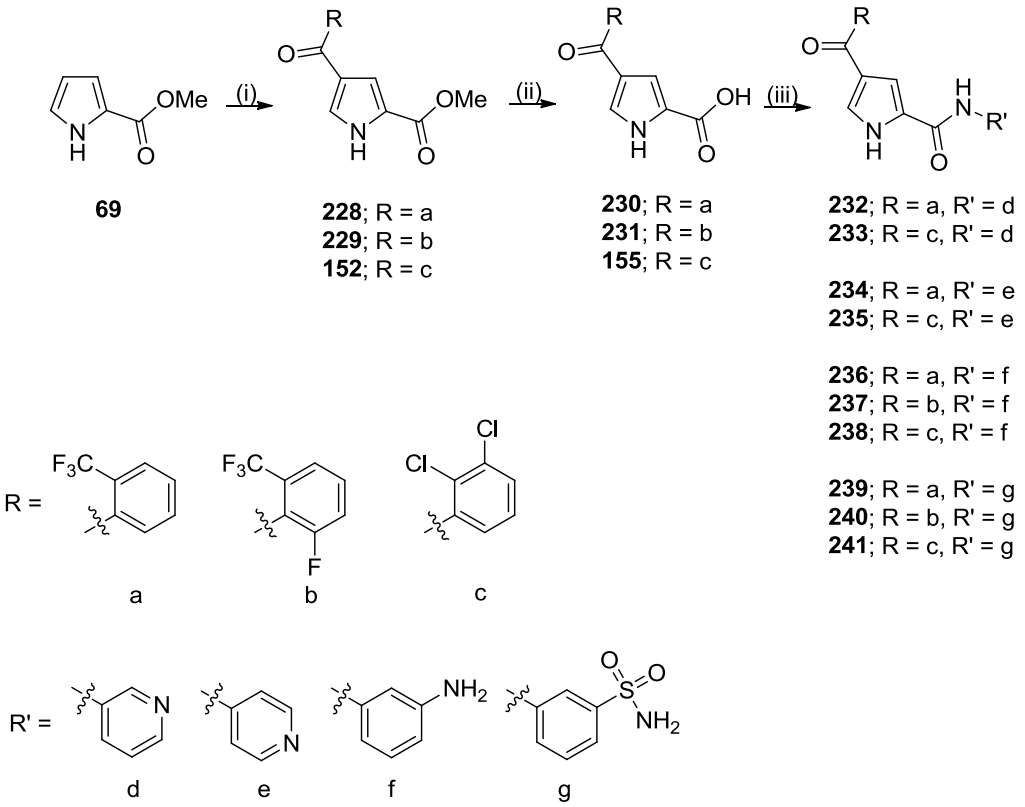


Figure 39. Target molecules proposed to improve potency for ERK5 and selectivity over p38α.

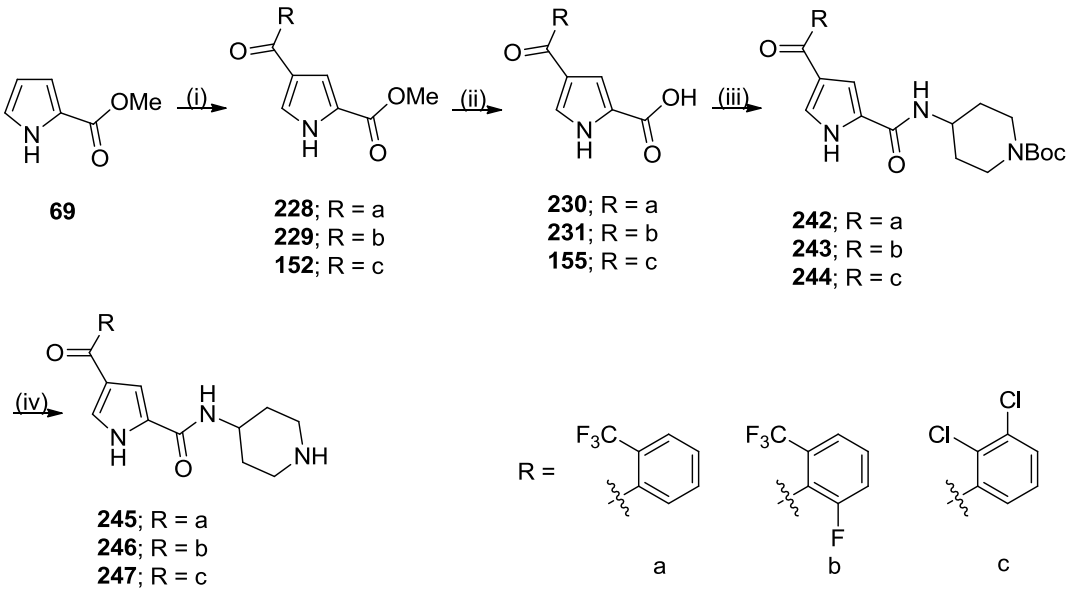
5.3.2 Synthesis of inhibitors with reduced p38α activity

Compounds with aromatic amine side chains were synthesised according to the general procedure shown in Scheme 46. Methyl pyrrole-2-carboxylate (**69**) was acylated with either 2-(trifluoromethyl)benzoyl chloride, 2-(fluoro-6-trifluoromethyl)benzoyl chloride or 2,3-dichlorobenzoyl chloride in good yield (**224**; 84%, **225**; 60% and **150**; 71%, respectively). Each methyl ester was then treated with aqueous LiOH to afford the corresponding carboxylic acids, **226**, **227** and **153** in excellent yield (all 99%). The appropriate aniline was then coupled using PCl_3 under microwave irradiation to give the 10 desired compounds (**228**-**237**) with yields ranging from 38-75%. The 3- and 4-pyridyl analogues in the 2-fluoro-6-trifluoromethyl series were synthesised by another group member.[130]



Scheme 46. *Reagents* and conditions: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) PCl_3, appropriate aniline, MeCN, MW, 150 ^oC, 5 min.

The three compounds with piperidinyl side chains were synthesised according to Scheme 47. Carboxylic acid intermediates **153**, **226** and **227** were coupled with 1-amino-1-boc-piperidine using CDI as the coupling agent to afford the Boc-protected compounds **238**, **239** and **240** in 82%, 75% and 80%, respectively. Coupling of 1-amino-1-boc-piperidine using the PCl_3 coupling method was attempted with each of the carboxylic acid intermediates, but was not as successful as using the CDI approach. The by-products of the PCl_3 coupling reaction, hydrochloric acid and phosphorus acid, were causing deprotection of the Boc-protected amino-piperidine. This meant that although some conversion to the correct product was observed, the reaction mixture was complex. Using the CDI method, compounds **242**, **243** and **244** were isolated in good yield. Deprotection of **242**, **243** and **244** was achieved using TFA in the presence of triethylsilane. Triethylsilane is used as a carbocation scavenger to remove the *t*-butyl carbocation generated in the reaction and prevent any side reactions that may occur with this reactive species.[131] The three deprotected compounds, **245**, **246** and **247** were obtained in good yield (97%, 65% and 80%, respectively).

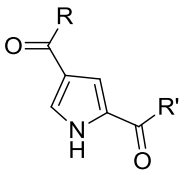


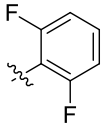
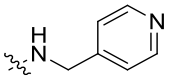
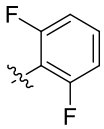
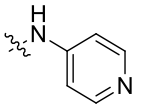
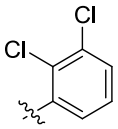
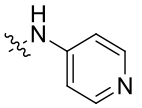
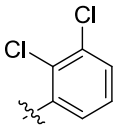
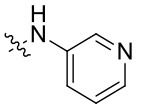
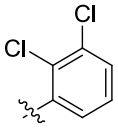
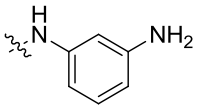
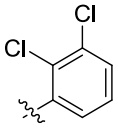
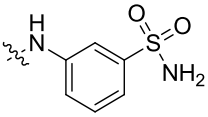
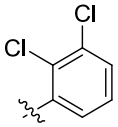
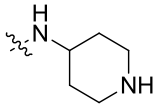
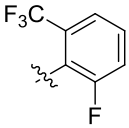
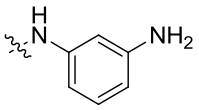
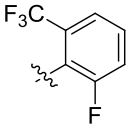
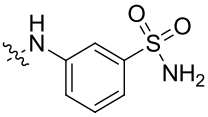
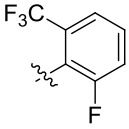
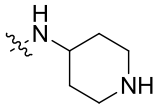
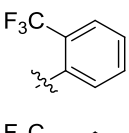
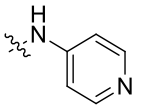
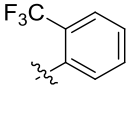
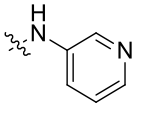
Scheme 47. *Reagents and conditions*: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) CDI, 4-amino-1-boc-piperidine, THF, 70 ^oC to RT, 18 h; (iv) TFA, Et_3SiH, DCM, RT, 1 h.

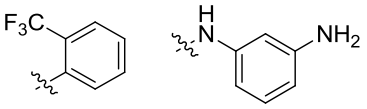
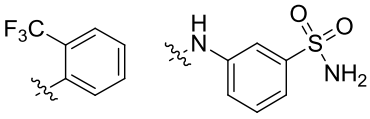
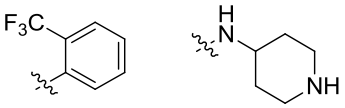
5.3.3 ERK5 inhibitory activity and p38α counter-screening data for compounds 232-247

The 15 compounds synthesised in this series were evaluated for ERK5 inhibitory activity and counter-screened against p38α in order to determine whether selectivity had been achieved. The cellular activity of four analogues was also assessed. These results are shown in Table 23 and all compounds are compared to original hit compound **53** and shortened amide side-chain analogue **127**.

Table 23. Biological results for compounds **232-247**.



Compound	R	R'	ERK5; IC_{50} (μM)	p38α; IC_{50} (μM)	Cellular activity (HEK293, μM)
53			2.4 ± 0.15 (n = 4)	0.58 ± 0.27 (n = 2)	-
127			0.90 ± 0.15 (n = 4)	100 ± 35 (n = 2)	-
235			5.5 ± 4.5 (n = 8)	>120	ND
233			1.5 ± 1.0 (n = 6)	22 ± 1.1 (n = 2)	24 ± 8.5 (n = 2)
238			1.6 ± 0.30 (n = 4)	>120	ND
241			0.87 ± 0.57 (n = 6)	>120	ND
247			1.7 ± 0.6 (n = 4)	94 ± 23 (n = 2)	30 (n = 1)
237			2.6 ± 0.32 (n = 4)	>120	ND
240			0.94 ± 0.14 (n = 4)	>120	ND
246			2.4 ± 1.1 (n = 4)	>120	17 (n = 1)
237			3.6 ± 0.96 (n = 2)	>120	ND
232			2.3 ± 0.8 (n = 4)	>120	ND

236			9.4 ± 0.37 (n = 2)	>120	ND
239			8.8 ± 8.5 (n = 4)	>120	ND
245			3.3 ± 1.7 (n = 4)	>120	30 (n = 1)

ND – Not Determined

In all cases, improved selectivity for ERK5 over p38α was observed compared to hit compound **53**, with most compounds having IC_{50} values >120 μM against p38α. The only exceptions were **233** and **247**, which had slightly improved activity against p38α, but were still 55-fold and 30-fold selective for ERK5, respectively. The most potent compounds were the two sulphonamides, **240** and **241** and all compounds had an IC_{50} value for inhibition of ERK5 below 10 μM. Four of the compounds were tested for cellular activity at the Babraham Institute in Cambridge using a MEF2D reporter gene assay in HEK293 cells. ERK5 IC_{50} values are determined *via* the measurement of phosphorylation of MEF2D, which is a transcription factor and downstream target of ERK5. The level of phosphorylation is measured by extent of luminescence detected. The values obtained for the four compounds are shown in Table 23. Interestingly, the cellular activities obtained were lower than the inhibitory activities measured in the cell-free assay in each case, and in order to explain this, the physicochemical properties and *in vitro* PK for selected compounds were reviewed.

5.3.4 Physicochemical properties and in vitro ADME profiling for compounds 233 and 235

The physicochemical properties of compounds **233** and **234** were analysed as a representative sample of the series (Table 24).

Table 24. Physicochemical properties of **233** and **235**.

Compound	MW	H-bond donors	H-bond acceptors	sLogP	TPSA
Lead criteria	<500	<5	<10	<5	75-100
233	360	2	5	3.7	75
235	360	2	5	3.7	75

Since **233** and **235** had fulfilled the physicochemcial properties of a lead candidate, the pharmacokinetic properties and drug metabolism profiles were evaluated at Cyprotex (Table 25).

Table 25. *In vitro* ADME profiling properties for **233** and **235**.

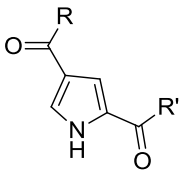
Compound	LE	Solubility L/U (µM)	PPB (%)	Mouse CLint (µL/min/mg)	Human CLint (µL/min/mg)
Lead criteria	>0.30	>50/50	<99	<48	<48
233	0.30	15/50	97	24	15
235	0.30	100/100	99	23	0

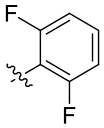
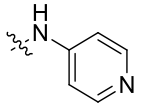
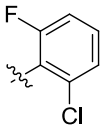
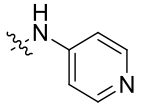
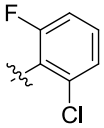
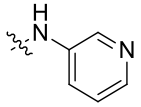
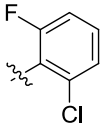
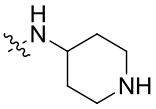
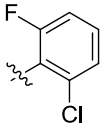
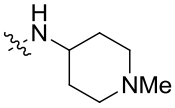
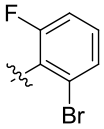
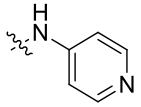
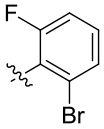
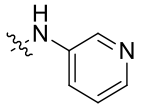
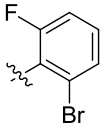
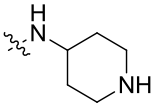
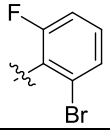
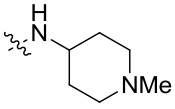
Compound **235** had excellent solubilty (S (L/U) = 100/100 µM) compared to **233**, which had reduced solubility of 15/50 µM. The poor solubility for compound **233** could be the reason for the lower activity observed in HEK293 cells, as it may suggest that the compound had poor cell permeability. In both cases the human and mouse microsomal clearance was good and plasma protein binding for both inhibitors was 99% or less, and therefore optimal.

5.3.5 Inhibitors with a 2-fluoro-6-chloro or 2-fluoro-6-bromoaroyl ring with improved potency and selectivity for ERK5 over p38α

Two alternative series of compounds to those described herein were synthesised by another member of the group.[132] These series incorporated aroyl rings with novel substitution patterns. Biological testing of these inhibitors identified compounds with improved ERK5 inhibitory activity and good selectivity over p38α. The results for these compounds are shown for reference in Table 26.

Table 26. Compounds with improved ERK5 potency and selectivity over p38α



Compound	R	R'	ERK5; IC_{50} (μM)	p38α; IC_{50} (μM)
127			0.90 ± 0.15 (n = 4)	100 ± 35 (n = 2)
150			0.60 ± 0.27 (n = 4)	>120
248			0.58 ± 0.24 (n = 4)	93 ± 13 (n = 2)
249			0.49 ± 0.04 (n = 4)	11 ± 1.0 (n = 2)
250			0.20 ± 0.07 (n = 4)	8.5 (n = 1)
251			0.68 ± 0.15 (n = 4)	>120
151			0.82 ± 0.07 (n = 4)	>120
252			0.39 ± 0.12 (n = 4)	19 ± 0.33 (n = 2)
253			0.28 ± 0.16 (n = 8)	11 ± 0.01 (n = 2)

*5.3.5.1 In vitro and in vivo pharmacokinetic studies with **151***

Compound **151** was selected for *in vitro* and *in vivo* pharmacokinetic studies. The physicochemical properties of **151** are shown in Table 27.

Table 27. Physicochemical properties of **151**.

Compound	MW	H-bond donors	H-bond acceptors	sLogP	TPSA
Lead criteria	<500	<5	<10	<5	75-100
151	388	2	5	3.1	75

In vitro pharmacokinetic studies were conducted at Cyprotex and the results are shown in Table 28. The solubility of **151** was measured as 100/100 μM (L/U), which is the optimum value for a lead compound. Both the human and mouse microsomal clearance were excellent. Caco-2 permeability was also evaluated and **151** was shown to be above the threshold (>10 x 10^{-6} cm/s) and the efflux ratio was low (0.82). In order to determine whether **151** would inhibit CYP450 enzymes, the compound was tested against a panel of CYP450 isoforms (2C19, 2C9, 2D6, 3A4). Encouragingly, inhibition of the CYP450 isoforms tested was measured as >5 μM in each case. In addition, compound **151** was tested in the MEF2D reporter gene assay and the IC_{50} was measured as 4.4 ± 2.1 μM. The GI_{50} for this compound was tested in Newcastle in PC3 cells as 44 ± 2.8 μM, which was not consistent with both the *in vitro* and cellular activity (HEK293). In addition, the selectivity of compound **151** was tested in a DiscovRx screen, which includes 456 kinases. Overall, the selectivity for ERK5 was good and MEK5 activity was observed. We obtained and IC_{50} against MEK5 by outsourcing compound **151** to Carna Bioscience (MEK5; IC_{50} = 3.4 μM). There was also evidence of **151** having activity against the JAK family of kinases. Overall **151** showed good drug-like properties and was entered into *in vivo* drug metabolism pharmacokinetics (DMPK) studies in mice.

Table 28. *In vitro* ADME profiling data for **151**.

Compound	Solubility (μM)	PPB (%)	Mouse CLint (μL/min/mg)	Human CLint (μL/min/mg)
Lead criteria	>50/50	<99	<48	<48
151	100/100	94	20	0

Two *in vivo* DMPK studies were conducted at Newcastle by Huw Thomas using CD1 mice. A 10 mg/kg dose of **151** was administered orally (PO) to mice bearing a HCT116 human colorectal tumour xenograft. The plasma of these mice was collected at five time

points following administration. A 100 mg/kg dose was also given to another set of mice bearing the same tumour xenograft and this time tumour tissue was removed at five time points following oral administration. The same experiments were also performed following intravenous (IV) and intraperitoneal (IP) administration in order to determine the pharmacokinetic properties associated with each dosing regimen. The results of these studies are summarised in Table 29. The oral bioavailability of **151** was measured as 68%, which above the desired oral bioavailability for a lead compound (>30%). The half life following oral dosing was determined to be 65 min. These values were not repeated following IV or IP administration, but the clearance in these cases was shown to be more desirable than in oral dosing when it was shown to be high (oral; 39 mL/min/kg, IV; 27 mL/min/kg and IP; 25 mL/min/kg).

Table 29. *In vivo* DMPK for **151**.

Administration	PO	IV	IP
Dose (mg/kg)	10	10	10
AUC inf (µg/mL.min)	254	372	398
Cmax (µM)	6	25	12
Vd (L/kg)	ND	1.2	ND
Tmax (min)	15	5.0	15
Half-Life (min)	65	38	43
Clearance (mL/min/kg)	39	27	25
Bioavailability, F (%)	68	ND	108

The *in vivo* efficacy of **151** was also tested at Newcastle by Huw Thomas using a matrigel plug assay and a human tumour xenograft model (using CD1 mice and A2780 human ovarian carcinoma cells). In both studies, the efficacy of **151** was compared to that of competitor compound, XMD8-92. The matrigel plug assay is a measure of angiogenesis and was performed using CD1 mice, which were subcutaneously treated with matrigel containing fibroblast growth factor (bFGF). This induces formation of a matrigel plug, which can be permeated by host cells and leads to formation of new blood vessels. In the presence of an anti-angiogenic agent the extent of new blood vessels formed would be reduced. 24 hours after the mice were treated with bFGF, they were randomised to receive either; vehicle, XMD8-82 (50 mg/kg, IP) or **151** (50 mg/kg, PO) bi-daily for 7 days. On day 7 the mice were sacrificed and the matrigel plugs

removed and snap frozen in liquid nitrogen. The concentration of haemoglobin present could then be determined as a measure for the extent of blood vessel formation. The results are shown in Figure 40, which shows that the administration of an ERK5 inhibitor has an anti-angiogenic effect.

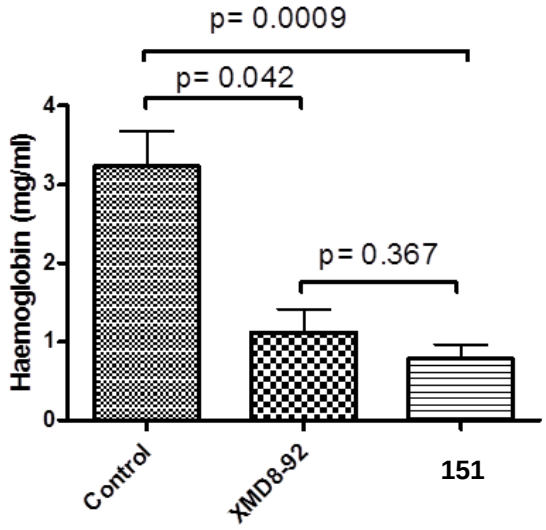


Figure 40. Haemoglobin levels determined in matrigel plug assay in CD1 mice treated with XMD8-92 or **151**.

In the human tumour xenograft model, CD1 mice were treated with A2780 human ovarian carcinoma cells and tumours were left to grow for 7 days. The mice were then randomised to receive either; vehicle, XMD8-82 (50 mg/kg, IP) or **151** (100 mg/kg, PO) bi-daily for 11 days. Tumour volumes were measured and the mice that had been treated with either XMD8-92 or **151** had tumours of a much reduced size compared with those in the control group (Figure 41). Nadir weight was used as an indicator of drug toxicity and in each case it remained at 98% for the mice treated with **151**. These initial *in vivo* DMPK studies are encouraging and show that an ERK5 inhibitor could have potential as an anti-angiogenic and anti-proliferative agent.

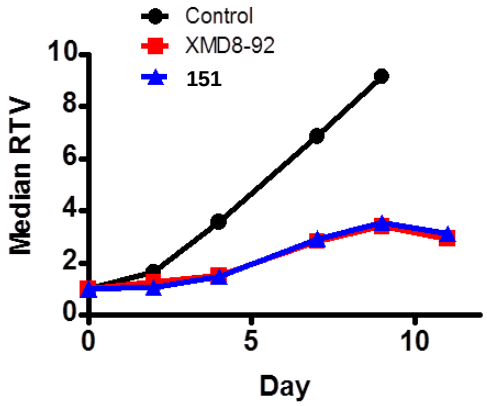


Figure 41. Treatment of CD1 mice bearing A2780 xenograft with XMD8-92 or **151**.

In order to determine the effect of **151** on cell migration, a scratch wound assay was performed at Newcastle by Lan Zhen Wang using a breast cancer cell line (MDA-MB-231). Cells were grown and a 'wound' was introduced by scratching a pipette tip through the centre of the cell culture. The cells at the 'wound' edge were then allowed to migrate into the space created by the scratch after having been treated with either XMD8-92 or **151** (at 10 µM and 30 µM). After 17 hours the level of migration can then be determined simply by viewing the extent with which the cells appear to have migrated into the space. The results of this assay are shown in Figure 42 and show that there is some effect evident with both ERK5 inhibitors compared to the control experiment, but considering the high concentrations of inhibitor used, the effect is not significant. It is not clear from this assay whether an ERK5 inhibitor would have an effect on tumour cell migration.

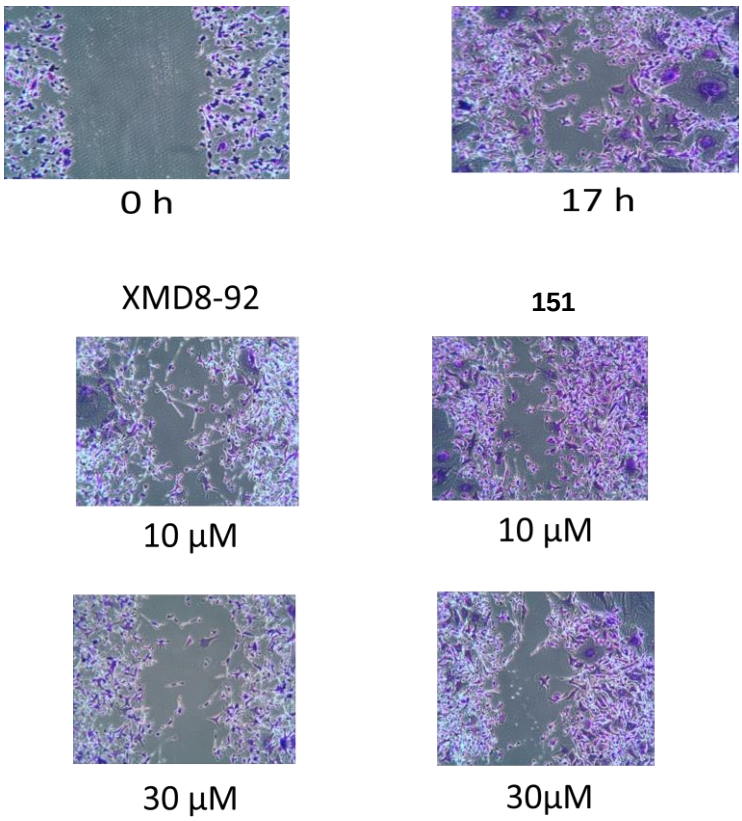
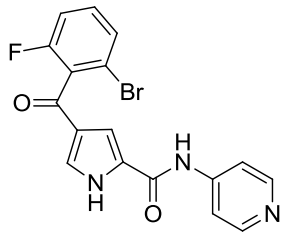


Figure 42. Scratch wound assay; control experiment and those performed with 10 and 30 µM of XMD8-92 and **151**, respectively.

*5.3.5.2 Docking studies with **251** in second-generation p38α-derived homology model*

As outlined in chapter 3, a second generation homology model of ERK5 based on p38α (43% sequence identity in active site domain) was developed at Newcastle University by Prof. Martin Noble. Compound **251** was used as a representative potent pyrrole-carboxamide inhibitor and was built into the ERK5 model using GROMACS (GROningen MAchine for Chemical Simulations). Figure 42 (A) shows compound **251** bound in the ERK5 model (magenta) and also bound in p38α crystal structure (cyan) for comparison. Key differences in the protein sequences of p38α (cyan) and ERK5 (magenta) have been highlighted to indicate where compound selectivity is most likely achieved. Figure 42 (B) shows the excellent fit of compound **251** into the new homology model.



251

ERK5; IC_{50} = 0.68 μM

p38α; IC_{50} > 120 μM

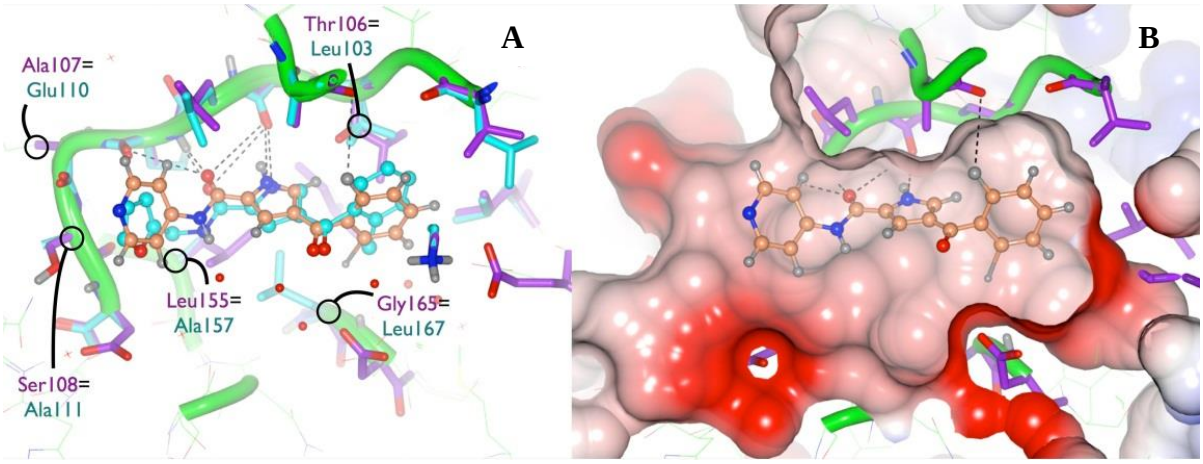


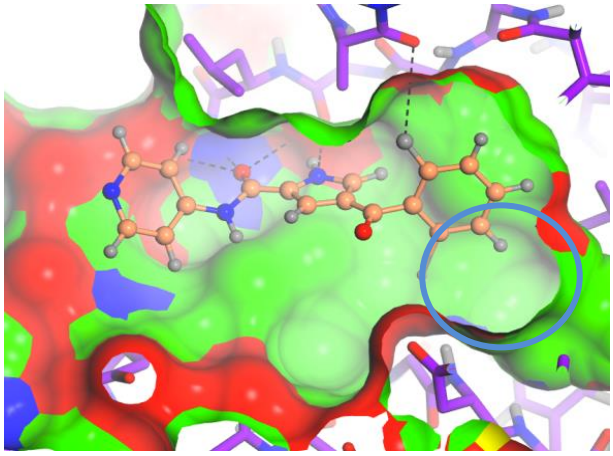
Figure 42. (A) ERK5 model (magenta) compared to p38α crystal structure (cyan), both shown in complex with compound **251**; (B) Compound **251** docked in the p38α-derived homology model.

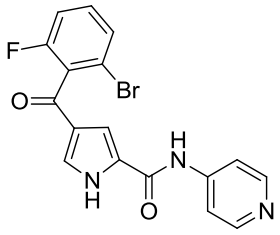
This homology model was utilised for the structure-based design of new inhibitors exploiting previously unexplored areas of the ERK5 ATP binding site.

5.4 Synthesis and biological evaluation of inhibitors with trisubstituted aroyl rings

5.4.1 Rationale

Docking of compound **251** led to the design of a new set of proposed ERK5 inhibitors incorporating a third substituent in the 3-position of the aroyl ring, to probe a putative lipophilic pocket highlighted in Figure 43, and ultimately improve binding interactions.





251

ERK5; IC_{50} = 0.68 μM

p38α; IC_{50} > 120 μM

Figure 43. p38α-derived homology model with **251** with potential lipophilic pocket to be exploited highlighted in blue.

The initial synthetic targets introduced a variety of 3-substituents onto the 2,6-difluoroaroyl motif. Following the success of the introduction of 2-chloro-6-fluoro-aroyl groups in improving selectivity for ERK5 over p38α, we decided to also synthesise 3-substituted analogues bearing the 2-chloro-6-fluoro substitution pattern. For each modification of the aroyl group, we introduced a 3-pyridyl, a *N*-methylpiperidyl or a pyrimidyl amide side-chain. At the time of synthesis, these side-chains combined optimal properties in terms of potency, aqueous solubility and reduced CYP450 inhibitory activity (Figure 44).

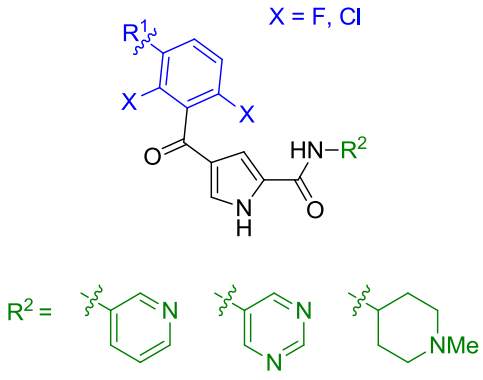
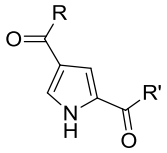
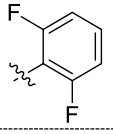
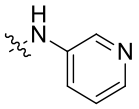
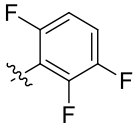
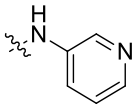
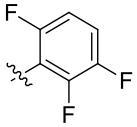
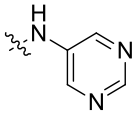
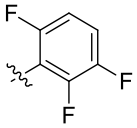
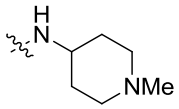
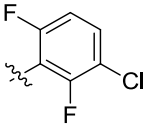
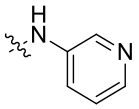
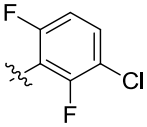
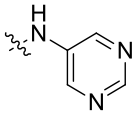
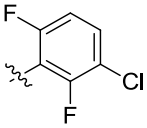
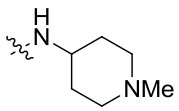


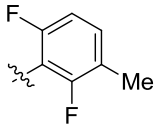
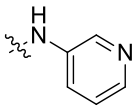
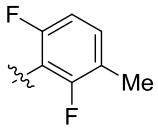
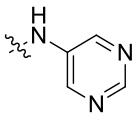
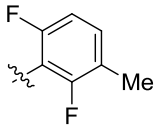
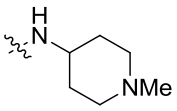
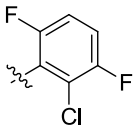
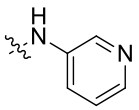
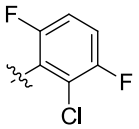
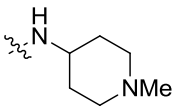
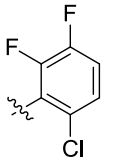
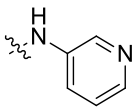
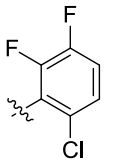
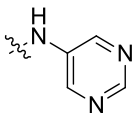
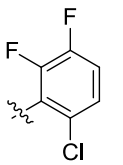
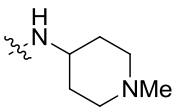
Figure 44. Trisubstituted aroyl target molecules to exploit a potential lipophilic pocket in the ERK5 ATP binding site.

The hypothesis was tested by another member of the group, who synthesised 14 compounds with substituents in the 3-position of the aroyl ring to determine if it would improve ERK5 inhibition.[132] These compounds and corresponding biological evaluation are shown in Table 30 for reference. It was evident from these preliminary results that addition of a third substituent onto the aroyl ring was tolerated and in some cases improved potency (e.g. **267**, ERK5; IC_{50} = 0.11 µM; 10-fold improvement compared to **128**). This suggests that further SARs into the incorporation of a 3-substituent onto the aroyl ring could improve potency further.

Table 30. Inhibitory activity of previously synthesised compounds incorporating a 3-substituent on the aroyl ring.



Compound	R	R'	ERK5; IC_{50} (µM)	p38α; IC_{50} (µM)
128			1.1 ± 0.27 (n = 4)	35 ± 0.90 (n = 2)
254			0.40 ± 0.10 (n = 4)	>120
255			0.94 ± 0.14 (n = 4)	>120
256			0.57 ± 0.10 (n = 4)	29 ± 0.50 (n = 2)
257			0.20 ± 0.05 (n = 4)	>120
258			0.24 ± 0.02 (n = 4)	>120
259			0.14 ± 0.02 (n = 4)	29 ± 5.2 (n = 2)

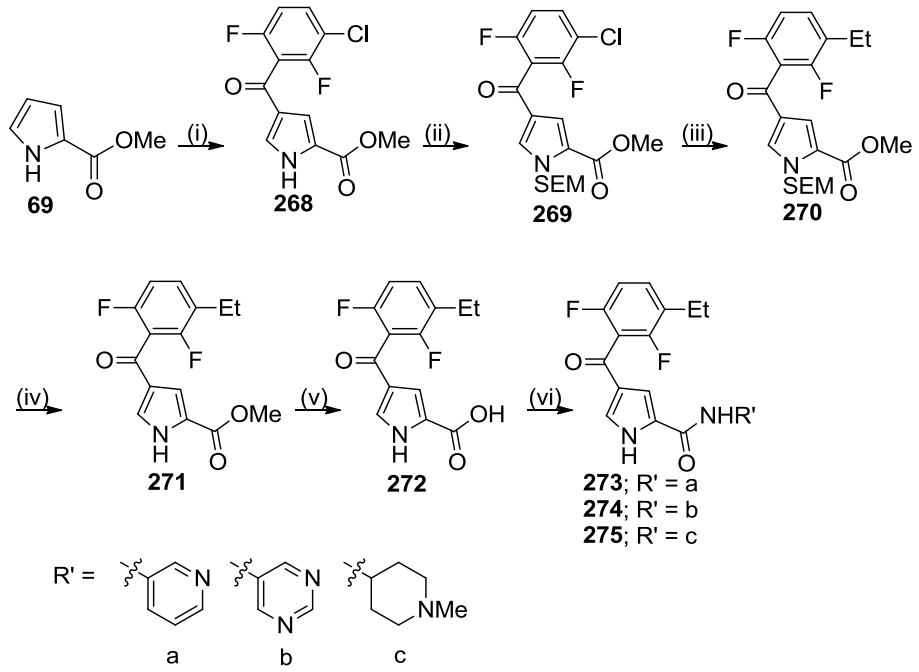
260			0.42 ± 0.10 (n = 4)	>120
261			0.76 ± 0.09 (n = 4)	>120
262			0.39 ± 0.02 (n = 4)	40 ± 3.3 (n = 2)
263			1.0 ± 0.28 (n = 4)	>120
264			0.80 (n = 1)	20 ± 0.7 (n = 2)
265			0.96 ± 0.15 (n = 4)	>120
266			0.71 ± 0.02 (n = 4)	>120
267			0.11 (n = 1)	50 ± 6.4 (n = 2)

5.4.2 Synthesis of tri-substituted aroyl-containing ERK5 inhibitors

5.4.2.1 Synthesis of analogues with a 3-alkyl substituent

In order to further validate the hypothesis that tri-substitution of the aroyl ring should improve potency against ERK5, further analogues were synthesised and tested for ERK5 inhibitory activity. The first set of compounds retained the 2,6-difluoro-substitution pattern and contained an alkyl group at the 3-position. Compounds with a methyl group in this position had already been tested (Table 30), and subsequent synthesis of compounds with larger alkyl groups was investigated. The synthesis of compounds with an ethyl group in the 3-position is shown in Scheme 48. The first step is the Friedel-Crafts acylation of **69** using 3-chloro-2,6-difluorobenzoyl chloride, which gave **268** in 94% yield. Protection of the pyrrole nitrogen with a SEM protecting group

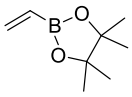
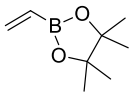
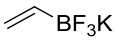
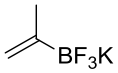
was performed in 95% yield. Protection of the pyrrole nitrogen was essential at this stage to prevent side reactions in the subsequent Suzuki-Miyaura reaction. The SEM group was chosen as it had proven stable when used previously by other members of the group under palladium-mediated cross-coupling reaction conditions.[132] The Suzuki-Miyaura reaction utilised ethylboronic acid and Pd(dtbpf)Cl_2 to produce **270** in 60% when the reaction was heated under microwave irradiation. The same yield could not be achieved under conventional heating. The optimal palladium catalyst for the transformation was Pd(dtbpf)Cl_2 (no reaction occurred with palladium tetrakis and de-chlorination was observed with Pd(dppf)Cl_2). Once the ethyl group was installed, the SEM group was removed using TBAF at 65 ^{o}C, producing **271** in 65% yield. Hydrolysis of the methyl ester gave carboxylic acid **272** in 98% yield. The coupling reactions were performed using the conditions shown in Scheme 48 and were relatively low yielding (**273**; 45%, **274**; 28% and **275**; 19%). The low yields were due to difficult purification of the final products, which all had to be purified by semi-preparative HPLC after several failed attempts to purify by MPLC.



Scheme 48. *Reagents and conditions*: (i) 3-chloro-2,6-difluorobenzoyl chloride, $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h; (ii) SEMCl, KO^{t}Bu, THF, RT, 18 h; (iii) ethylboronic acid, Pd(dtbpf)Cl_2, Cs_2CO_3, MW, 150 ^{o}C, 1.5 h; (iv) TBAF, THF, 65 ^{o}C, 18 h; (v) LiOH (aq), THF, 60 ^{o}C, 18 h; (vi) where R' = pyridyl or pyrimidyl; (a) cyanuric fluoride, pyridine, MeCN, RT, 45 min (b) appropriate amine, 18 h, RT; where R' = *N*-methylpiperidine; (a) CDI, THF, 70 ^{o}C, 3 h; (b) *N*-methyl-4-aminopiperidine, RT, 18 h.

The same Suzuki-Miyaura cross-coupling reaction conditions were attempted using isopropylboronic acid, but without any success. The main product from the reaction was the de-chlorinated starting material. It was thought that the isopropylboronic acid may not be stable and therefore installation of an isopropenyl moiety, which could then be reduced to the isopropyl, was deemed a more viable approach. The best Suzuki-Miyaura conditions for the introduction of an isopropenyl group were tested using vinylboronic acid pinacol ester and are shown in Table 31. The same Suzuki-Miyaura conditions that had been applied to the ethyl analogues did not provide the vinyl analogues and de-chlorination of the starting material was again observed. Using $Pd(dppf)Cl_2$ as the catalyst produced the same result. After reviewing the literature, it was discovered that use of potassium vinyltrifluoroborate in place of a traditional boronic acid or pinacol ester was often successful for installation of a vinyl group.[133] Using the literature conditions (entry 3, Table 31) the product was obtained in 80% yield (compound **276**). The reaction was repeated using potassium isopropenyltrifluoroborate and the correct product was synthesised in 42% yield (compound **277**).

Table 31. Suzuki-Miyaura conditions trialled for installation of vinyl and isopropenyl groups.

Entry	1	2	3	4
Boron coupling partner				
Catalyst	$Pd(dtbpf)Cl_2$	$Pd(dtbpf)Cl_2$	$PdCl_2$	$PdCl_2$
Ligand	-	-	XPhos	XPhos
Solvent	Dioxane	Dioxane	THF/H_2O (9:1)	THF/H_2O (9:1)
Base	Cs_2CO_3	Cs_2CO_3	Cs_2CO_3	Cs_2CO_3
Time (min)	90	90	60	60
Temp. (^oC)	150	150	85	85
Heating method	MW	MW	Conventional	Conventional
Result	De-chlorination	De-chlorination	80% yield	42% yield

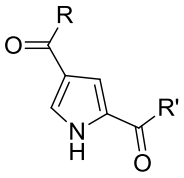
The subsequent steps were the same as those shown in Scheme 48 for the ethyl analogues, (deprotection, vinyl, **278**; deprotection, isopropenyl, **279**; hydrolysis, vinyl,

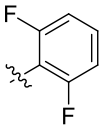
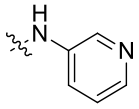
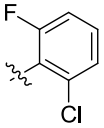
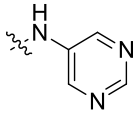
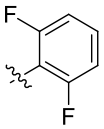
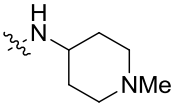
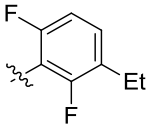
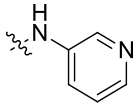
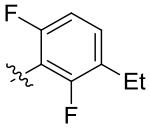
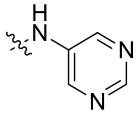
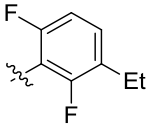
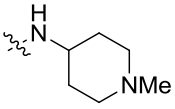
280; hydrolysis, isopropenyl, **281**). The final coupling steps for the vinyl analogues gave final compounds **282**, **283** and **284** in low yield (16%, 19% and 20%, respectively). Again, all compounds were purified by semi-preparative HPLC and were sent for evaluation of ERK5 inhibitory activity. For the isopropenyl analogues, only the pyridyl analogue (**285**) was synthesised in 40% yield, using CDI as the coupling agent, and sent for biological evaluation.

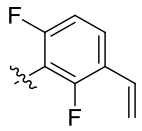
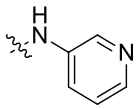
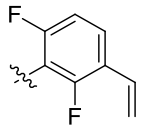
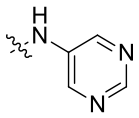
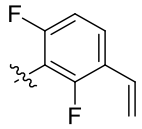
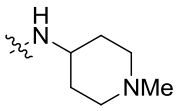
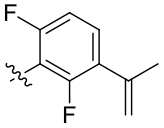
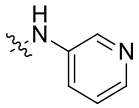
5.4.2.2 Biological evaluation of inhibitors with a 3-alkyl substituent

The inhibitory activity against ERK5 and p38α for each compound is shown in Table 32, and is compared to disubstituted compounds **128**, **182** and **149**.

Table 32. Biological evaluation of compounds incorporating a 3-alkyl substituent on the aroyl ring.



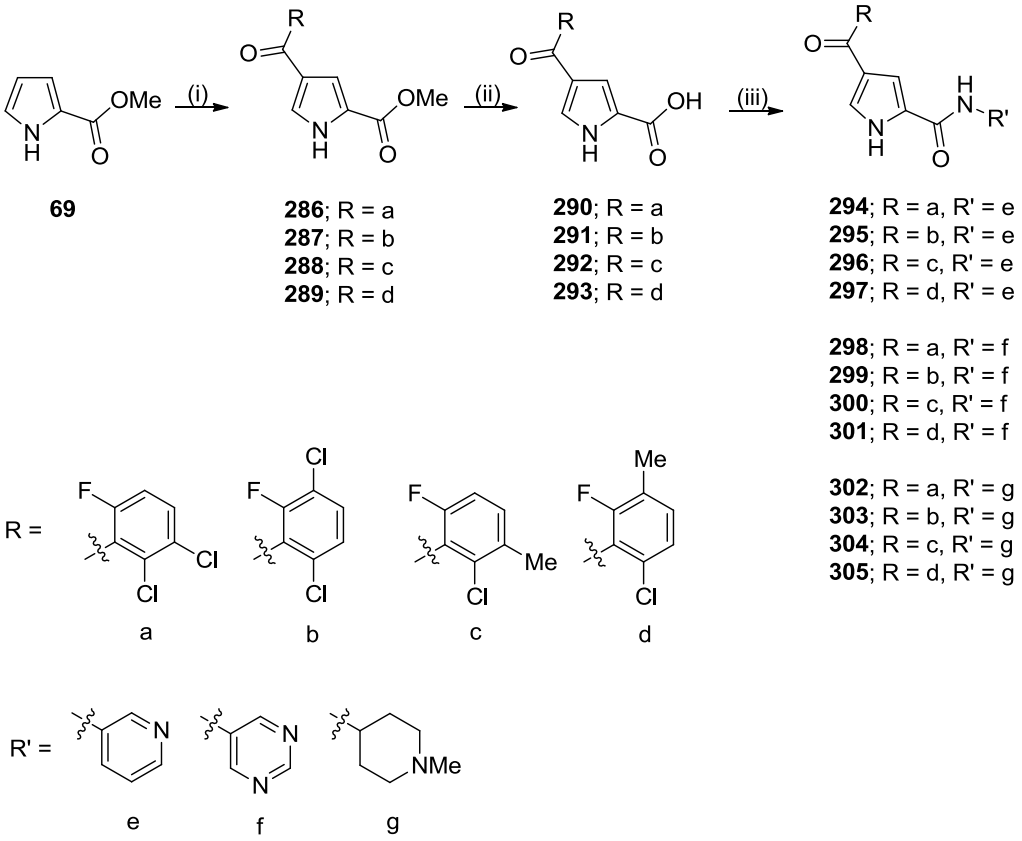
Compound	R	R'	ERK5; IC_{50} (μM)	p38α; IC_{50}(μM)
128			1.1 ± 0.27 (n = 4)	35 ± 0.90 (n = 2)
182			0.36 ± 0.10 (n = 4)	>120
149			1.0 ± 0.10 (n = 4)	6.8 ± 0.10 (n = 2)
273			1.3 ± 0.15 (n = 4)	>120
274			3.4 ± 0.14 (n = 4)	>120
275			0.61 ± 0.12 (n = 4)	>120

282			1.6 ± 0.35 (n = 4)	>120
283			1.4 (n = 1)	46 ± 4.2 (n = 2)
284			0.64 ± 0.14 (n = 4)	>120
285			2.4 ± 0.40 (n = 4)	>120

In each case, there was no improvement in inhibitory activity against ERK5 compared to compounds **128**, **182** and **149**, although selectivity for ERK5 over p38α was retained. These observations suggest that larger alkyl groups are tolerated, but are not necessary for ERK5 inhibition and that the size of the optimal substituent to fill the lipophilic pocket identified may be more limited than suggested by the homology model.

5.4.2.3 Re-introduction of the 2-chloro-substituent into tri-substituted aroyl analogues

The introduction of 2-chloro-6-fluoroaroyl groups onto the pyrrole carboxamide scaffold improved potency and selectivity for ERK5 over p38α (i.e. **148**, ERK5; IC_{50} = 0.60 μM, p38α; IC_{50} >120 μM). Synthesis of trisubstituted derivatives with a 2-chloro-6-fluoro substitution pattern and a substituent in the 3-position was therefore deemed desirable. Analogues with a 3-fluoro-substituent were synthesised by a previous group member and the results for these are shown in Table 30.[132] In order to test whether a larger substituent would be tolerated at the 3-position, 3-chloro- and 3-methyl-analogues were synthesised according to the procedures outlined in Scheme 49. The synthetic route began with the optimised Friedel-Crafts acylation of **69** to give methyl esters **286**-**289** in good yield (60-99%). Hydrolysis to the corresponding carboxylic acids **290**-**293** was performed in almost quantitative yield (98-99%). All of the amide couplings were performed using PCl_3 as the coupling agent and were performed in moderate to good yield (47-80%) to give compounds **294**-**305**.

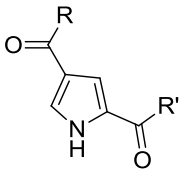


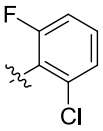
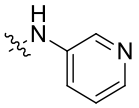
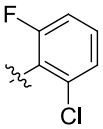
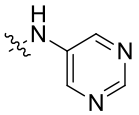
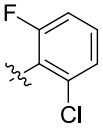
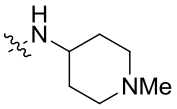
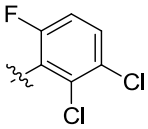
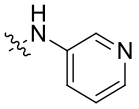
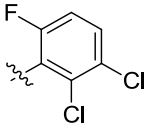
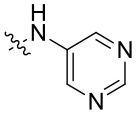
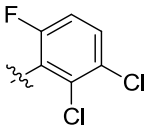
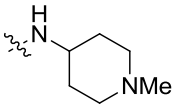
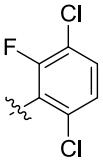
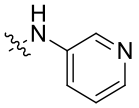
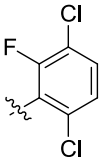
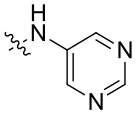
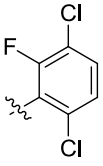
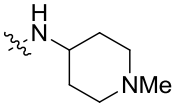
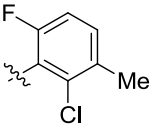
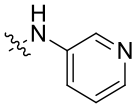
Scheme 49. *Reagents and conditions*: (i) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (ii) LiOH (aq), THF, 60 ^oC, 18 h; (iii) PCl_3, appropriate aniline, MeCN, MW, 150 ^oC, 5-30 min.

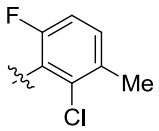
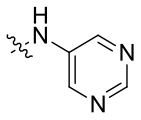
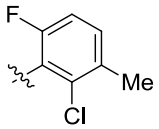
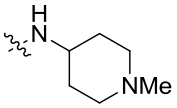
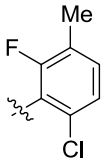
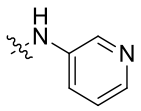
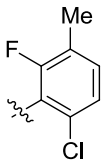
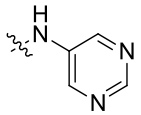
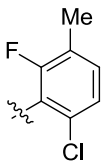
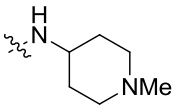
*5.4.2.4 ERK5 inhibitory activity and p38α counter-screening data for **294-305***

The ERK5 and p38α inhibitory activity for compounds **294**-**305** is shown in Table 33. In each case, the results can be compared to 2-chloro-6-fluoro compounds **248**, **182** and **250**.

Table 33. Biological Evaluation of **294-305**



Compound	R	R'	ERK5; IC_{50} (μM)	p38α; IC_{50}(μM)
248			0.58 ± 0.24 (n = 4)	93 ± 13 (n = 2)
182			0.36 ± 0.10 (n = 4)	>120
250			0.20 ± 0.07 (n = 4)	8.5 (n = 1)
294			0.27 ± 0.10 (n = 4)	>120
298			0.35 ± 0.14 (n = 4)	>120
302			0.28 ± 0.10 (n = 4)	53 ± 7.8 (n = 2)
295			0.07 ± 0.03 (n = 7)	>120
299			0.32 ± 0.37 (n = 4)	>120
303			0.11 (n = 1)	110 (n = 1)
296			0.65 ± 0.37 (n = 4)	>120

300			1.8 ± 0.03 (n = 4)	>120
304			0.47 ± 0.08 (n = 4)	>120
297			0.51 ± 0.28 (n = 4)	>120
301			0.26 ± 0.13 (n = 4)	>120
305			0.49 ± 0.06 (n = 4)	>120

The results show that both chloro- and methyl- substituents are well tolerated in the 3-position. Compound **295** showed an almost 9-fold improvement in ERK5 inhibitory activity compared with **248** (ERK5; IC_{50} = 0.58 μM), and was the first inhibitor tested to have an IC_{50} value below 100 nM in the pyrrole carboxamide series. Importantly, inhibitor **295** and all of the other compounds in this series, also retain selectivity for ERK5 over p38α. It is evident that introduction of a methyl group, although tolerated, does not provide the same increase in potency as that observed for the introduction of a chloro group as in compound **295**.

*5.4.2.5 Physicochemical and in vitro ADME profiling for compounds **295**, **299** and **303***

Review of the physicochemical properties of compounds **295**, **299** and **303** showed that they met the criteria for a lead candidate, although the TPSA for compound **303** was slightly low (Table 34).

Table 34. Physicochemical properties of compounds **295**, **299** and **303**.

Compound	MW	H-bond donors	H-bond acceptors	sLogP	TPSA
Lead criteria	<500	<5	<10	<5	75-100
295	378	2	5	3.8	75
299	379	2	6	4.0	88
303	398	2	5	3.4	65

The solubility and *in vitro* pharmacokinetic properties of inhibitors **295**, **299** and **303** were measured at Cyprotex and are shown in Table 35. Compounds **295** and **303** have poor aqueous solubility compared to **299**. Clearance in both human and mouse liver microsomes is high for **295**, but not above the desired threshold for a lead candidate. Plasma protein binding was good for all three compounds. Caco-2 permeability was also tested for **295** and was shown to be above the threshold (Papp A2B = 25 x 10^{-6} cm/s) and the efflux ratio was low (0.66) suggesting the compound is not a substrate for efflux *via* P-glycoprotein (Pgp). In addition, **295** was counter-screened against MEK5 in an assay ran by Carna Bioscience. MEK5 inhibitory activity was measured as 0.79 μM indicating that **295** is ~10 fold selective for ERK5 over closely related MEK5. Investigations are underway to identify inhibitors that are equipotent with **295** with improved aqueous solubility.

Table 35. *In vitro* ADME profiling for **295**, **299** and **303**.

Compound	LE	Solubility L/U (μM)	Plasma Protein Binding (%)	Mouse CLint (μL/min/mg)	Human CLint (μL/min/mg)
Lead criteria	>0.3	>50/50	<99	<48	<48
295	0.39	10/65	98	48	32
299	0.36	100/100	95	0	0
303	0.37	3/20	77	0	0

In order to explain the increase in ERK5 inhibitory activity of **295**, docking studies have been conducted at Newcastle by Prof. Martin Noble using the recently published structure of monomeric ERK5 (Figure 45). An initial bound pose was deduced by superimposing the carboxamide-bound structure of p38α onto the ERK5 template, and modifying the drug molecule to match the structural formula of **295** using tools from the RDKIT toolkit. Bond restraints and partial charges for the ligand were calculated using PRODRG, and the complex was energy minimised using GROMACS. The model

shows how well the tri-halogenated ring fits into pockets in the ERK5 ATP binding site and this could account for the increased inhibitory activity of **295**.

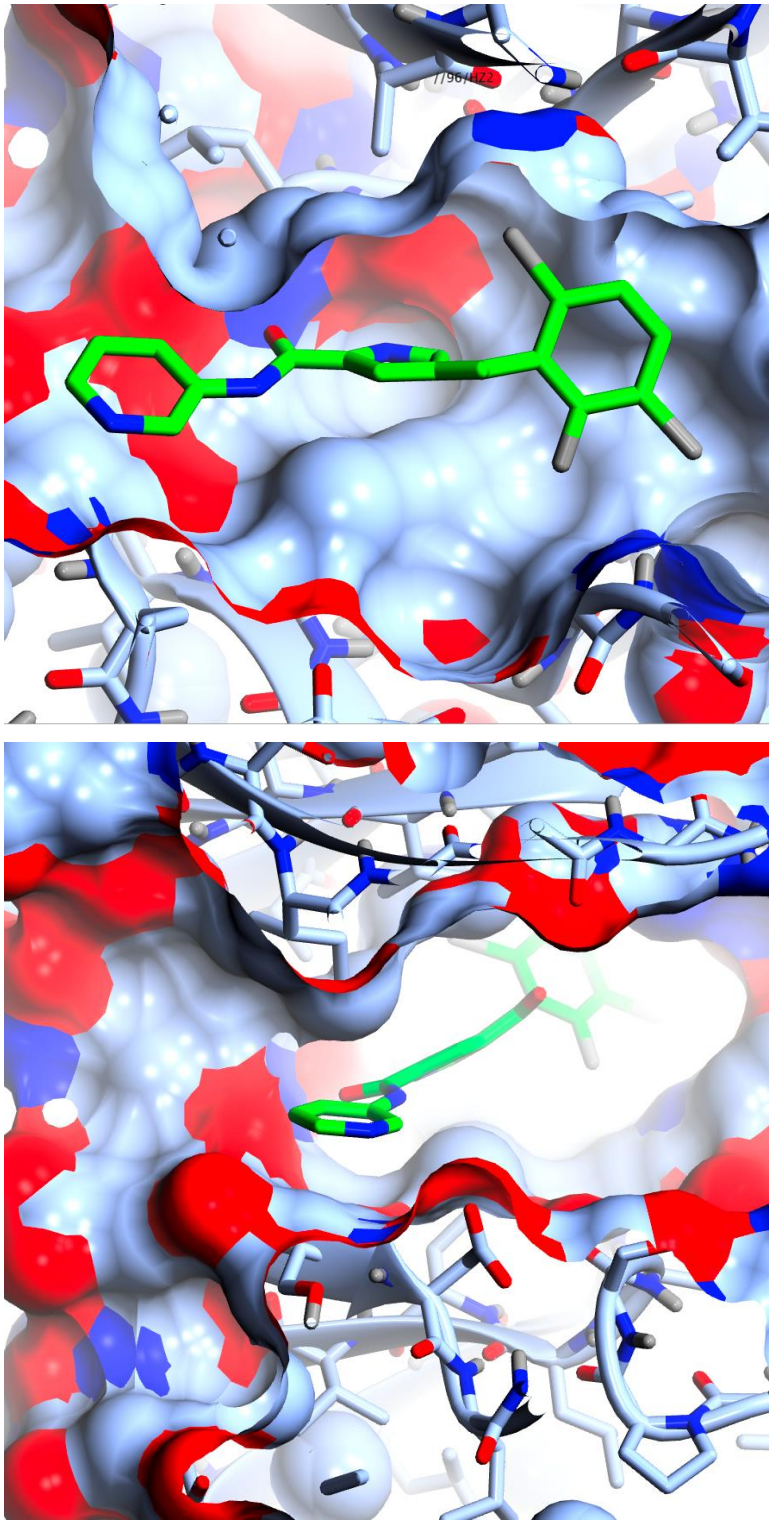
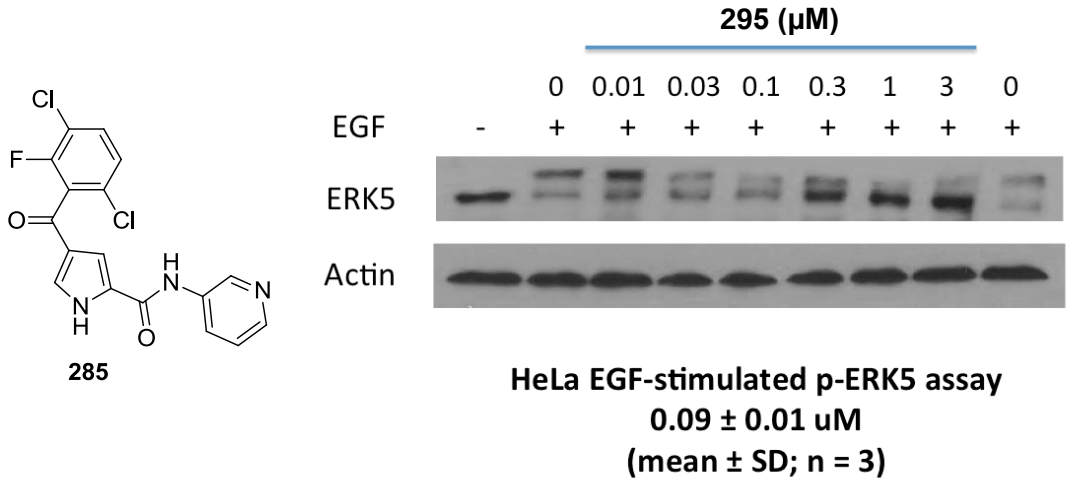


Figure 45. Docking of **295** using published ERK5 X-ray crystal structure.

*5.4.2.6 Cellular activity of **295** in an Epidermal Growth Factor (EGF)-stimulated HeLa cell assay*

In order to assess the cellular activity of compound **295**, an EGF-stimulated HeLa cell assay was conducted at Newcastle by Lan-Zhen Wang. HeLa cells were treated with increasing concentrations of inhibitor **295** followed by EGF stimulation for 10 min before being harvested. Samples were run on 6% Tris–glycine gel and transferred to nitrocellulose, before Western blot analysis was conducted with ERK5 antibody (Figure 46). The IC_{50} value was determined from densitometry of top bands, and was calculated as 0.09 ± 0.01 μM. The IC_{50} value obtained is consistent with that obtained in the cell-free IMAP assay (ERK5; IC_{50} = 0.07 μM), suggesting that despite the low aqueous solubility of **295**, the compound is cell permeable. Further *in vivo* PK/PD studies with compound **295** are underway.



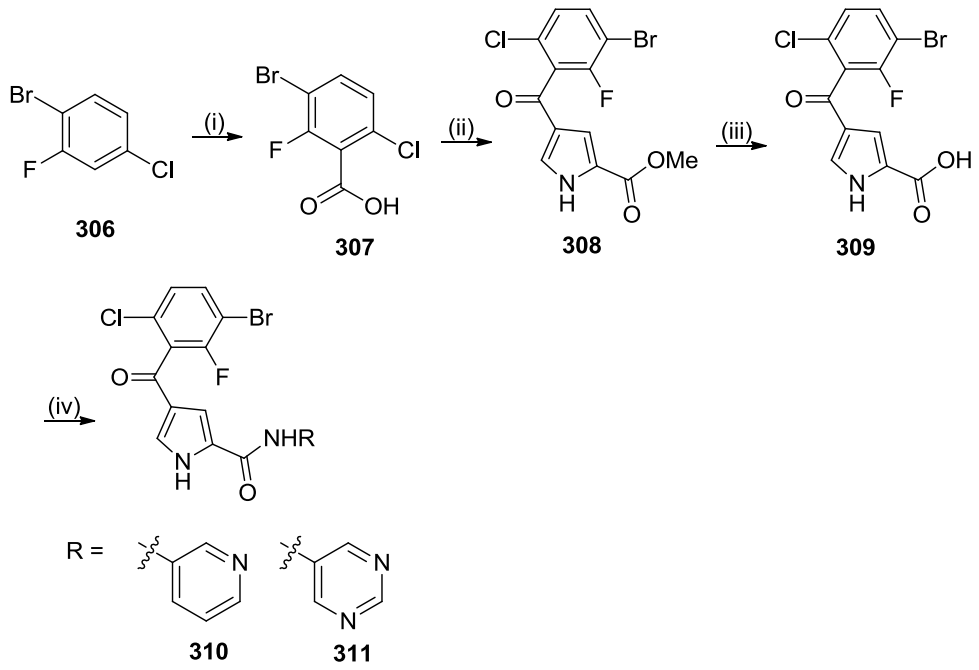
285

Figure 46. Western blot analysis showing inhibition of p-ERK5 by **295** at ≤100 nM. Figure shows the resolution of a phospho-ERK5 band (top) from non-phosphorylated ERK5 (lower band).

5.4.2.7 Introduction of a bromo- substituent into the 3-position of the aroyl ring

Following the success of **295**, synthesis of analogues with a bromo- substituent in the 3-position was undertaken. These analogues were synthesised for evaluation of their ERK5 inhibitory activity, but also as potential intermediates for the synthesis of further analogues since the bromo- substituent could potentially be used in a variety of

subsequent reactions (Scheme 50). Synthesis began with a literature procedure to afford 3-bromo-6-chloro-2-fluorobenzoic acid (**307**) from 1-bromo-4-chloro-2-fluorobenzene (**306**) *via* deprotonation of the proton between the fluoro- and chloro- substituents using LDA and introduction of the carboxylic acid using solid CO_2.[134] This reaction worked well in 90% yield. Carboxylic acid **307** was converted to the acid chloride using thionyl chloride, which was subsequently used *in situ* in the Friedel-Crafts acylation of methyl pyrrole 2-carboxylate, giving **308** in 87% yield. Hydrolysis of **308** using LiOH gave carboxylic acid **309** in 99% yield. Coupling of 3-aminopyridine and 5-aminopyrimidine was performed using PCl_3 affording **310** and **311** in 58% and 30% yield, respectively.



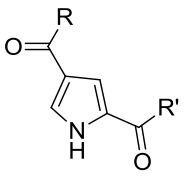
Scheme 50. *Reagents and conditions*: (i) (a) LDA, THF, -70 ^{o}C, 2 h; (b) CO_2, Et_2O, -70 ^{o}C to RT, 4 h; (ii) (a) $SOCl_2$, THF, RT, 3 h; (b) methyl pyrrole 2-carboxylate, $AlCl_3$, DCM, RT, 18 h; (iii) LiOH (aq), THF, 60 ^{o}C, 18 h; (iv) PCl_3, appropriate aniline, MeCN, MW, 150 ^{o}C, 5-30 min.

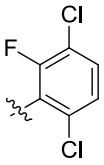
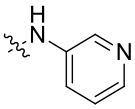
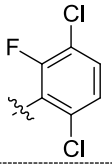
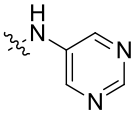
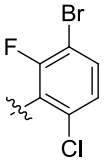
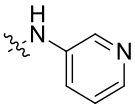
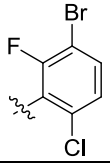
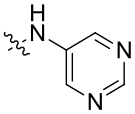
Synthesis of the *N*-methylpiperidyl analogue was hampered by purification problems and a pure sample of this compound has, as yet, not been synthesised.

*5.4.2.8 Biological evaluation of compounds **310** and **311**.*

The ERK5 and p38α inhibitory activities of compounds **310** and **311** are shown in Table 36 and can be compared to compounds **295** and **299**.

Table 36. Biological activity of **310** and **311**.



Compound	R	R'	ERK5; IC_{50} (μM)	p38α; IC_{50}(μM)
295			0.07 ± 0.03 (n = 7)	>120
299			0.32 ± 0.37 (n = 4)	>120
310			0.067 ± 0.03 (n = 4)	>120
311			0.61 ± 0.23 (n = 4)	>120

Increasing the size of the 3-chloro- substituent of **295** and **299** to a 3-bromo- substituent was well tolerated, although we did not observe an improvement in ERK5 inhibitory activity. As stated previously, intermediate **308** will be utilised as an intermediate for the synthesis of inhibitors with further variation at the 3-position of the aroyl ring. For example, upon protection of the pyrrole nitrogen, the bromo- substituent could be used as a coupling partner for a variety of palladium-mediated coupling reactions enabling further elaboration at this stage of the synthesis. This would help to further expand SARs around the introduction of tri-substituted aroyl rings into pyrrole carboxamide inhibitors of ERK5.

Chapter 6. Structure-activity relationship studies (SARs) around the pyrrole core

6.1 Introduction of substituents at the pyrrole 3-position

6.1.1 Rationale

Homology modelling based on p38α and conducted at Newcastle University identified a lipophilic pocket, which could potentially be exploited *via* substitution at the 3-position of the pyrrole ring (Figure 46).

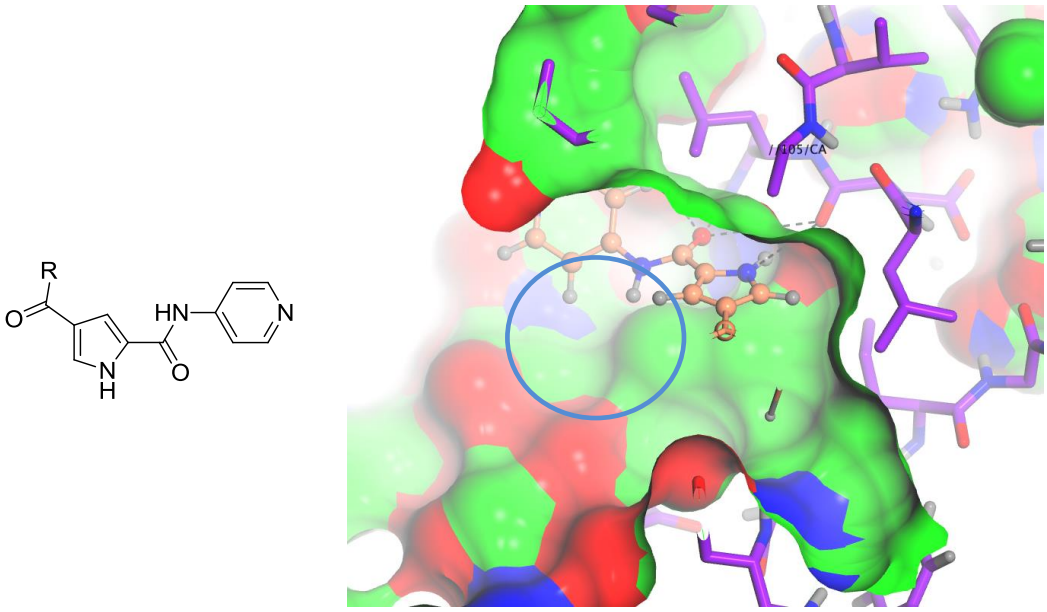


Figure 46. p38α-derived homology model with potential lipophilic pocket highlighted in blue.

The initial set of compounds to be synthesised had small alkyl groups at the 3-position of the pyrrole in order to see if substitution would be tolerated and ERK5 inhibitory activity retained or improved. The aroyl group in each case was either the 2-chloro-6-fluorobenzoyl or the 2-bromo-6-fluorobenzoyl as these groups had been identified as providing good selectivity over p38α. The potency of the trisubstituted aroyl rings had not been discovered at the time we started the synthesis of these analogues. For each modification of the pyrrole core and aroyl ring, three amide side-chains were to be incorporated; 3-pyridyl, *N*-methylpiperidyl and pyrimidyl (Figure 47). These amide

side-chains were chosen for their optimal properties (potency, aqueous solubility and improved CYP450 inhibition profile, respectively).

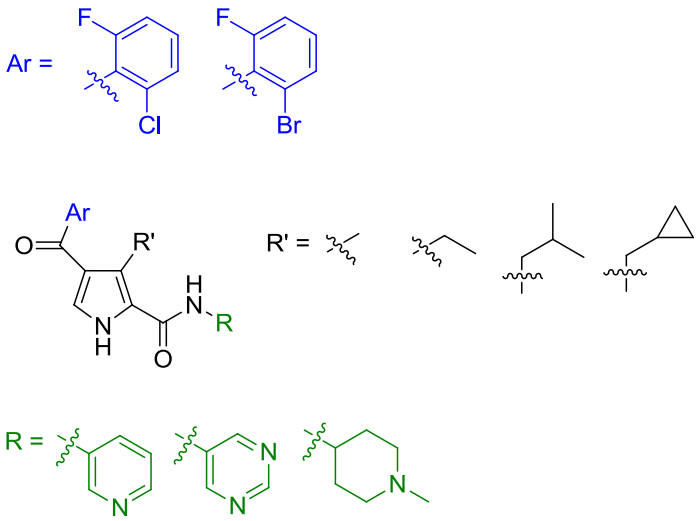
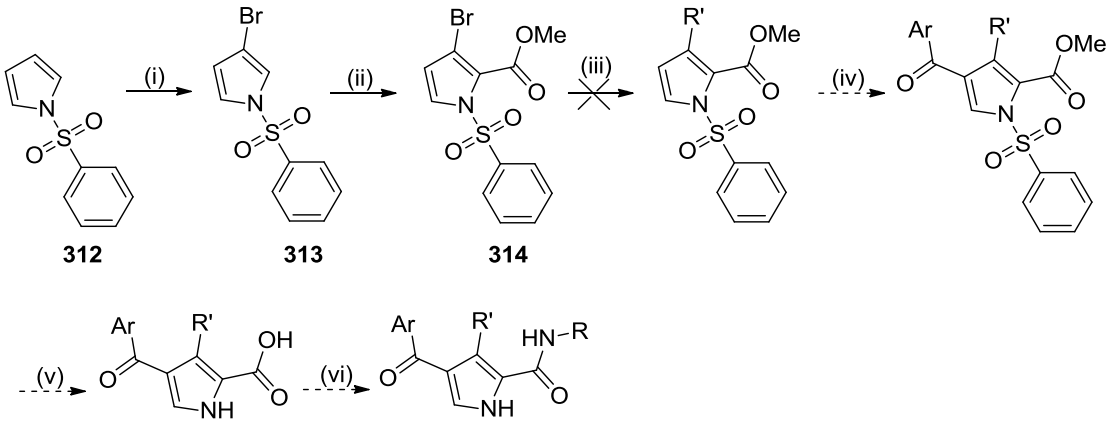


Figure 47. Compounds to be synthesised with substituents at the 3-position of the pyrrole ring.

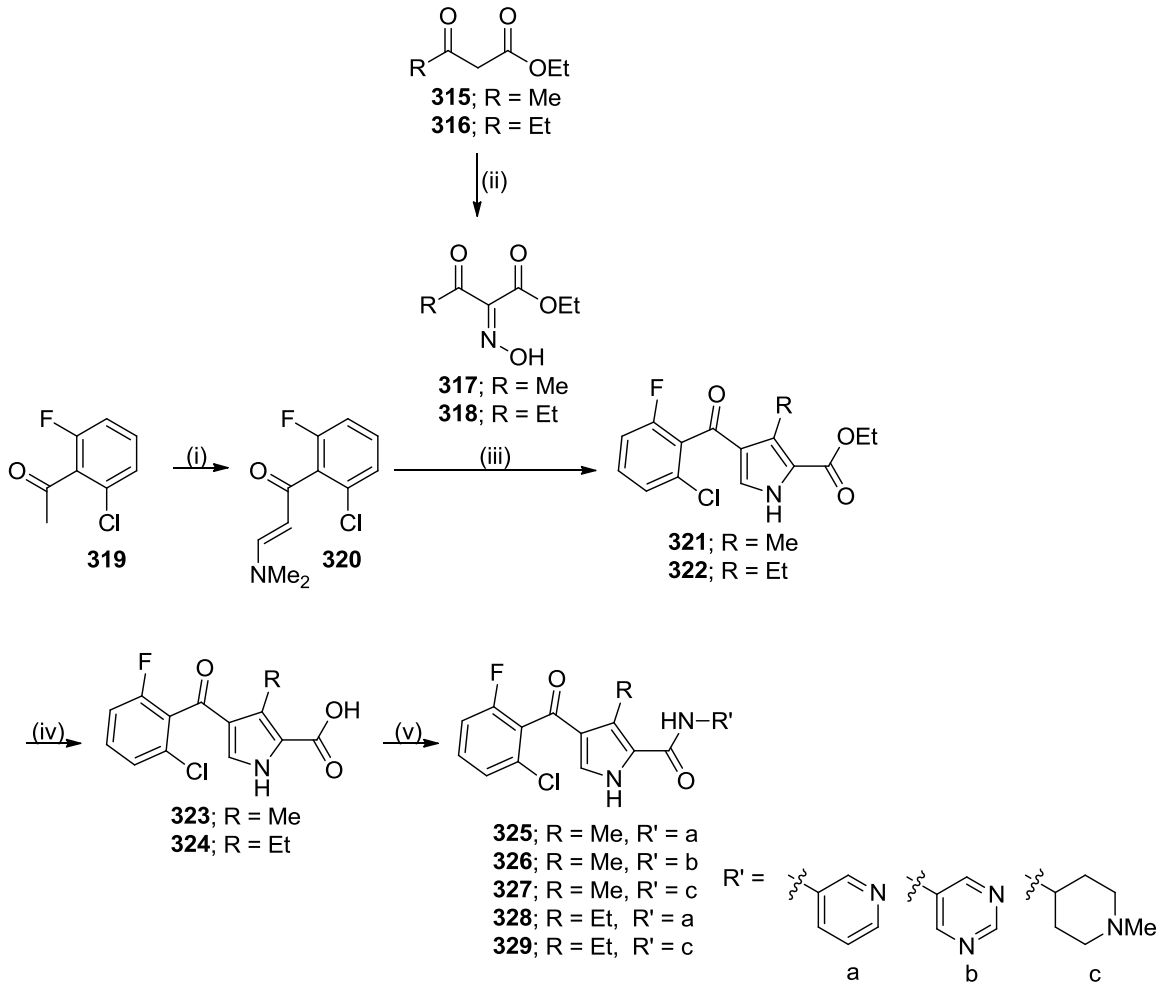
6.1.2 Synthesis of 3-substituted pyrrole analogues

In order to introduce a substituent at the 3-position of the pyrrole ring, a literature procedure was followed, initially introducing a bromo-substituent at this position (Scheme 51).[135] The aim was to use this intermediate to perform Suzuki-Miyaura reactions and introduce the desired alkyl groups. Reaction of *N*-phenylsulfonylpyrrole (**312**) with bromine in acetic acid under reflux for 1 h produced 3-bromopyrrole derivative **313** in 69% yield. Deprotonation of the 2-position of pyrrole **313** using LDA followed by addition of methyl chloroformate afforded compound **314** in 75% yield. The subsequent Suzuki-Miyaura reaction was attempted using trimethylboroxine, palladium tetrakis and sodium carbonate under conventional heating overnight. Unfortunately, the reaction was unsuccessful and starting material was recovered. The reaction was also attempted using ethylboronic acid, but was equally unsuccessful. Both reactions were trialled under microwave heating, but to no avail. The coupling conditions used were based on literature procedures, whereby an aryl group had been introduced into the 3-position of intermediate **314.**[135-136] At the time, there were no published syntheses that had installed an alkyl group into the 3-position *via* a Suzuki-Miyaura cross-coupling reaction.



Scheme 51. *Reagents and conditions*: (i) Br_2, AcOH, reflux, 1 h; (ii) (a) diisopropylamine, *n*-BuLi, -78 ^oC, 1 h; (b) **313**, -78 ^oC, 1 h then methyl chloroformate, THF, -78 ^oC to RT, 2 h; (iii) trimethylboroxine or appropriate boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane:water (9:1), 110 ^oC, 18 h; (iv) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (v) LiOH (aq), THF, 60 ^oC, 18 h; (vi) appropriate amine, PCl_3, MeCN, MW, 150 ^oC, 5-30 min.

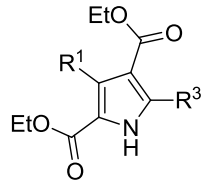
An alternative synthetic strategy required the convergent synthesis of two starting materials and was based on a literature procedure for the synthesis of the core pyrrole ring substituted in the 2-, 3- and 4-positions with the required groups (Scheme 52).[137-138] The synthetic route was trialled for the synthesis of inhibitors with 3-methyl and 3-ethyl substituents and a 2-chloro-6-fluorobenzoyl group in the 4-position of the pyrrole ring.



Scheme 52. *Reagents and conditions*: (i) *N*,*N*-dimethylformamide dimethyl acetal, toluene, reflux, 18 h; (ii) $NaNO_2$ (aq), AcOH, H_2O, 0 ^oC, 4 h; (iii) NaOAc, AcOH, Zn dust, 140 ^oC, 6 h; (iv) LiOH (aq), THF, 100 ^oC, 18 h; (v) appropriate amine, PCl_3, MeCN, MW, 150 ^oC, 5 min.

The first starting material was synthesised on reaction of commercially available 2'-chloro-6'-fluoroacetophenone (**319**) with *N*,*N*-dimethylformamide dimethyl acetal providing enamine **320** in 80% yield.[137] Reaction of ethyl acetoacetate (**315**) or ethyl propionylacetate (**316**) with sodium nitrite in acetic acid and water provided the second building block, oximes **317** or **318** in 85% yield (Scheme 52). Reaction of **320** with **317** or **308** in a reductive cyclisation reaction in the presence of zinc provided **321** and **322** both in 30% yield.[138] Initial attempts at the cyclisation were much lower yielding and it was found that portion-wise addition of eight equivalents of zinc dust over 6 hours gave the best results, although the yield was still modest. The benefit of this route is that the

reductive cyclisation step provides a key intermediate with a 3-alkyl substituent (**321**; methyl or **322**; ethyl), a 4-benzoyl substituent and a 2-carboxylate in place. One disadvantage is if any of the ethyl acetoacetate (**315**) or ethyl propionylacetate (3**16**) remain after formation of oximes **317** and **318**, the two can react together in the presence of zinc to form Knorr's pyrrole (**330**) or the ethyl equivalent (**331**) as side products (Figure 48).



330; R^1, R^3 = Me
331; R^1, R^3 = Et

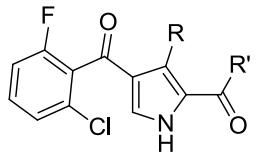
Figure 48. Potential side-products formed in step (iii) (Scheme 52) in the presence of any remaining ethyl acetoacetate (**315**) or ethyl propionylacetate (**316**).

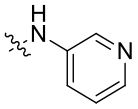
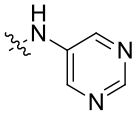
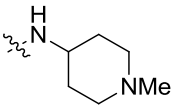
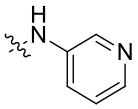
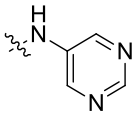
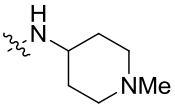
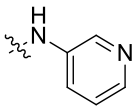
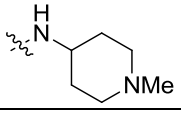
The ethyl ester of **321** and **322** was hydrolysed using lithium hydroxide to give carboxylic acids **323** and **324** in 78% and 75% respectively. The final amide couplings were all performed using PCl_3 and gave compounds **325-329** in moderate yields (20-30%). Unfortunately the pyrimidyl analogue with a 3-ethyl substituent was not successfully isolated due to problems with purification.

6.1.3 Biological Evaluation of 3-substituted pyrroles 325-329

Compounds **325-329** were sent for biological evaluation and the results are shown in Table 37. The ERK5 inhibitory activity and p38α counter-screening data for compounds **325-329** are compared to unsubstituted pyrrole derivatives **248**, **182** and **250**.

Table 37. ERK5 and p38α inhibitory activity for compounds **325**-**329**.



Compound	R	R'	ERK5; IC_{50} (μM)	p38α; IC_{50} (μM)
248	**H**		0.58 ± 0.24 (n = 4)	93 ± 13 (n = 2)
182	**H**		0.36 ± 0.10 (n = 4)	>120
250	**H**		0.20 ± 0.07 (n = 4)	8.5 (n = 1)
325	Me		40 ± 0.9 (n = 4)	43 ± 1.7 (n = 2)
326	Me		69 ± 1.1 (n = 4)	>120
327	Me		45 ± 4.1 (n = 4)	36 ± 7.3 (n = 2)
328	Et		38 ± 0.78 (n = 4)	50 ± 1.0 (n = 2)
329	Et		59 ± 3.4 (n = 4)	24 ± 2.7 (n = 2)

In each case the ERK5 inhibitory activity was reduced compared to inhibitors **248**, **182** and **250**. As with all inhibitors synthesised in this project, these compounds were counter-screened for p38α activity. For most compounds, the selectivity for ERK5 over p38α was also abolished with compounds being equipotent for both kinases. With this in mind, synthesis of any further analogues with larger alkyl groups in the 3-position or different aroyl groups in the 4-position was ceased.

These results indicate that the introduction of a substituent at the 3-position of the pyrrole ring is not tolerated. The lack of activity is most likely due to the change in conformation a substituent in the 3-position could impose on both, the benzoyl ring and the amide side chain. A change in conformation could mean that essential interactions between the inhibitor and ERK5 binding site are no longer being fulfilled (i.e. changing

the conformation of the amide group could disrupt hinge binding). Figure 49 shows the energy minimised structures of inhibitors with and without substituents at the 3-position of the pyrrole ring. It is evident that the introduction of a methyl or ethyl group at the 3-position has an effect on both the aroyl ring and amide group conformations, with the 3-ethyl group imposing the greatest change. This does not explain how the compounds would bind in the ERK5 binding site and obtaining a co-crystal of each compound bound to ERK5 would be the only way to definitively demonstrate the effect a substituent at the pyrrole 3-position would have on binding.

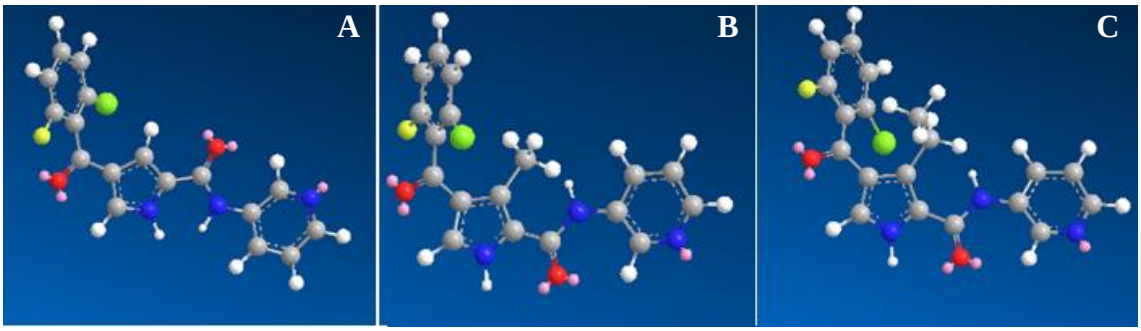


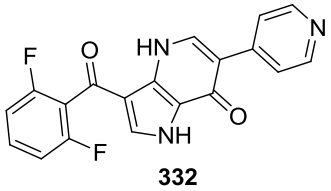
Figure 49. Energy minimised conformations of pyrrole carboxamide inhibitors; (A) with no 3-substituent, (B) with a 3-methyl substituent and (C) with a 3-ethyl substituent. Energy minimised using Chem3D.

6.2 Development of inhibitors containing a 1*H*-pyrrolo[3,2-*b*]pyridin-7(4*H*)-one core

6.2.1 Rationale

In order to further probe the ERK5 ATP binding site, the synthesis of novel inhibitors with a core 1*H*-pyrrolo[3,2-*b*]pyridin-7(4*H*)-one bicycle was investigated. The compounds were to incorporate either a 2,6-difluoro-, 2,3-dichloro-, 2-chloro-6-fluoro- or 2-bromo-6-fluoro benzoyl group and a 3- or 4-pyridyl side chain, based on previous SARs. These compounds were docked in the first generation ERK2-based homology model (Figure 50). They all introduce rigidity around the core pyrrole ring as well as removing the amide NH. It has been shown previously that the amide NH is not necessary for inhibitory activity against ERK5, as compounds synthesised without this moiety retained potency. Removal of a methylene group from the amide side chain had improved potency against ERK5 (chapter 4, section 4.2). This suggested that increasing

the rigidity of inhibitors is beneficial and hence, the introduction of rigidity *via* incorporation of a new planar bicyclic core was postulated to be a valid strategy for improvement of ERK5 inhibitory activity. Initially, inhibitors with a methylpyridyl group and without the methylene group were proposed for synthesis in order to compare ERK5 inhibitory activity.



332

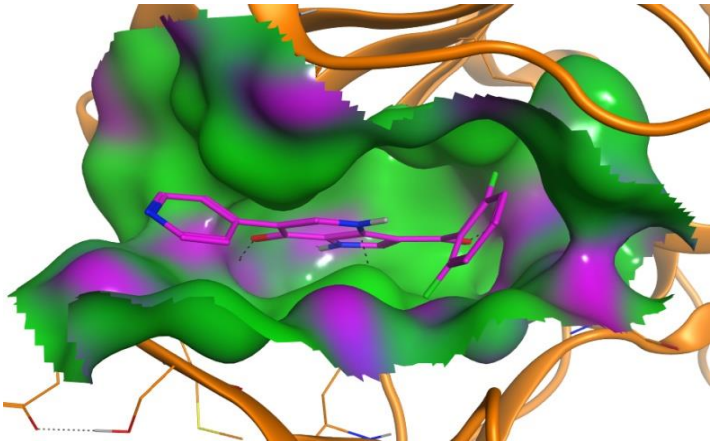
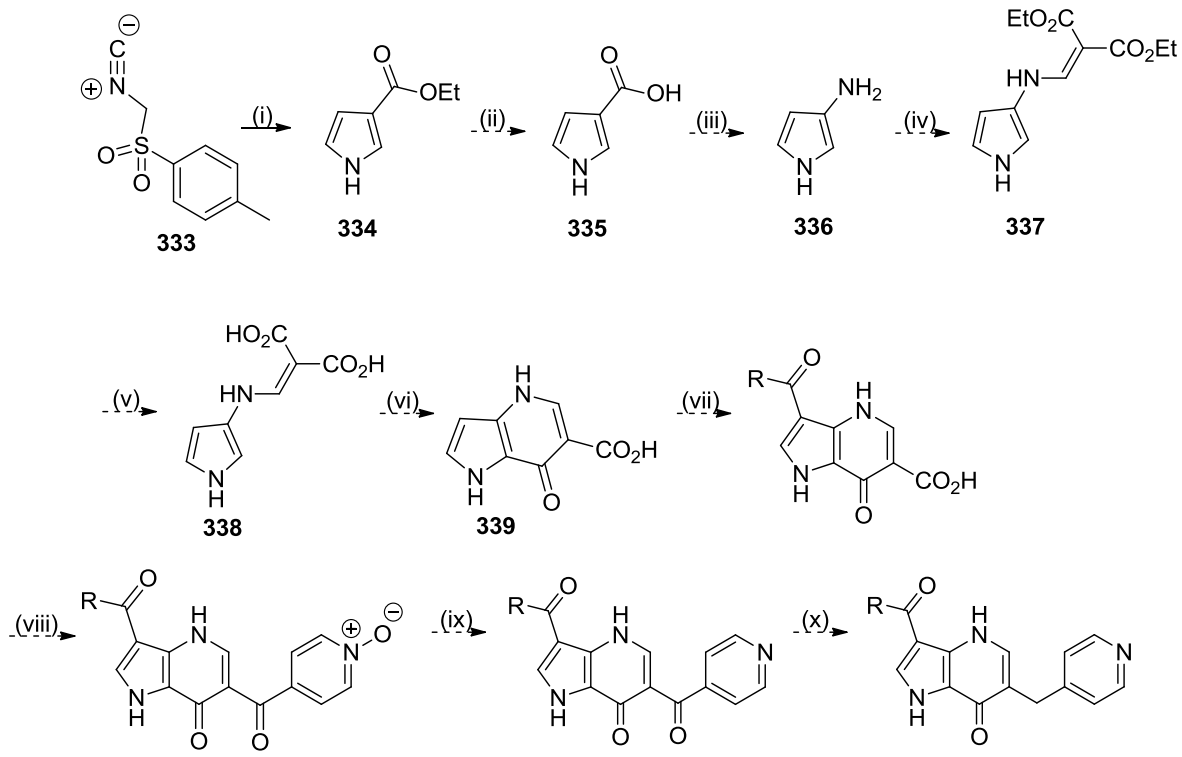


Figure 50. Compound **332** docked into the ERK2-derived homology model developed by CRT-DL.

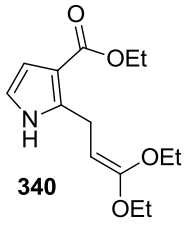
6.2.2 Synthesis of 1H-pyrrolo[3,2-b]pyridin-7(4H)-one inhibitors

The first synthetic step was based on a literature procedure using toluenesulfonylmethyl isocyanide (TosMIC) (**333**) in a reaction with ethyl acrylate to give ethyl 1*H*-pyrrole-3-carboxylate **334** (Scheme 53).[139]



Scheme 53. *Reagents and conditions*: (i) ethyl acrylate, NaH, Et_2O, RT, 3 h; (ii) LiOH, THF, 60 ^oC, 18 h; (iii) DPPA, NEt_3, ^tBuOH, 30 ^oC, 18 h; (iv) diethyl 2-(bromomethylene)malonate, $Pd_2(dba)_3$, Xantphos, K_3PO_4, toluene, 120 ^oC, 18 h; (v) LiOH (aq), THF, 60 ^oC, 18 h; (vi) $POCl_3$, reflux, 3 h; (vii) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^oC to RT, 18 h; (viii) pyridine *N*-oxide, conditions TBC; (ix) conditions TBC; (x) conditions TBC.

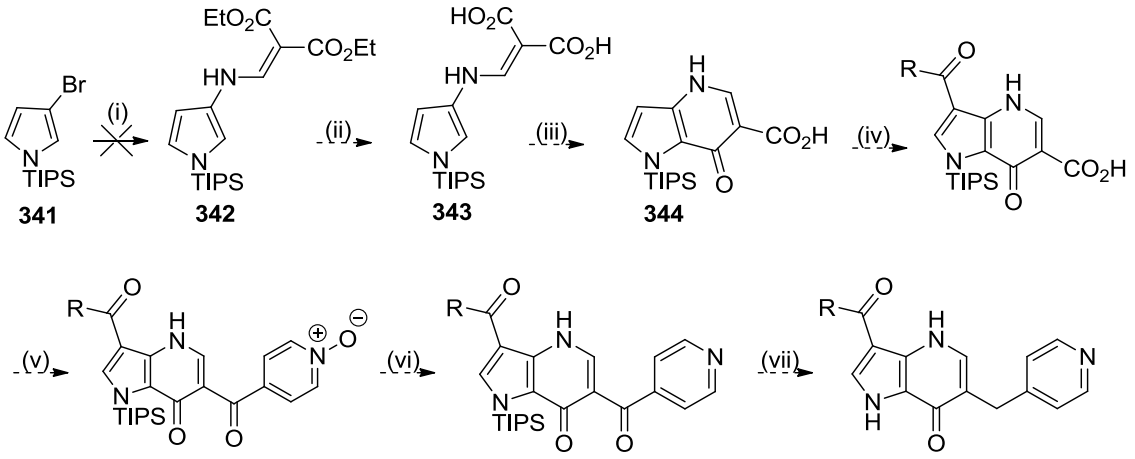
The reaction appeared to work well, but despite purification by chromatography, an impurity remained. The impurity was thought to be the Michael addition product (**340**; Figure 51) from the reaction of **334** with remaining ethyl acrylate as confirmed by the detection of the correct mass by LC-MS. Due to this problem, and also the potential issues that may have arisen with the Curtius rearrangement in step (iii), an alternative approach was conducted.



340

Figure 51. Michael addition product from reaction of **334** with ethyl acrylate.

The first step of the alternative route was the substitution of the 3-bromo group of TIPS protected 3-bromopyrrole **341** using diethyl 2-(aminomethylene)malonate (Scheme 54).



341 342 343 344

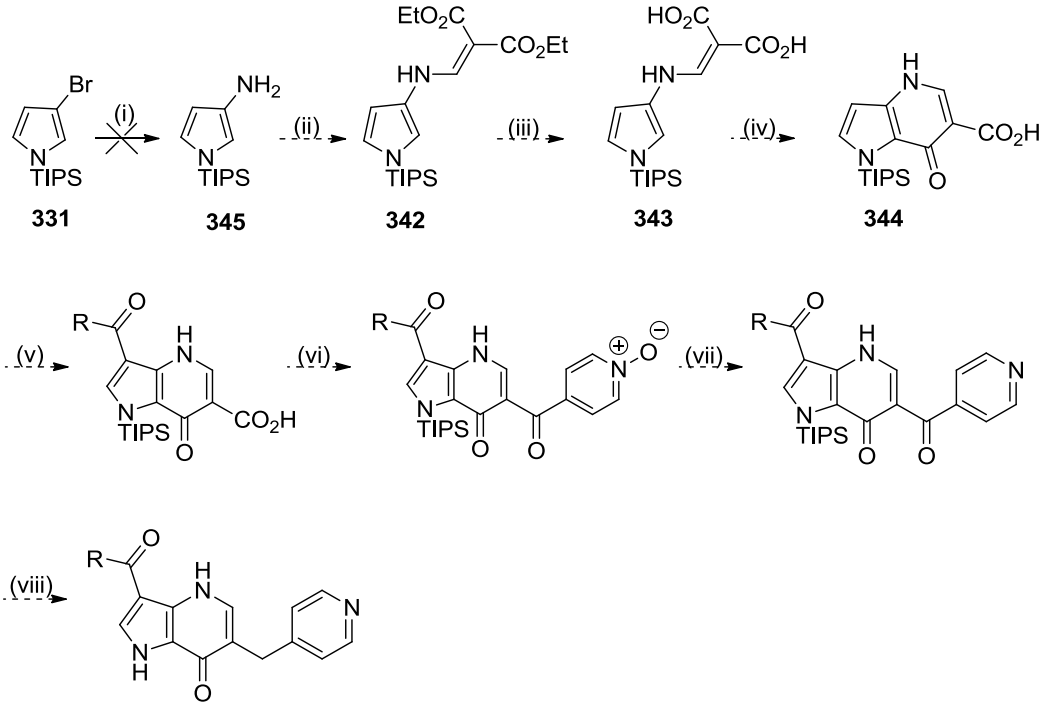
Scheme 54. *Reagents and conditions*: (i) diethyl 2-(aminomethylene)malonate, see Table 38; (ii) LiOH (aq), THF, 60 ^{o}C, 18 h; (iii) $POCl_3$, reflux, 3 h; (iv) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h; (v) pyridine *N*-oxide, conditions TBC; (vi) conditions TBC; (vii) conditions TBC.

The substitution with diethyl 2-(aminomethylene)malonate was attempted using a variety of conditions, including acid-catalysed aromatic substitution in TFE (Table 38). The reaction was unsuccessful when performed in TFE alone, and when TFA was used to catalyse the reaction. The next attempted approach was to use Buchwald coupling conditions.[140] This was attempted using both conventional and microwave heating, and in each case **342** was not obtained.

Table 38. Attempted conditions for step (i) Scheme 54.[140]

Entry	Heating Method	Temp. (°C)	Solvent	Time (h)	Catalyst	Ligand	Base	Result
1	MW	160	TFE	0.25	-	-	-	No rxn
2	MW	160	TFE	0.25	TFA	-	-	No rxn
3	MW	100	Dioxane	0.5	$Pd(OAc)_2$	BINAP	Cs_2CO_3	No rxn
4	MW	150	Dioxane	0.5	$Pd(OAc)_2$	BINAP	Cs_2CO_3	Degradation
5	Conv.	100	Dioxane	18	$Pd(OAc)_2$	BINAP	Cs_2CO_3	Degradation
6	Conv.	110	Toluene	18	$Pd_2(dba)_3$	BINAP	Cs_2CO_3	No rxn

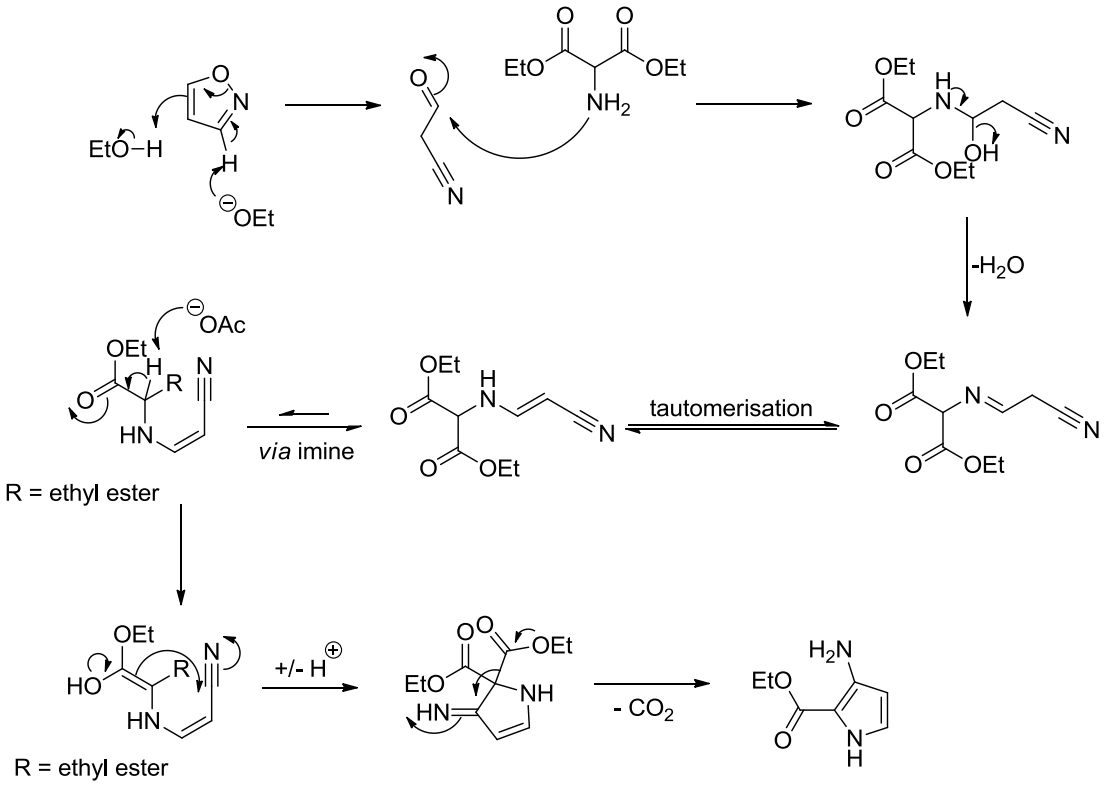
Problems had been encountered previously with Buchwald coupling of 2-bromopyrrole with amines (Chapter 4), therefore reversal of the coupling partners was investigated. Starting with 3-bromopyrrole **341** (Scheme 55), Buchwald coupling with benzophenone imine was performed. The Buchwald coupling in this case was postulated to be more favourable due to the electron-rich nature of the amine coupling partner. Once coupled, the imine could be hydrolysed to leave the free amine in the 3-position of the pyrrole ring. 3-Aminopyrrole **345** could then be coupled with diethyl 2-(bromomethylene)malonate. Unfortunately the coupling with benzophenone imine was not successful and an alternative method of accessing the 3-aminopyrrole was sought.



Scheme 55. *Reagents and conditions*: (i) benzophenone imine, $Pd(OAc)_2$, Cs_2CO_3, BINAP, 100 °C, 5 h; (ii) 1 M HCl, THF, RT, 15 min; (iii) diethyl 2-(bromomethylene)malonate, $Pd_2(dba)_3$, Xantphos, K_3PO_4, toluene, 120 °C, 18 h; (iv) LiOH (aq), THF, 60 °C, 18 h; (v) $POCl_3$, reflux, 3 h; (vi) appropriate benzoyl chloride,

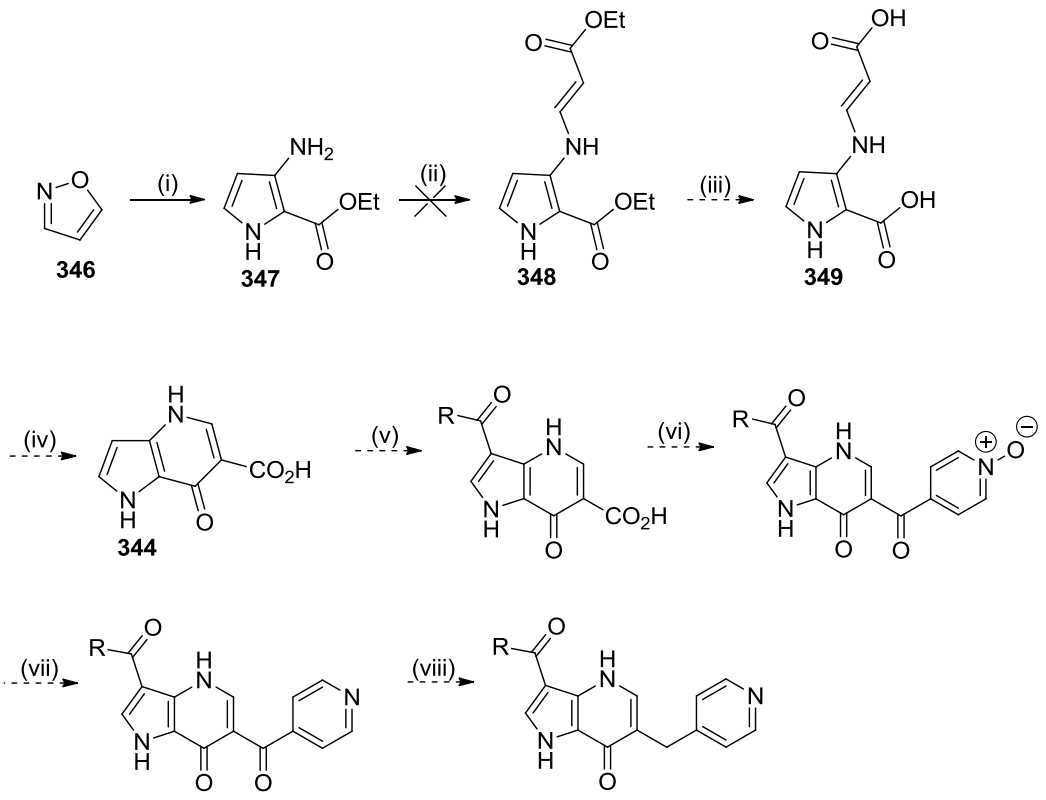
$AlCl_3$, DCM, 0 °C to RT, 18 h; (vii) pyridine *N*-oxide, conditions TBC; (viii) conditions TBC; (ix) conditions TBC.

A literature procedure using isoxazole (**346**) and diethyl 2-aminomalonate hydrochloride to form ethyl 3-amino-1*H*-pyrrole-2-carboxylate (**347**), which goes *via* the mechanism shown in Scheme 56 was conducted.[141] This route was attractive since the 3-amino group was installed directly for use in the subsequent Buchwald reaction. The 2-ethylcarboxylate group could also be utilised at a later stage to form the bicycle.



Scheme 56. Proposed mechanism of reaction of isoxazole with diethyl 2-aminomalonate.[141]

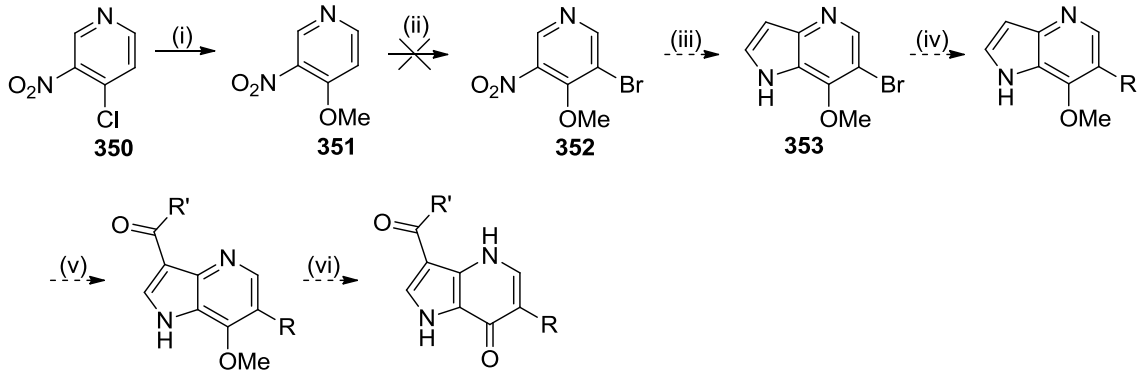
Ethyl 3-amino-1*H*-pyrrole-2-carboxylate (**347**) was synthesised in 85% yield (Scheme 57). Buchwald reaction with ethyl 3-bromoacrylate was attempted using both conventional heating at 120 °C for 18 h and microwave irradiation at 120 °C for 30 min. Unfortunately, the reaction was unsuccessful in both cases.



Scheme 57. *Reagents and conditions*: (i) (a) EtOH, NaOEt, 0 ^{o}C, 45 min; (b) AcOH, diethyl 2-aminomalonate hydrochloride, NaOAc, RT, 18 h; (c) EtOH, NaOEt, RT, 18 h (d) AcOH, RT, 5min; (ii) ethyl 3-bromoacrylate, $Pd_2(dba)_3$, Xantphos, K_3PO_4, toluene, 120 ^{o}C, 18 h; (iii) LiOH (aq), THF, 60 ^{o}C, 18 h; (iv) $POCl_3$, reflux, 3 h; (v) appropriate benzoyl chloride, $AlCl_3$, DCM, 0 ^{o}C to RT, 18 h; (vi) pyridine *N*-oxide, conditions TBC; (vii) conditions TBC; (viii) conditions TBC.

Due to the lack of success with the synthesis and the discovery of potent inhibitors without the methylene group in the amide side chain (Chapter 4), a new synthetic strategy was employed focussing on bicyclic inhibitors without a methylene group. An alternative synthetic route was attempted as shown in Scheme 58, starting with commercially available 4-chloro-3-nitropyridine (**350**). The first step involved substitution of the chloro- group using sodium methoxide and proceeded smoothly to produce **351** in 96% yield. Attempts to introduce a bromo- substituent into the 5-position of the pyridine ring were unsuccessful. The first conditions attempted were NBS in MeCN and heating under microwave irradiation, based on a literature procedure for bromination of 4-methoxypyridine.[142] The reaction failed to produce **352**, and starting material **351** was recovered. Reaction of **351** with bromine in H_2O and heating to 50 ^{o}C for 2 h was also unsuccessful. This procedure was based on a literature method

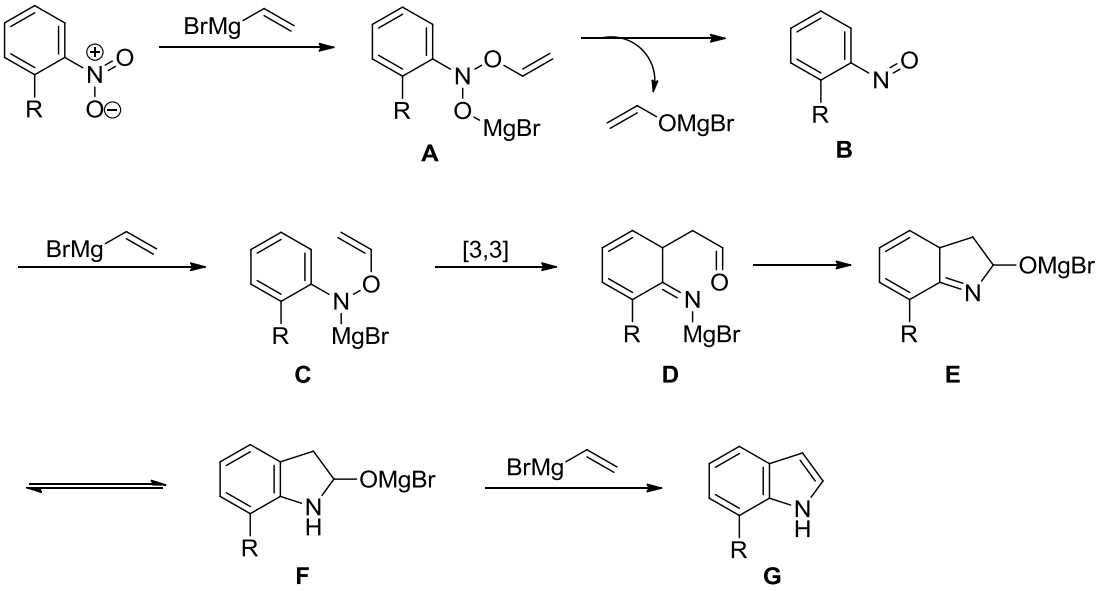
for bromination of 4-hydroxy-3-nitropyridine.[143] The reason for the lack of success in brominating 4-methoxy-3-nitropyridine (**351**) could be the directionality of the bulky methoxide substituent influenced by the 3-nitro- substituent impeding the introduction of a large bromine atom. In 4-methoxypyridine, the methoxide substituent won't prevent bromination since there is no substituent in the 3-position of the ring. In 4-hydroxy-3-nitropyridine, bromination is able to occur since the 4-hydroxy- group is less sterically hindered than a 4-methoxy substituent.



Scheme 58. *Reagents and conditions*: (i) Na, MeOH, dioxane, reflux, 2.5 h; (ii) NBS, MeCN, MW, 100 ^{o}C, 1 h or Br_2, H_2O, 50 ^{o}C, 2 h; ((iii) vinylmagnesium bromide, THF, -78 ^{o}C, 5 h; (iv) appropriate boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane:water (9:1), 110 ^{o}C, 18 h; (v) appropriate benzoyl chloride, $AlCl_3$, DCM, RT, 18 h; (vi) TMSI, DCM, RT, 3 h.

A slightly modified synthetic route was employed starting from 4-hydroxy-3-nitropyridine (**354**) (Scheme 60). Bromination according to a literature procedure using bromine in water afforded **355** in 90% yield.[143] Reaction of **355** with $POCl_3$ under microwave irradiation at 140 ^{o}C for 15 min produced **356** in 89% yield. Substitution of the 4-chloro- substituent of **356** using sodium methoxide provided **352** in 86% yield. Although starting from 4-hydroxy-3-nitropyridine and progressing through 3 steps to obtain 3-bromo-4-methoxy-5-nitropyridine (**352**) added an extra step into the synthesis of pyrrolopyridone targets, the steps were high yielding and proceeded without the need for purification. The next step of the synthesis utilised a Bartoli-type cyclisation to form azaindole **353** from reaction of **352** with vinyl magnesium bromide. The Bartoli cyclisation was originally reported for the synthesis of indoles from nitrobenzene derivatives. It was reported that reaction of a variety of 2-substituted nitrobenzenes

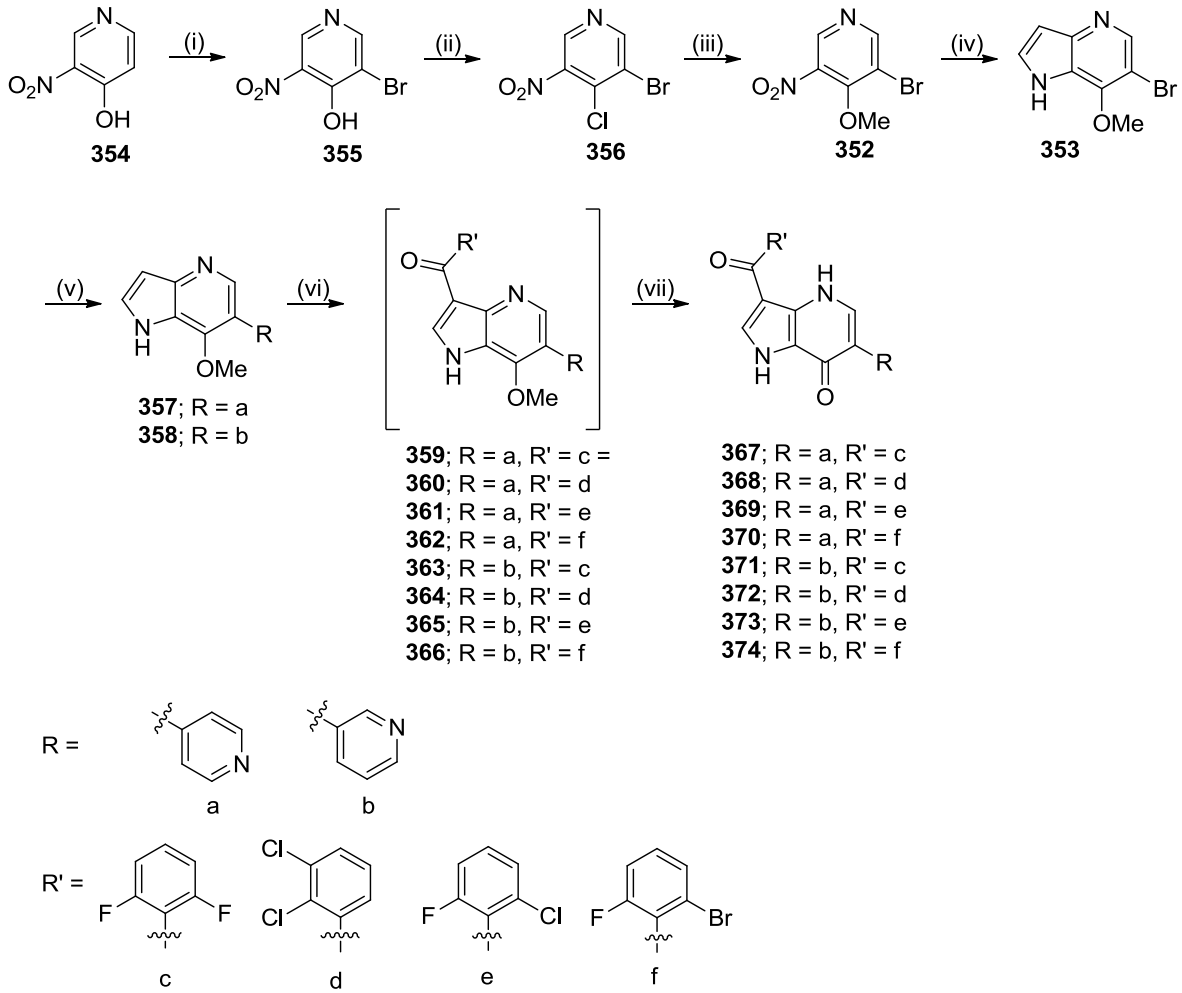
with 3 equivalents of vinylmagnesium bromide afforded the corresponding 7-substituted indoles in good yield.[144] The postulated mechanism of the reaction is shown in Scheme 59. In the first step of the reaction, the nitro group of the nitroarene is attacked by the Grignard reagent at one of the oxygen atoms to produce intermediate **A**. Elimination of the magnesium enolate affords the nitrosoarene **B**, which undergoes an inverse 1,2-addition by a second equivalent of Grignard reagent. The resulting *N*-aryl-*O*-vinyl hydroxylamino magnesium salt **C** is then set up for a [3,3]-sigmatropic rearrangement leading to intermediate **D**. Ring closure of **D** leads to intermediate **E**, which tautomerises to **F**. The third and final equivalent of the Grignard reagent acts as a base to afford the final indole product **G**.[145-146] Following on from the success of the Bartoli indole synthesis, it was postulated that the reaction could be utilised to synthesise azaindoles using the appropriate nitropyridine starting material. It has been demonstrated that this approach is successful in producing a range of 4- and 6-azaindoles in modest yield, and interestingky, larger substituents adjacent to the nitro-group improved reaction yield.[147]



Scheme 59. Postulated mechanism of Bartoli indole synthesis.[145-146]

The Bartoli approach was applied to 3-bromo-4-methoxy-5-nitropyridine (**352**) which was reacted with vinyl magnesium bromide to afford azaindole **353**. Initial attempts followed the literature method, which involved addition of an excess of vinyl magnesium bromide at -78 ^{o}C and then warming to -20 ^{o}C for 8 h.[147] The desired azaindole **353** was produced in 15% yield, which was comparable to the closest analogue in the literature (4-methoxy-5-nitropyridine, which gave the corresponding

azaindole in 11% yield).[147] Attempts to improve the reaction yield by slow addition of vinyl magnesium bromide over 3 h with the use of a syringe pump were not successful. However, performing the reaction at -78 ^{o}C over 5 h without warming to -20 ^{o}C, increased the reaction yield to 40%. Reaction of azaindole **353** with either 4-pyridyl- or 3-pyridyl- boronic acid under Suzuki-Miyaura conditions afforded either **357** or **358** in 75% yield each. Friedel-Crafts acylation of intermediates **357** and **358** was attempted with the four desired benzoyl chlorides using 2.5 equivalents of aluminium trichloride. This was unsuccessful, and it was found that similar reactions had been performed in the literature, but required a larger number of equivalents of aluminium trichloride. At least 3-5 equivalents were required since the aluminium could chelate to the two nitrogen atoms in the azaindole ring and would not be available for the Friedel-Crafts reaction.[148] Repeating the reactiosn with 5.5 equivalents of aluminium trichloride and 5 equivalents of benzoyl chloride was successful in producing the required products (**359-366**). Purification of compounds **359-366** was difficult since the methoxy group was partly demethylated during the Friedel-Crafts reaction, and the two products were difficult to separate *via* chromatography. In light of this, it was decided not to isolate intermediates **359-366** and to perform the subsequent demethylation reaction with trimethylsilyl iodide to give the final products **367-374**. Again, purification problems were encountered due to the presence of the methoxy intermediates remaining after the demethylation reaction. As such, the final products were purified by semi-preparative HPLC. Overall, compounds **367-374** were produced in low yields (21-30%) over two steps.

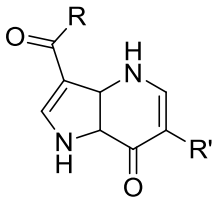


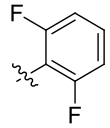
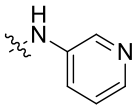
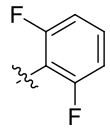
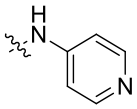
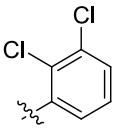
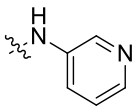
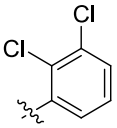
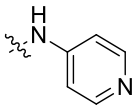
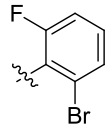
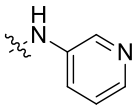
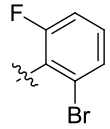
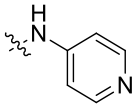
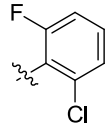
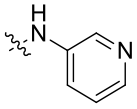
Scheme 60. *Reagents and conditions*: (i) Br_2, H_2O, 50 ^oC, 2 h; (ii) $POCl_3$, *N*,*N*-diethylaniline, MW, 140 ^oC, 15 min; (iii) Na, MeOH, RT, 2.5 h; (iv) vinylmagnesium bromide, THF, -78 ^oC, 5 h; (v) appropriate boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, dioxane:water (9:1), 110 ^oC, 18 h; (vi) appropriate benzoyl chloride, $AlCl_3$, DCM, RT, 18 h; (vii) TMSI, DCM, RT, 3 h.

6.3.3 ERK5 Inhibitory activity of 1H-pyrrolo[3,2-b]pyridin-7(4H)-one inhibitors

Compounds **367-374** were sent for biological evaluation, and the results are shown in Table 39.

Table 39. Biological Results for **367-374**.



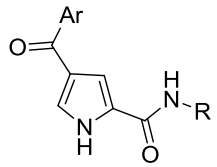
Compound	R	R'	ERK5; IC_{50} (μM)	p38α; IC_{50} (μM)
371			38 ± 4.0 (n = 2)	14 ± 0.79 (n = 2)
367			>120	6.7 ± 0.67 (n = 2)
372			46 ± 5.8 (n = 2)	42 ± 1.1 (n = 2)
368			7.4 ± 3.4 (n = 2)	ND
374			11 ± 1.2 (n = 2)	5.7 ± 0.16 (n = 2)
370			6.2 ± 0.05 (n = 2)	4.8 ± 0.94 (n = 2)
373			>120	ND

Disappointingly, ERK5 inhibition was reduced compared to inhibitors in the pyrrole carboxamide series. In addition, selectivity over p38α was diminished. It had been postulated that the incorporation of a bicyclic core may increase rigidity and lock the 2 carbonyl groups into favourable conformations for binding, but the lack of potency and selectivity exhibited for ERK5 suggests that this is not the case. The lack of activity and loss of selectivity observed for these compounds meant further investigation into bicyclic analogues was ceased. The final analogue, **369** was produced in too small quantity to be purified by semi-preparative HPLC and was therefore not sent for biological evaluation.

Chapter 7. Conclusions and future work

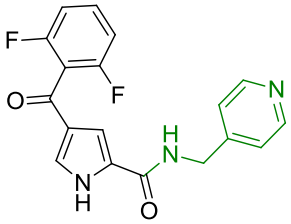
7.1 Conclusions

SAR studies around the pyrrole carboxamide hit scaffold (**A**) were undertaken in order to improve ERK5 inhibitory activity. Homology modelling based on both the ERK2 and p38α crystal structures was used to guide structure-based design in the absence of a crystal structure of ERK5.



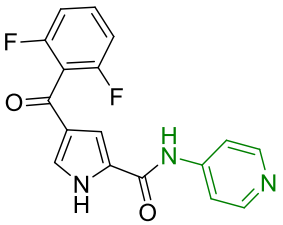
A

Modification of the amide moiety led to the discovery that removal of a methylene group from the side chain resulted in an almost 3-fold improvement in ERK5 inhibitory activity compared to the initial hit compound (Figure 52). Shortening of the amide side chain was also shown to improve selectivity for ERK5 over closely related MAPK p38α.



53

ERK5; IC_{50} = 2.4 ± 0.09 μM
p38α; IC_{50} = 0.58 ± 0.27 μM



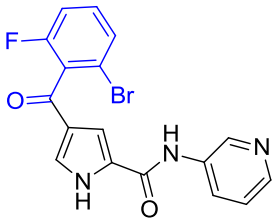
127

ERK5; IC_{50} = 0.90 ± 0.15 μM
p38α; IC_{50} = 100 ± 35 μM

Figure 53. Increase in potency and selectivity observed for **127** compared to **53.**

Modification of the aroyl group also led to an improvement in selectivity for ERK5 over p38α. Introduction of a different halogen atom (i.e. chloro or bromo) into the 2-position of the aromatic ring conferred good potency and retained selectivity for ERK5 over p38α. Compound **151** was selected for *in vitro* and *in vivo* pharmacokinectic (PK) studies with promising results [*in vitro* PK; solubility >100 μM, PPB = 94% and *in vivo* PK; oral bioavailability = 68% and $t_{1/2}$ = 65 min (p.o. mice)]. *In vivo* efficacy studies

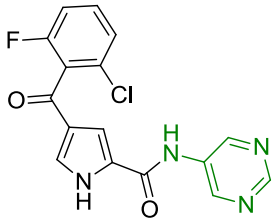
were also encouraging, suggesting that compound **151** may have potential as an anti-angiogenic and anti-proliferative agent.



151

ERK5; IC_{50} = 0.82 ± 0.07 μM
p38α; IC_{50} >120 μM

Despite the promising *in vitro* and *in vivo* PK results obtained for compound **151**, inhibition of CYP450 enzymes was identified when **151** was tested against a panel of five isoforms. Replacement of the pyridyl moiety was investigated in order to improve CYP450 inhibition profiles. Synthesis of a series of compounds with various substituted pyridyl, pyrimidyl or pyridazyl rings was conducted. The SARs revealed compounds with a pyrimidyl moiety to be potent inhibitors of ERK5 (IC_{50} <5 μM) with excellent CYP450 inhibition profiles when tested against a panel of CYP_{450} isoforms, exemplified by compound **182**.

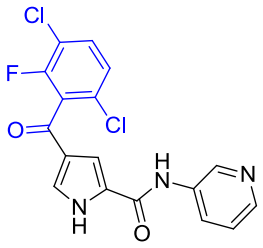


182

ERK5; IC_{50} = 0.36 ± 0.10 μM
p38α; IC_{50} >120 μM

CYP1A, CYP2C9, CYP2C19, CYP2D6; IC_{50} >20 μM

More recent modifications to the aroyl substituent found that introduction of a 2,3,6-tri-substituted aromatic ring led to a 12-fold increase in potency against ERK5. Modifications were made based upon the identification of a lipophilic pocket in the second-generation homology model (based on p38α). Interestingly, compound **295** was identified as the first inhibitor with a sub-100 nM ERK5 inhibitory activity.



295

ERK5; IC_{50} = 0.07 ± 0.03 μM
p38α; IC_{50} >120 μM

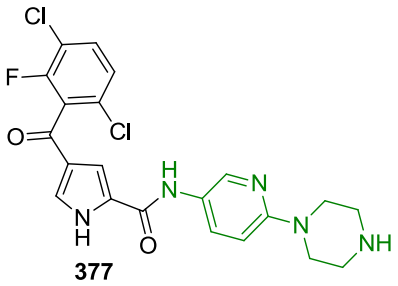
Attempts to access a lipophilic pocket in the ERK5 binding site *via* substitution at the 3-position of the core pyrrole ring were not successful. The ERK5 inhibitory activity of all compounds containing a substituent at the 3-position of the pyrrole ring was reduced (all IC_{50} >30 μM) and selectivity for ERK5 over p38α diminished.

The replacement of the pyrrole by a pyrrolopyridone bicyclic core was attempted to increase the rigidity and reduce entropy of the ERK5 inhibitors. Unfortunately, although the bicyclic inhibitors were synthetically challenging, they were not active against ERK5 (all IC_{50} >5 μM) and showed complete loss of selectivity for ERK5 over p38α.

The pyrrole carboxamide series has progressed from the hit-to-lead to the lead optimisation phase of drug development with a significant 30-fold improvement in ERK5 potency. The CYP450 inhibition profile has also been improved along with the selectivity of inhibitors for ERK5 over p38α. Further studies are currently being undertaken in order to find an inhibitor suitable for pre-clinical evaluation.

7.2 Future work

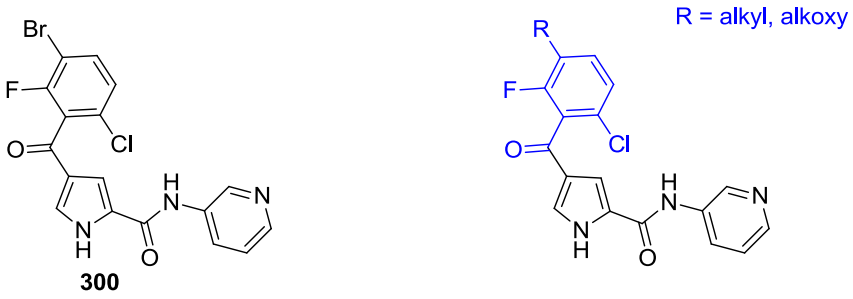
In order to identify a compound suitable for pre-clinical evaluation, studies are ongoing to further improve ERK5 potency and ADME properties. Compound **295** was shown to be an extremely interesting lead compound (ERK5; IC_{50} = 0.07 μM), but suffered from low aqueous solubility. In order to address this problem, future work will investigate the incorporation of water-solubilising groups (e.g. piperidine) onto the amide side-chain of inhibitors containing the 2,4,6-trisubstituted aroyl motif (Figure 53).



377

Figure 53. Introduction of water-solubilising groups onto the amide side-chain of **295**.

Introduction of a 3-bromo substituent onto the aroyl ring was tolerated (**300**, ERK5; IC_{50} = 0.07 μM). Further investigation into the incorporation of larger substituents in the position will be carried out. The bromo substituent can be utilised for the synthesis of further analogues *via* palladium-mediated coupling reactions (Figure 54). It may also be possible to introduce more polar, water-solubilising groups into this position using the bromo- substituent as a precursor.



300

Figure 54. Introduction of substituents into the 3-position of the aroyl ring using the 3-bromo substituent.

A p38α-derived homology model identified a lipophilic pocket in the ERK5 ATP binding site. However, attempts to access this pocket *via* substitution at the 3-position of the core pyrrole ring were unsuccessful. It may be possible to access the identified pocket *via* substitution of the amide NH or substitution of the *ortho* position of the pyridyl ring in the amide side-chain (Figure 55). Inhibitors will be synthesised in order to investigate this hypothesis.

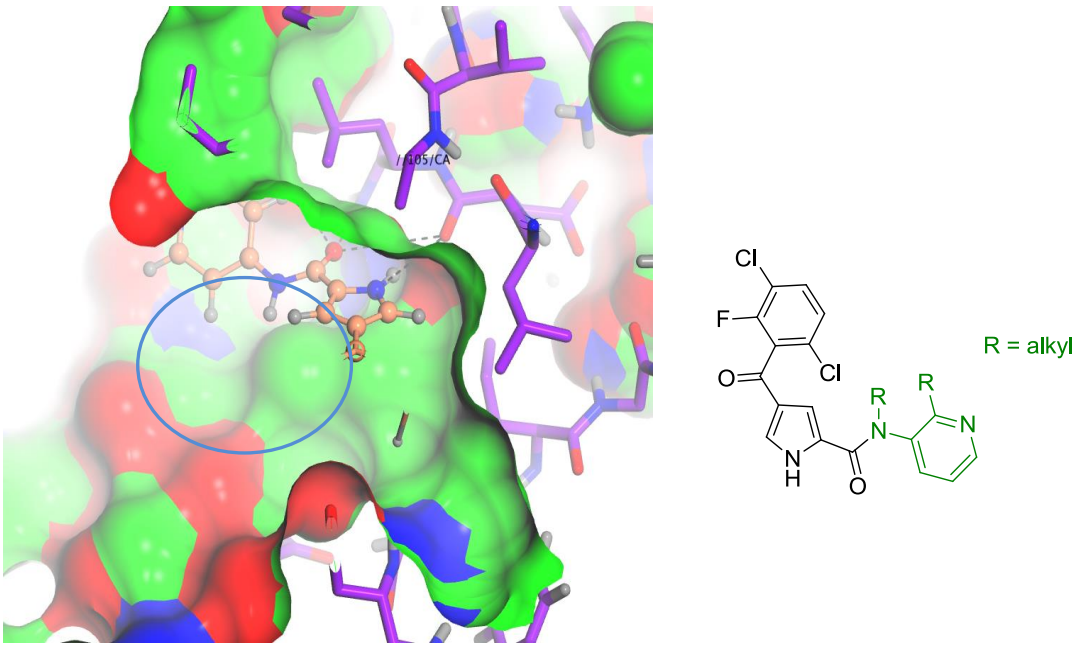


Figure 55. p38α-derived homology model highlighting the presence of a lipophilic pocket (in blue) and potential inhibitors to be synthesised to exploit this region of the ERK5 binding site.

Chapter 8. Experimental

8.1 Safety

All procedures were conducted in line with the school of Chemistry safety policy. COSHH risk assessments were completed prior to beginning any experiment.

8.2 Solvents and Reagents

Chemicals were purchased from Sigma-Aldrich, Alfa Aesar and Apollo Scientific unless otherwise stated. SureSeal™ or Acroseal™ bottles of anhydrous solvents were purchased from Sigma-Aldrich or Acros, respectively. Deuterated solvents used for the determination of NMR spectra were purchased from Sigma-Aldrich. Unless otherwise stated, reactions were carried out under an inert atmosphere of nitrogen.

8.3 Column chromatography

Purification using column chromatography was achieved using a Biotage automated flash purification system with UV monitoring at 298 nm and collection at 254 nm. Biotage automated chromatography pre-packed silica cartridges were used in most cases. Where stated, the purification of some compounds was performed using Biotage C18 reversed phase silica columns, which have octadecyl (end-capped) functionalised silica or Biotage KP-NH cartridges were used for the separation of highly polar compounds.

Where necessary semi-preparative HPLC was carried out using one of the following machines: (i) Varian Prostar Modular HPLC system with a binary pumping system, UV detector and fraction collector, controlled by Varian Star software, or (ii) Agilent 1200 HPLC system with a binary pump, autosampler, fraction collector and diode array detector, controlled by Agilent ChemStation software.

8.4 Microwave assisted synthesis

Where stated, reactions were carried out under microwave irradiation in sealed microwave vials with the use of a Biotage Initiator Sixty with a robotic sample bed. Reactions were irradiated at 2.45 GHz, and were able to reach temperatures between 60 and 250 ^{o}C. Heating was at a rate of 2-5 ^{o}C/s and the pressure was able to reach 20 bar.

8.5 Analytical Techniques

Melting points were measured using a Stuart Scientific SMP3 apparatus. ^{1}H NMR spectra were obtained using a Bruker Avance III 500 spectrometer using a frequency of 500 MHz. ^{13}C and ^{19}F NMR spectra were acquired using the Bruker Avance III 500 spectrometer operating at a frequency of 125 MHz, and 470 MHz, respectively. The abbreviations for spin multiplicity are as follows: s = singlet; d = doublet; t = triplet; q = quartet, quin = quintet, sept = septet and m = multiplet. Combinations of these abbreviations are employed to describe more complicated splitting patterns (e.g. dd = doublet of doublets) and where broadening of the peak is observed, spin multiplicity is accompanied by the prefix br = broad.

LC-MS analyses were conducted using a Waters Acquity UPLC system with PDA and ELSD. When a 2 min gradient was used, the sample was eluted on Acquity UPLC BEH C18, 1.7μm, 2.1 x 50mm, with a flow rate of 0.6 mL/min using 5-95% 0.1% formic acid/MeCN. Analytical purity of compounds was determined using Waters XTerra RP18, 5 μm (4.6 × 150 mm) column at 1 mL/min using either 0.1% aqueous ammonia and acetonitrile or 0.1% aqueous formic acid and acetonitrile with a gradient of 5-100% over 15 min.

FTIR spectra were measured using a Bio-Rad FTS 3000MX diamond ATR apparatus. UV spectra were recorded on a Hitachi U-2800A spectrophotometer and were performed in ethanol. HRMS were provided by the ESPRC National Mass Spectrometry Service, University of Wales, Swansea.

8.6 Biological assays

8.5.1 ERK5 IMAP^{TM} assay (This assay was performed by Ai Ching Wong at CRT-DL)

The assay buffer was prepared using 0.01% Tween®-20 5x stock, supplied as part of IMAP™ FP Progressive Binding System Kit (Molecular Devices R7436) and diluted to 1x using miliQ H_2O. 1 µL of a 1M DTT stock was added for every 1 mL of 1x assay buffer to give a final concentration of 1 mM DTT.

ERK5 was expressed and purified at CRT-DL by Leon Pang and Sue Young. Aliquots were stored at –80 °C. The ERK5 working solution was used at a 1 in 1 in 350 final dilution in assay buffer. A 1:175 dilution of ERK5 stock solution was performed in 1x assay buffer. For 1 plate, 13 µL of ERK5 stock was added to 2262 µL of 1x assay buffer.

To prepare the ATP/substrate working solution for one plate, ATP disodium salt (90 µL, 20 mM) (Sigma A7699) and FAM-EGFR-derived peptide (15 µL, 100 µM) (LVEPLTPSGEAPNQ(K-5FAM)-COOH) (Molecular Devices RP7129; reconstituted in miliQ H_2O to a stock concentration of 100 µL; stored at -20°C) was added to 2295 µL of 1x assay buffer.

To prepare the IMAP™ binding solution for one plate 20.5 µL of IMAP™ binding reagent stock, 1476 µL of 1x binding buffer A (60%), and 984 µL of binding buffer B (40%) (IMAP™ FP Progressive screening express kit (Molecular Devices R8127) was added to 9819.5 µL of milliQ H_2O.

1 µL of inhibitor (in 60:40 H_2O/DMSO) or control/blanks (60:40 H_2O/DMSO) were dry-spotted into the relevant wells of a 384-well assay plate using the MATRIX PlateMate® Plus. 5 µL of ERK5 working solution was added to test and control wells, and 5 µL of 1x assay buffer added to blanks; 4 µL of ATP/substrate working solution was added to all wells using a Matrix multichannel pipette. The plate was sealed using DMSO resistant clear seal and incubated for 2 h at 37 °C. 1 µL of the kinase reaction mixture from the first plate was dry spotted into a second 384-well assay plate using the MATRIX PlateMate® Plus. 9 µL of assay buffer was added, followed by 30 µL of

IMAP™ binding solution using a multichannel pipette. The plate was incubated at RT in darkness for 2 h. The assay plate was then read on an Analyst HT plate reader (Molecular Devices) using the settings described below:
Measurement mode = Fluorescence polarisation; Method ID = ERK5; Integration time = 100 ms; Excitation filter = Fluorescein 485-20; Emission filter = 530-25; Dichroic mirror = 505 nm; Plate definition file = Corning 384 black fb; Z-height = 5.715 mm (middle); G-factor = 1; Attenuator = out; Detector counting = Smartread+; Sensitivity = 2.

8.5.2 p38α LANCE assay (This assay was performed by Ai Ching Wong at CRT-DL)

1x assay buffer was prepared consisting of the following reagents; 250 mM tris(hydroxymethyl)aminomethane (Tris) pH 7.5, 25 mM $MgCl_2$, 2.5 mM ethylene glycol tetraacetic acid (EGTA), 10 mM DTT and 0.05% Triton X100 in milliQ H_2O (NB: 1x buffer final assay concentrations were 5x lower than stated above).

The p38α/SAPK2 working solution was prepared using active N-terminal GST-tagged recombinant full length protein (Millipore 14-251) supplied as a 10 µg/4 µL stock. This was diluted to a 10 µg/40 µL (1 µM) concentration by addition of 156 µL of Tris/HCl (pH 7.5, 50 mM), NaCl (150 mM), EGTA (0.1 mM), Brij-35 surfactant (0.03%), glycerol (50%) and 0.1% 2-mercaptoethanol (0.1%). The final dilution was dependent on activity of the enzyme batches. The p38α concentration used in the assay was 1 nM. A 2x working stock solution (2 nM, 500 fold dilution of 1 µM stock) in 1x assay buffer was prepared. For one plate, 9.4 µL of p38α (1 µM) was added to 1870.6 µL of milliQ H_2O.

The ATP/substrate working solution for one plate used ATP disodium salt (17.5 µL, 200 mM stock), (Sigma A7699) and U*light*-MBP Peptide (50 µL, 5 µM stock) (Perkin Elmer TRF0109), which were added to 400 µL of 5x assay buffer and 1532.5 µL of milliQ H_2O.

The EDTA/antibosy detection reagrent for one plate consisted of; 84 µL of ethylenediaminetetraacetic acid (EDTA) (0.5 M) (Sigma E4378-100G) and 27 µL of

Europium-anti-phospho-MBP antibody (0.625 µM) (Perkin Elmer) added to 420 µL of LANCE detection buffer (1x) and 3669 of milliQ H_2O.

1 µL of compound (in 80:20 H_2O/DMSO) or control/blank (80:20 H_2O/DMSO) was dry-spotted into the relevant wells of a 384-well assay plate using the MATRIX PlateMate® Plus. 5 µL of p38α working solution was added to test and control wells, and 5 µL of assay buffer added to blanks; 4 µL of ATP/substrate working solution was added to all wells using a Thermo Multidrop Combi or Matrix multichannel pipette. The plate was sealed using DMSO resistant clear seal and incubated for 1 h at 37 °C. 10 µL of the EDTA/antibody working solution was added to all wells using a Thermo Multidrop Combi or Matrix multichannel pipette. The plate was incubated at RT in darkness for 2 h. The assay plate was then read on a PheraStar microplate reader using the settings described below:
Pherastar: Measurement mode = TRF; Method ID = LANCE HTRF ERK5; Optic Module: 337, 665, 620 nm. Focal Height = 6.0, Positioning delay, 0.1 sec, Number of flashes per well = 100, Integration start = 60 µs, Integration time = 200 µs, Simultaneous dual emission, Ratio multiplicator = 1000.

8.5.3 Dual-luciferase reporter assay (This assay was performed by Dr Pamela Lochhead at the Babraham Institute)

Agar plates for bacterial colony growth were prepared using 250 mL of lysogeny broth (LB) agar spiked with 250 µL of an antibacterial agent (kanomycin or ampicillin), and, using a sterile pipette, 25 mL of spiked LB agar was transferred into 12 petri dishes.
Transformed bacterial colonies were grown as follows: to a polypropylene tube was added 48 µL of *E. coli* in glycerol stock (prepared in-house at the Babraham Institute), and 2 µL of either the Gal4-Luciferase or MEK5D DNA plasmid. The resulting mixture was incubated at 0 ^oC for 15 min. Bacteria were subjected to stress by heating at 42 ^oC for 30 sec, then returning to 0 ^oC. 200 µL of LB agar was added at 0 ^oC, and the resulting mixture was incubated with shaking for 30 min to 1 h at 37 ^oC.

At 37 ^oC, 250 µL of the solution containing Gal4-Luciferase transformed bacteria was transferred onto one LB agar/kanomycin plate. 250 µL of the solution containing

MEK5D transformed bacteria was transferred onto one LB agar/ampicillin plate. Agar plates were incubated for 24 h at 37 ^{o}C for bacterial colony growth.

To a conical flask was added 100 mL LB agar, 100 µL of the relevant antibiotic, and a sample of the bacterial colonies from the relevant petri dish. The flasks were incubated for a further 24 h at 37 ^{o}C, with stirring (150-170 rpm).

For DNA purification, 2 x 50 mL portions of the incubated bacteria in agar obtained were transferred into 50 mL Falcon tubes and subjected to centrifugation at 6000 G for 15 min. DNA plasmids were isolated in aqueous media from the remaining bacterial pellet using a Qiagen Plasmid Plus Midi Kit, according to kit instructions.

In order to quantify the DNA, concentration of the purified aqueous DNA was determined using a NanoDrop 1000 spectrophotometer. DNA stock solutions were at 2419.4 ng/µL and 1965.6 ng/µL for Gal-4-Luciferase and MEK5D plasmid stocks respectively. All other DNA plasmid stocks used were prepared in house at the Babraham Institute by Dr Pamela Lochhead.

Cell Maintenance

HEK293 cells were grown as adherent colonies on tissue culture dishes in Dulbecco's Modified Eagle Medium (DMEM) tissue culture medium (Gibco® 41966) containing L-glutamine (2 mM, Gibco® 1499), penicillin/streptomycin (100 µg/mL, PAA P11-010), foetal bovine serum (FBS) (10%, PAA A15-151) as additives.

Penicillin and streptomycin are antibacterial agents which minimise the risk of bacterial infection occurring in cell culture. FBS is a serum supplement, which contains growth factors that promote eukaryotic cell growth. L-glutamine is a nutrient which supports the growth of cells which have high energy demands, such as cells which synthesise large amounts of proteins and/or nucleic acids or those which use glucose inefficiently.

Trypsinizing of Adherent Cell Colonies

Cells were regularly passaged in order to maintain optimum colony sizes on plates. In order to do this, and to harvest cells for use in the dual-luciferase reporter assay, adherent cells were lifted from tissue culture dishes. Cell media was removed *via* aspiration, and the cell monolayer was washed with phosphate buffered saline (PBS)

solution (10 mL), which was then aspirated. The cells were lifted from the plate through incubation at 37 ^{o}C for 5-10 min with 1 mL trypsin (0.5% trypsin-EDTA (1x), Gibco® 15400054).

PBS (10x): NaCl (4% w/v), KCl (0.1% w/v), $Na_2HPO_4.7H_2O$ (0.6% w/v), KH_2PO_4 (0.1% w/v), NaN_3 (0.01% w/v).

Reverse Transfection of HEK293 cells

Preparation of EGFP Control (C3) Working Solution (For 1 x 96 well plate)

To a 50 mL Falcon tube was added 250 µL Opti-MEM® growth media (reduced serum medium, Invitrogen 31985) and the following DNA plasmids: MEF2D-Gal4 (2.5 µL, 0.25 mg/mL stock), Gal4-Luc (2.5 µL, 1.25 mg/mL stock), Renilla Luciferase (2.5 µL, 0.1 mg/mL stock), EGFP construct, containing no MEK5D (0.25 µL, 0.5 mg/mL stock), and HA-ERK5 wt (2.5 µL, 0.5 mg/mL stock). To this mixture was then added 5 µL of Lipofectamine™ 2000 transfection reagent (Invitrogen 11668) and complexation allowed for 15-20 min.

Preparation of EGFP-MEK5D (5D) Working Solution (For 1 x 96 well plate)

To a 50 mL Falcon tube was added 1500 µL Opti-MEM® growth media (reduced serum medium, Invitrogen 31985), and the following DNA plasmids: MEF2D-Gal4 (15 µL, .25 mg/mL stock), Gal4-Luc (15 µL, 1.25 mg/mL stock), Renilla Luciferase (15 µL, 0.1 mg/mL stock), EGFP-MEK5D construct (1.5 µL, 0.5 mg/mL stock), HA-ERK5 wt (15 µL, 0.5 mg/mL stock). To this mixture was then added 30 µL of Lipofectamine™ 2000 transfection reagent (Invitrogen 11668) and complexation allowed for 15-20 min.

Preparation of HEK293 Cells for Reverse Transfection

Trypsinized HEK293 cells were re-suspended in 19 mL of fresh cell media and counted using haemocytometry. Cells were then diluted with fresh cell media to achieve a final cell concentration of 2 x 10^5 cells/mL. 1237.5 µL and 7425 µL of this cell media was added to the C3 and 5D working solutions respectively. 100 µL of the transfected cells were then aliquoted into the appropriate wells in a 96-well opaque sided tissue culture plate, and incubated at 37 ^{o}C for 6 h.

Addition of Compounds to HEK293 Cells

Each compound supplied was prepared as a 10 mM working solution in DMSO, from which the following stocks were prepared in DMSO: 3.33 mM, 1 mM, 0.11 mM and 10

µM. 4 compounds was assayed per 96-well plate. For each compound, 1 mL of growth media was added to eight 1.5 mL Eppendorf® tubes. 6 µL of DMSO was added to two tubes for C3 and blank 5D wells, and 6 µL of the relevant compound stock solution was added to the 6 remaining tubes. BIX02189 was added to each plate in varying concentrations as an indicator of assay reliability. Solutions of drug in media were thoroughly mixed, and 100 µL of the relevant compound was transferred (in triplicate) to the 96-well plate containing transfected HEK293 cells using an electronic multichannel pipette with variable tip spacing. Sealed plates were incubated at 37 ^{o}C for 24 h. Final compound concentrations were as follows; 30 µM, 10 µM, 3 µM, 1 µM and 0.3 µM.

Assay Plate Lysis

15 mL of 1x passive lysis buffer (PLB) was prepared using 5x PLB (Dual-Luciferase Reporter Assay System, Promega® E1960), diluting with H_2O. Assay plates prepared were removed from the incubator and the growth media removed by aspiration. 20 µL of 1x PLB was dispensed into each well and the plates shaken for 10 min. Assay plates were sealed and stored at -80 ^{o}C overnight.

Quantification of Cellular Inhibition of ERK5

Cellular inhibition of ERK5 was quantified using the Dual-Luciferase® Reporter Assay System (Promega® E1960). Luciferase assay buffer/Luciferase assay substrate and Stop and Glo® assay buffer/Stop and Glo® substrate were prepared according to kit instructions. 100 µL of the Luciferase system was added to each well using a multichannel pipette, before analysis using an EG&G Berthold Microlumat Plus luminometer. 100 µL of the Stop and Glo® system was subsequently added, and the plate was analysed once again by luminometer. Raw data was processed using Microsoft Excel, enabling generation of IC_{50} values for each compound. IC_{50} values obtained are based on the means of 3 experiments. Each data point is the mean of 3 values ± standard deviation to produce one curve using Microsoft Excel. IC_{50} values were determined by eye.

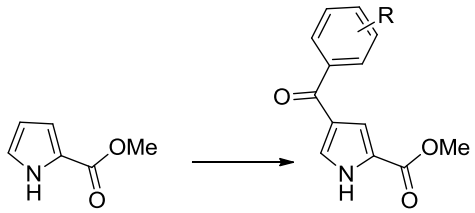
8.5.4 EGF-stimulated HeLa cell assay (This assay was performed by Lan-Zhen Wang at the NICR)

HeLa cells were serum starved overnight followed by treatment with ERK5 inhibitors for 1 h. Cells were then stimulated with 100 ng/ml EGF for 10 min. The cells were harvested and lysed at 4^{o}C for 5–10 min in Laemmli buffer containing Halt protease and phosphate inhibitors (Pierce). The lysates were boiled for 10 min at 100^{o}C. Twenty microliters sample was run on 6% Tris–glycine gels and transferred to nitrocellulose. Western blotting was done with ERK5 antibody (Cell signalling #3372S). The IC_{50} was calculated from densitometry of top bands.

8.7 General Procedures

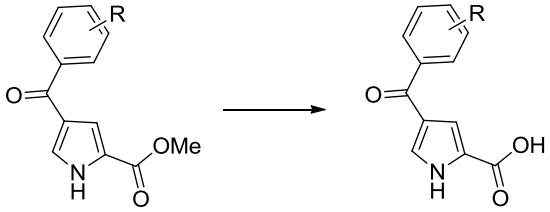
The following general procedures were employed for the syntheses of structurally similar compounds.

General Procedure A – Friedel Crafts acylation



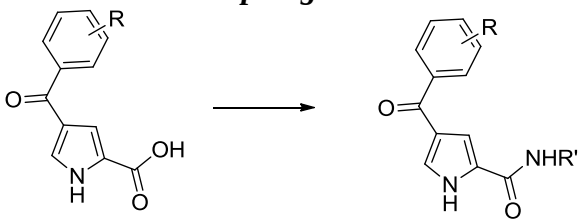
Aluminium chloride (2.5 equiv.) was added to DCM (2.5 mL/mmol pyrrole) at 0 ^{o}C, followed by the appropriate benzoyl chloride or acid anhydride (2.0 equiv.). Methyl 2-pyrrole carboxylate (1.0 equiv.) was then added, and the resulting mixture was stirred at 0 ^{o}C for 1 h before being warmed to RT and left to stir overnight. The mixture was quenched with a 1.0 M aqueous solution of HCl until effervescence ceased, and the resulting mixture was extracted using DCM (5 mL/mmol pyrrole). The combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$, followed by water and a saturated brine solution (5 mL/mmol pyrrole) respectively. The organic layer was then dried over Na_2SO_4, filtered, and the filtrate concentrated *in vacuo*. The crude product was purified by medium pressure flash chromatography (MPLC).

General Procedure B – Ester hydrolysis



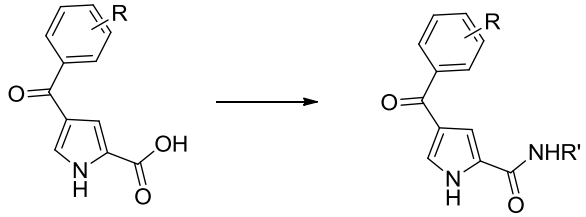
The pyrrole ester (1.0 equiv.) was dissolved in THF (8 mL/mmol pyrrole) and solution of LiOH (20 equiv.) in water (13.0 mL/mmol pyrrole) was added. The resulting mixture was heated at 60 ^{o}C overnight. The mixture was then cooled and acidified using a 1.0 M aqueous solution of HCl to pH 3-4, causing a precipitate. The solid was extracted into EtOAc (2 x 50 mL/mmol pyrrole), and the combined organic layers were washed with water followed by brine (50 mL/mmol pyrrole respectively) before being dried over Na_2SO_4. The filtrate was concentrated *in vacuo.*

General Procedure C - Amide Coupling with CDI



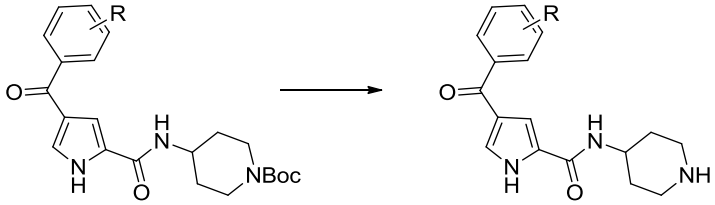
The pyrrole carboxylic acid (1.0 equiv.) was dissolved in THF (5 mL/mmol pyrrole) before carbonyldiimidazole (2.0 equiv.) was added. The reaction mixture was then heated at reflux for 3 h. After this time, the reaction mixture was cooled to 50 ^{o}C before the amine (2.5 equiv.) was added and the mixture heated for a further 3 h. The reaction mixture was then cooled to RT (20 ^{o}C) and left to stir overnight. The mixture was then diluted with EtOAc (50 mL/mmol pyrrole), and subsequently washed with water, and brine (50 mL/mmol pyrrole) respectively and extracted with EtOAc (3 x 30 mL/mmol pyrrole). The combined organic extracts were dried over Na_2SO_4 and the filtrate concentrated *in vacuo*. Purification was achieved using MPLC.

General Procedure D - Amide coupling with PCl_3



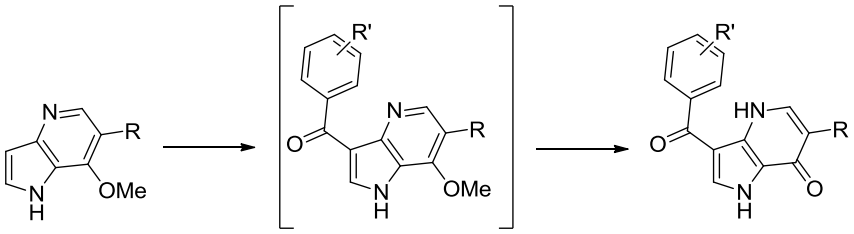
The carboxylic acid (1.0 equiv.) was dissolved in MeCN (5 mL/mmol pyrrole) before the relevant amine (2.5 equiv.) was added followed by phosphorus trichloride (1.0 equiv.). The mixture was then heated using microwave irradiation at 150 ^{o}C for 5 min. The reaction was then quenched with a few drops of H_2O and the solvent removed *in vacuo.* The residue was re-dissolved in EtOAc (50 mL/mmol pyrrole) and washed with a saturated aqueous solution of $NaHCO_3$ (50 mL/mmol pyrrole) before being extracted into EtOAc (3 x 30 mL/mmol pyrrole). The combined organic extracts were dried over Na_2SO_4, and the filtrate concentrated was *in vacuo* to afford the crude product, which was purified by MPLC.

General Procedure E – Boc deprotection



The Boc-protected piperidine (1.0 equiv.) was dissolved in DCM (6 mL/mmol pyrrole) before TFA (6 mL/mmol pyrrole) was added, followed by Et_3SiH (2.5 equiv.) The reaction mixture was then allowed to stir at RT for 1 h before the solvent was removed *in vacuo.* The residue was then dissolved in EtOAc (50 mL/mmol pyrrole) before being washed with saturated aqueous solution of $NaHCO_3$ (3 x 50 mL/mmol pyrrole) and extracted into EtOAc (3 x 30 mL/mmol pyrrole). The organic extracts were combined, dried over Na_2SO_4 and concentrated *in vacuo*.

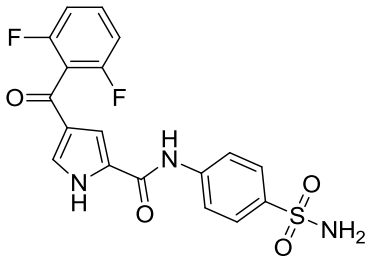
General Procedure F – Friedel-Crafts acylation of pyrrolopyridines followed by TMSI demethylation



The azaindole (1.0 equiv.) was dissolved in DCM (5 mL/mmol pyrrole) before the appropriate benzoyl chloride (5.0 equiv.) was added followed by $AlCl_3$ (5.5 equiv.) and the resulting mixture was then stirred at 0 ^oC for 1 h before being warmed to RT (20 ^oC) and left to stir overnight. The mixture was then quenched with MeOH until effervescence ceased, and then the solvent was removed *in vacuo*. The resulting residue was re-dissolved in EtOAc and purified by MPLC. After purification a mixture of methoxypyridine and pyridone was isolated and this was taken up in DCM (5 mL/mmol pyrrole) before TMSI was added (6.0 equiv.) and the mixture was then left to stir at RT (20 ^oC) for 3 h. MeOH was added and the solvent removed *in vacuo*. EtOAc (50 mL/mmol pyrrole) was added and the organic fraction was washed with water (2 x 50 mL/mmol pyrrole), dried over Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by semi-preparative HPLC.

8.8 Experimental data

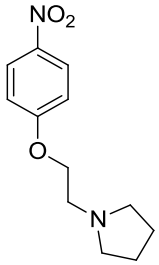
4-(2,6-Difluorobenzoyl)-*N*-(4-sulphamoylphenyl)-1*H*-pyrrole-2-carboxamide (61)



4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (100 mg, 0.40 mmol) was dissolved in acetonitrile (5 mL). Pyridine (32 µL, 0.40 mmol) was then added followed by cyanuric trifluoride (14 µL, 0.16 mmol). The mixture was allowed to stir for 30 min at RT before sulphanilamide (172 mg, 1.00 mmol) was added and the reaction mixture was left to stir at RT overnight. Brine was added and the product was extracted into EtOAc (2 x 10 mL). The combined organic extracts were dried over Na_2SO_4 and the

solvent removed *in vacuo*. Purification was achieved by MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a white solid (62 mg, 38%); R_f = 0.82 (10% MeOH in EtOAc); M.p.: 310-311 ^oC; λ_{max} (EtOH)/nm: 284, 233; ν_{max}/cm^{-1}: 3362, 3253, 3123, 2636, 2045, 1669 (CO), 1635 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.26-7.30 (4H, m, H-3', H5' and SO_2NH_2), 7.54-7.56 (2H, m, H-3 and H-5), 7.64 (1H, dddd, *J* = 6.8, 6.9, 8.4 and 8.5 Hz, H-4'), 7.80 (2H, d, *J* = 8.9 Hz, *p*-subs. H-Ar), 7.90 (2H, *J* = 8.9 Hz, *p*-subs. H-Ar), 10.34 (1H, s, CONH), 12.72 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.0 (C-Ar), 111.9 (C-Ar), 114.0 (C-Ar), 117.8 (C-Ar), 118.5 (C-Ar), 120.5 (C-3), 125.4 (C2 and C-5), 129.0 (C-4), 132.5 (C-Ar), 133.6 (C-Ar), 158.7 (J_{CF} = 245.5 Hz, CF), 164.1 (CONH), 185.6 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -115.4; LRMS (ES^+) *m/z* 406.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{14}F_2N_3O_4S$ $[M+H]^+$ 406.0658, found 406.0658.

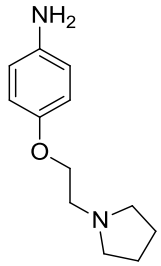
1-(2-(4-Nitrophenoxy)ethyl)pyrrolidine (68)



4-Nitrophenol (1.00 g, 7.19 mmol) was dissolved in DMF (20 mL). K_2CO_3 (2.78 g, 20.1 mmol) was then added and the resulting yellow mixture was stirred for 15 min at RT. 1-(2-Chloroethyl)pyrrolidine hydrochloride (2.20 g, 12.94 mmol) was added and the reaction was heated to 80 ^oC for 2 h, before the solvent was removed *in vacuo*. Water (100 mL) was added to the resulting residue, which was extracted with DCM (4 x 40 mL). The combined organic layers were washed with a saturated aqueous solution of $NaHCO_3$ (3 x 30 mL), dried over Na_2SO_4 and the solvent removed *in vacuo*. The title compound was obtained as a yellow oil (1.10 g, 65%); R_f = 0.10 (100% EtOAc); λ_{max} (EtOH)/nm 305, 221; ν_{max}/cm^{-1} 2951, 2787, 2449, 1921, 1599 (NO_2); ^1H NMR (500 MHz, DMSO-d_6) δ 1.45 (4H, pent., *J* = 3.1 Hz, (CH_2)$_2$-pyrrolidine), 2.25-2.29 (4H, m, N(CH_2)$_2$ -pyrrolidine), 2.58 (2H, t, *J* = 5.8 Hz, CH_2), 3.98 (2H, t, *J* = 5.8 Hz, CH_2), 6.92 (2H, d, *J* = 7.1 Hz, H-Ar), 7.96 (2H, d, *J* = 7.1 Hz, H-Ar); ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.2 (CH_2)$_2$-pyrrolidine), 54.0 N(CH_2)$_2$-pyrrolidine), 54.1 (NCH_2), 67.9

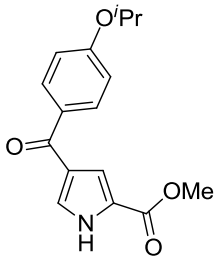
(CH_2O), 115.1 (C-Ar), 118.1 (C-Ar), 125.8 (C-Ar), 127.0 (C-Ar), 163.9 (C-Ar); LRMS (ES^+) *m/z* 237.1 $[M+H]^+$.

4-(2-(Pyrrolidin-1-yl)ethoxy)aniline (66)



1-(2-(4-Nitrophenoxy)ethyl)pyrrolidine (**68**) (400 mg, 1.69 mmol) was dissolved in AcOH (10 mL) before zinc powder (1.10 g, 16.9 mmol) was added and the reaction was left to stir at RT overnight. The mixture was then filtered through a pad of Celite, and the AcOH was removed *in vacuo* before water was added and the solution neutralised using ammonia. The product was extracted using EtOAc (3 x 20 mL) before the organic layers were combined, dried over Na_2SO_4 and the solvent removed *in vacuo* to give the title compound as a brown oil (320 mg, 92%); R_f = 0.23 (30% MeOH in DCM); λ_{max} (EtOH)/nm 294, 233; ν_{max}/cm^{-1} 3414 (NH_2), 3015, 2600, 2162, 1573; ^1H NMR (500 MHz, MeOD) δ 1.74-1.77 (4H, m, (C*H*$_2$)$_2$-pyrrolidine), 2.64-2.66 (4H, m, N(C*H*$_2$)$_2$-pyrrolidine), 2.82 (2H, t, *J* = 5.5 Hz, CH_2), 3.95 (2H, t, *J* = 5.5 Hz, CH_2), 6.60 (2H, d, *J* = 7.1 Hz, H-Ar), 6.65 (2H, d, *J* = 7.1 Hz, H-Ar); ^{13}C NMR (125 MHz, MeOD) δ 23.0 (*C*H_2)$_2$-pyrrolidine), 53.9 (N(*C*H_2)$_2$-pyrrolidine), 54.1 (NCH_2), 66.4 (CH_2O), 114.9 (C-Ar), 115.6 (C-Ar), 142.6 (C-Ar), 149.6 (C-Ar); LRMS (ES^+) *m/z* 207.2 $[M+H]^+$.

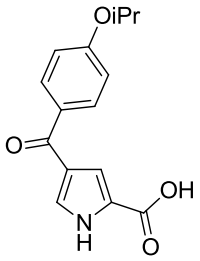
Methyl 4-(4-isopropoxybenzoyl)-1*H*-pyrrole-2-carboxylate (72)



4-Isopropoxybenzoic acid (500 mg, 2.8 mmol) was dissolved in THF (7 mL). $SOCl_2$ (0.61 mL, 8.4 mmol) was then added followed by DMF (20 µL, 0.28 mmol). The mixture was then stirred at RT for 3 h, before being concentrated *in vacuo*. The residue

was dissolved in DCM (4 mL) and the reaction was carried out according to general procedure A, using methyl pyrrole-2-carboxylate (**69**) (175 mg, 1.4 mmol), and $AlCl_3$ (467 mg, 3.5 mmol). The crude residue was purified by MPLC (silica, 2-100% EtOAc in petrol) followed by an additional $NaHCO_3$ wash to give the title compound as an orange solid (270 mg, 67%); R_f = 0.41 (10% EtOAc in petrol); M.p 112-113 ^oC; λ_{max} (EtOH)/nm 293, 229; ν_{max}/cm^{-1} 3293, 2979, 2560, 2159, 2017, 1720 (CO_2Me), 1599 (CO), 1552; ^1H NMR (500 MHz, DMSO-d_6) δ 1.10 (6H, d, *J* = 6.0 Hz, $CH(CH_3)_2$), 3.58 (3H, s, OCH_3), 4.53 (1H, sept., *J* = 6.0 Hz, $CH(CH_3)_2$), 6.82 (2H, d, *J* = 6.9 Hz, H-Ar), 6.92 (1H, dd, *J* = 1.6 and 3.2 Hz, H-3), 7.34 (1H, dd, *J* = 1.6 and 3.2 Hz, H-5), 7.57 (2H, d, *J* = 6.9 Hz, H-Ar), 12.45 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.7 ($CH(CH_3)_2$), 51.2 (CH_3), 69.5 ($CH(CH_3)_2$), 114.9 (C-3), 116.3 (C-Ar), 124.6 (C-2 and C-5), 130.6 (C-4), 130.9 (C-Ar), 131.3 (C-Ar), 160.4 (C-Ar), 161.3 (CO_2Me), 187.4 (CO); LRMS (ES^+) *m/z* 288.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{19}NO_4$ $[M+H]^+$ 288.1230, found 288.1235.

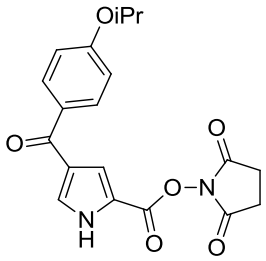
4-(4-Isopropoxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (73)



Compound **73** was synthesised according to general procedure B, using methyl 4-(4-isopropoxybenzoyl)-1*H*-pyrrole-2-carboxylate (**72**) (200 mg, 0.70 mmol), LiOH (335 mg, 14.00 mmol) in water (9 mL) and THF (8 mL) to give the title compound as a pale yellow solid (187 mg, 98%); R_f = 0.42 (5% MeOH in EtOAc); M.p. 185 ^oC (dec.); λ_{max} (EtOH)/nm 293, 254, 231; ν_{max}/cm^{-1} 3661, 3036, 2607, 2357, 2084, 1619 (CO_2H), 1598 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 1.37 (6H, d, *J* = 6.1 Hz, $CH(CH_3)_2$), 4.80 (1H, sept., *J* = 6.1 Hz, $CH(CH_3)_2$), 7.10 (2H, d, *J* = 8.9 Hz, H-Ar), 7.15 (1H, dd, *J* = 1.7 and 3.2 Hz, H-3), 7.56 (1H, dd, *J* = 1.7 and 3.2, H-5), 7.84 (2H, d, *J* = 8.9 Hz, H-Ar), 12.54 (1H, br s, OH), 12.84 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.7 ($CH(CH_3)_2$), 69.5 ($CH(CH_3)_2$), 115.0 (C-3), 115.5 (C-Ar), 124.5 (C-2 and C-5), 128.3 (C-4), 130.9 (C-Ar), 131.0 (C-Ar), 160.6 (C-Ar), 161.6 (C-Ar), 187.6 (CO_2H); LRMS

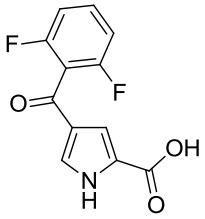
(ES$^+$) *m/z* 274.1 [M+H]$^+$; HRMS *m/z* calcd for $C_{15}H_{13}NO_4$ [M-H]$^-$ 272.0928, found 272.0932.

2,5-Dioxopyrrolidin-1-yl 4-(4-isopropoxybenzoyl)-1*H*-pyrrole-2-carboxylate (74)



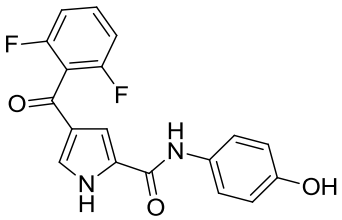
4-(4-Isopropoxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (**73**) (100 mg, 0.37 mmol) was dissolved in EtOAc (5 mL) at 0 ^{o}C. DCC (82 mg, 0.40 mmol) was then added followed by *N*-hydroxysuccinimide (46 mg, 0.40 mmol). The resulting yellow mixture was left to stir at 0 ^{o}C for 2 h before being warmed to RT and being left to stir for 18 h. The cream suspension was filtered through a pad of Celite and the filtrate concentrated *in vacuo* to give the title compound as an orange oil (135 mg, 99%); R_f = 0.47 (50% EtOAc in Petrol); λ_{max} (EtOH)/nm 277, 229; ν_{max}/cm^{-1} 3242, 2981, 2594, 1728 (CON), 1597 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.30 (6H, d, *J* = 6.1 Hz, CH(C*H*$_3$)$_2$), 2.82 (4H, s, 2 x CH_2), 4.59 (1 H, sept., *J* = 6.1 Hz, C*H*(CH_3)$_2$), 6.86 (2H, d, *J* = 8.9 Hz, H-Ar), 7.49 (1H, br s, H-3), 7.63 (1H, br s, H-5), 7.76 (2H, d, *J* = 8.9 Hz, H-Ar), 10.36 (1 H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.8 (CH(*C*H_3)$_2$), 25.6 (2 x CH_2), 70.2 (*C*H(CH_3)$_2$), 115.5 (C-3), 120.5 (C-Ar), 125.5 (C-2 and C-5), 128.8 (C-4), 131.6 (C-Ar), 155.8 (C-Ar), 161.6 (C-Ar), 169.6 (C-Ar), 172.2 (2 x CO), 188.6 (CON); LRMS (ES$^+$) *m/z* 371.1 [M+H]$^+$; HRMS *m/z* calcd for $C_{19}H_{19}N_2O_6$ [M+H]$^+$ 371.1243, found 371.1241.

4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (75)



Compound **75** was synthesised according to general procedure B, using methyl 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**76**) (450 mg, 1.70 mmol), LiOH (0.81 g, 34.00 mmol) in water (22 mL) and THF (14 mL) to give the title compound as a pale orange solid (424 mg, 99%); R_f = 0.41 (10% EtOAc in petrol); M.p. 197-198 ^oC; λ_{max} (EtOH)/nm 283, 232; ν_{max}/cm^{-1} 3772, 3024, 2779, 2612, 2539, 1705 (CO_2H), 1624 (CO) ; ^1H NMR (500 MHz, DMSO-d_6) δ 7.05 (1H, br s, H-3), 7.29-7.33 (2H, m, H-Ar), 7.57 (1H, dd, *J* = 1.4 and 3.0 Hz, H-5), 7.64-7.70 (1H, dddd, *J* = 6.7, 6.8, 8.4 and 8.5 Hz, H-Ar), 12.77 (1H, br s, NH), 12.97 (1H, br s, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ, 112.1 (C-Ar), 114.2 (C-3), 125.8 (C-2 and C-5), 129.8 (C-4), 132.3 (C-Ar), 158.8 (d, J_{CF} = 245.5 Hz, CF), 159.6 (CO_2H), 181.7 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.2; LRMS (ES^+) *m/z* 371.1 $[M+H]^+$; HRMS *m/z* calcd for $C_{12}H_6F_2NO_4$ $[M-H]^-$ 250.0321, found 250.0325.

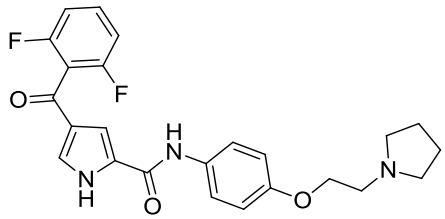
4-(2,6-Difluorobenzoyl)-*N*-(4-hydroxyphenyl)-1*H*-pyrrole-2-carboxamide (76)



Compound **76** was synthesised according to general procedure C, using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (100 mg, 0.40 mmol), carbonyldiimidazole (129 mg, 0.80 mmol), 4-aminophenol (109 mg, 1.0 mmol) and THF (4 mL). The crude residue was purified by MPLC (silica, 2-60% EtOAc in petrol) followed by recrystallisation from EtOAc to give the title compound as a beige solid (73 mg, 54%); R_f = 0.78 (5% MeOH in EtOAc); M.p 232-233 ^oC; λ_{max} (EtOH)/nm 245, 216; ν_{max}/cm^{-1} 3673, 3297 (OH), 2520, 2159, 2017, 1621 (CO), 1543 (CONH); ^1H NMR (500 MHz, MeOD) δ 6.60 (2 H, d, *J* = 8.9 Hz, NH-*H*-Ar), 6.94 -6.98 (2H, m, H-Ar) 7.23 (1H, br s, H-3), 7.25 (2 H, d, *J* = 8.9 Hz, NH-*H*-Ar), 7.29 (1H, br s, H-5), 7.39 (1H,

dddd, J = 6.4, 6.5, 8.5 and 8.6 Hz, H-4'); ^{13}C NMR (125 MHz, MeOD) δ 111.8 (C-Ar), 113.1 (C-Ar), 116.3 (C-Ar), 123.4 (C-3), 124.1 (C-2 and C-5), 127.9 (C-4), 130.2 (C-Ar), 131.2 (C-Ar), 133.1 (C-Ar), 155.7 (C-Ar), 160.7 (d, J_{CF} = 251.0 Hz, CF), 161.8 (CONH), 184.8 (CO); ^{19}F NMR (470 MHz, MeOD) δ -115.4; LRMS (ES^+) *m/z* 343.0 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{11}F_2N_2O_3$ $[M-H]^-$ 341.0743, found 341.0747.

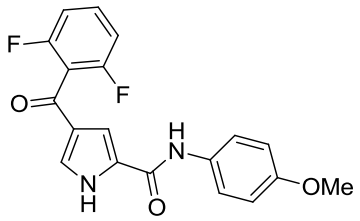
4-(2,6-Difluorobenzoyl)-*N*-(4-(2-(pyrrolidin-1-yl)ethoxy)phenyl)-1*H*-pyrrole-2-carboxamide (77)



4-(2,6-Difluorobenzoyl)-*N*-(4-hydroxyphenyl)-1*H*-pyrrole-2-carboxamide (**76**) (50 mg, 0.15 mmol) was dissolved in DMF (1 mL) before potassium carbonate (58 mg, 0.42 mmol) was added. The mixture was left to stir at RT for 15 min before 1-(2-chloroethyl)pyrrolidine.HCl (50 mg, 0.27 mmol) was added. The mixture was placed in the microwave at 100 ^{o}C for 30 min, before further 1-(2-chloroethyl)pyrrolidine.HCl (13 mg, 0.075 mmol) and potassium carbonate (10 mg, 0.075 mmol) were added and the reaction was placed back in the microwave for a further 15 min. The mixture was diluted with EtOAc (8 mL) and washed with water followed by brine (8 mL/mmol pyrrole, respectively) before the organic extract was dried over Na_2SO_4, and the filtrate concentrated *in vacuo*. The crude product was purified by MPLC (NH silica, 2-30% MeOH in EtOAc) to give the pure compound as a cream solid (23 mg, 35%); R_f = 0.57 (NH silica, 10% MeOH in EtOAc); M.p.: 146-147 ^{o}C; λ_{max} (EtOH)/nm: 274, 231; ν_{max}/cm^{-1}: 3353, 3326, 2354, 2075, 1766 (CO), 1632 (CONH); ^{1}H NMR (500 MHz, MeOD) δ 1.76-1.83 (4H, m, 2 x pyrrolidine CH_2), 2.73 (4H, br s, 2 x pyrrolide N-CH_2), 3.07 (2H, t, J = 6.6 Hz, CH_2), 4.51 (2H, t, J = 6.6 Hz, CH_2), 6.66 (2H, d, J = 8.9 Hz, *p*-subs. H-Ar), 6.99-7.04 (2H, m, H-3' and H-5'), 7.28 (1H, br s, H-3), 7.30 (2H, d, J = 8.9 Hz, *p*-subs. H-Ar), 7.45 (1H, dddd, J = 6.4, 6.5, 8.5 and 8.6 Hz, H-4'), 7.52 (1H, d, J = 1.45 Hz, H-5); ^{13}C NMR (125 MHz, MeOD) δ 24.2 (2 x pyrrolidine CH_2), 55.5 (2 x pyrrolide N-CH_2), 57.35 (CH_2), 113.0 (CH_2), 114.7 (C-Ar), 116.3 (C-Ar), 124.3 (C-3), 125.9 (C-5 and C-2), 129.6 (C-4), 131.2 (C-Ar), 133.3 (C-Ar), 135.5 (C-Ar), 155.8 (C-Ar), 159.8 (C-Ar), 159.9 (C-Ar), 161.3 (C-Ar), 161.9 (CONH), 184.1 (CO); ^{19}F NMR

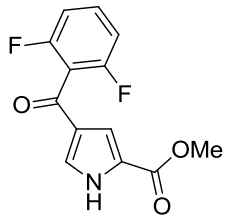
(470 MHz, MeOD) δ -115.1; LRMS (ES^+) *m/z* 440.19 $[M+H]^+$; HRMS *m/z* calcd for $C_{24}H_{25}F_2N_3O_3$ $[M+H]^+$ 440.1782, found 440.1783.

4-(2,6-Difluorobenzoyl)-*N*-(4-methoxyphenyl)-1*H*-pyrrole-2-carboxamide (78)



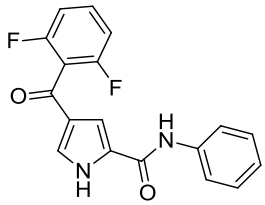
Compound **78** was synthesised according to general procedure C, using 4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (100 mg, 0.40 mmol), carbonyldiimidazole (129 mg, 0.80 mmol), *p*-anisidine (123 mg, 1.0 mmol) and THF (4 mL). The reaction mixture was diluted with EtOAc, acidified to pH 6 using a 1.0 M aqueous solution of HCl and the resulting mixture washed according to general procedure C. The crude residue was purified by MPLC (silica, 0-1% MeOH in EtOAc) followed by recrystallisation from EtOAc to give the title compound as a beige solid (73 mg, 54%); R_f = 0.88 (5% MeOH in EtOAc); M.p. 241-242 ^oC; λ_{max} (EtOH)/nm 285, 259, 233; ν_{max}/cm^{-1} 3352, 3193, 1997, 1621 (CO), 1504 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 3.75 (3 H, s, CH_3), 6.93 (2 H, d, *J* = 9.1 Hz, NH-*H*-Ar), 7.28 (2 H, t, *J* = 7.7 Hz, H-Ar), 7.48 (2 H, br s, H-3 and H-5), 7.59-7.67 (3 H, m, H-Ar + NH-*H*-Ar), 9.96 (1H, s, CONH), 12.61 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.2 (CH_3), 110.7 (C-Ar), 112.1 (C-Ar), 113.8(C-Ar), 117.7 (C-Ar), 118.0 (C-Ar) 121.7 (C-3), 125.8 (C2 and C-5), 128.7 (C-4), 131.8 (C-Ar), 132.1 (C-Ar), 157.7 (J_{CF} = 248.5 Hz, CF), 159.7 (CONH), 181.8 (CO); ^{19}F NMR (470 MHz, MeOD) δ -114.2; LRMS (ES^+) *m/z* 357.1 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{15}F_2N_2O_2$ $[M+H]^+$ 357.1043, found 357.1043.

Methyl 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylate (79)



Compound 7**9** was synthesised according to general procedure A, using methyl pyrrole-2-carboxylate (**69**) (400 mg, 3.2 mmol), DCM (7 mL), 2,6-difluorobenzoyl chloride (0.8 mL, 6.4 mmol) and $AlCl_3$ (1.07 g, 8.0 mmol). The crude compound was purified by MPLC (silica, 2-100% EtOAc in petrol) followed by a final wash with $NaHCO_3$ to remove remaining starting material. The title compound was obtained as an orange solid (558 mg, 66%); R_f = 0.46 (10% EtOAc in petrol); M.p. 131-132 ^oC; λ_{max} (EtOH)/nm 281, 232; ν_{max}/cm^{-1} 3335, 2919, 2563, 2224, 2021, 1733 (CO_2Me), 1628 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 3.81 (3H, s, CH_3), 7.04-7.06 (1H, m, H-3), 7.24-7.28 (2H, m, H-Ar), 7.59-7.65 (2H, m, H-5 and H-Ar), 12.92 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.7 (CH_3), 112.1 (C-Ar), 114.7 (C-Ar), 124.6 (C-3), 125.9 (C-2 and C-5), 130.3 (C-4), 132.3 (C-Ar), 159.5 (d, J_{CF} = 259.3 Hz, CF), 160.4 (CO_2Me), 181.6 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.2; LRMS (ES^+) *m/z* 266.0 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_{10}F_2NO_3$ $[M+H]^+$ 266.0623, found 266.0629.

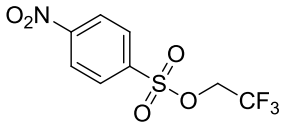
4-(2,6-Difluorobenzoyl)-*N*-phenyl-1*H*-pyrrole-2-carboxamide (80)



Compound **80** was synthesised according to general procedure C, using 4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (100 mg, 0.40 mmol), carbonyldiimidazole (129 mg, 0.80 mmol), aniline (91 µL, 1.0 mmol) and THF (4 mL). The reaction mixture was diluted with EtOAc, acidified to pH 3-4 using a 1.0 M aqueous solution of HCl and the resulting mixture washed according to general procedure C. The crude product was purified by MPLC (silica, 2-60% EtOAc in petrol) followed by recrystalllisation from EtOAc to give the title compound as a white solid (65 mg, 50%); R_f = 0.85 (5% MeOH in EtOAc); M.p. 272-273 ^oC; λ_{max} (EtOH)/nm 259;

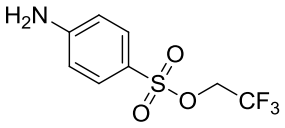
ν_{max}/cm^{-1} 3345, 3218, 2805, 2352, 1621 (CO), 1534 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.1 (1 H, t, *J* = 7.5 Hz, NH-*H*-Ar), 7.23-7.25 (2H, m, NH-*H*-Ar), 7.33-7.37 (2 H, m, NH-*H*-Ar), 7.27 (1 H, br s, H-3), 7.50 (1 H, br s, H-5), 7.63 (1H, dddd, *J* = 6.5, 6.8, 8.4 and 8.5 Hz, H-4'), 7.71 (2H, d, *J* = 8.4 Hz, H-Ar), 10.03 (1H, s, CONH), 12.62 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.1 (C-Ar), 112.3 (C-Ar), 117.8 (C-Ar), 120.1 (C-3), 125.8 (C-2 and C-5), 128.7 (C-4), 129.1 (C-Ar), 132.2 (C-Ar), 138.8 (C-Ar), 158.3 (d, J_{CF} = 250.1 Hz, CF), 159.6 (CONH), 181.8 (CO); ^{19}F NMR (470 MHz, MeOD) δ -114.2; LRMS (ES^{+}) *m/z* 327.0 [M+H]$^{+}$; HRMS *m/z* cald for $C_{18}H_{13}F_2N_2O_2$ [M+H]$^{+}$ 327.0940, found 327.0942.

2,2,2-Trifluoroethyl 4-nitrobenzenesulfonate (82)



DMAP (28 mg, 0.23 mmol) and NEt_3 (0.96 mL, 6.9 mmol) were dissolved in TFE (2 mL) at RT, before 4-nitrobenzene-1-sulfonyl chloride (0.50 g, 2.3 mmol) was added. The reaction was left to stir for 1 h before the solvent was removed *in vacuo*. The resulting residue was dissolved in DCM (10 mL) and washed with an aqueous solution of HCl (0.05 M, 10 mL) followed by water (10 mL). The organic extract was dried over Na_2SO_4 and the solvent removed *in vacuo* to give the pure product as a pale yellow solid (0.57 g, 87%); R_f = 0.70 (50% EtOAc in Petrol); M.p.: 88-89 ^{o}C (lit.[99] 80-81 ^{o}C) λ_{max} (EtOH)/nm: 248; ν_{max}/cm^{-1}: 3115, 1782, 1543 (NO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.43 (2H, q, *J* = 7.9 Hz CH_2), 8.08 (2H, d, *J* = 8.4 Hz, H-Ar), 8.37 (2H, d, *J* = 8.4 Hz, H-Ar), ^{1}H NMR (500 MHz, MeOD) δ 65.0 (OCH_2CF_3), 122.7 (CF_3), 129.4 (C-Ar), 140.8 (C-Ar), 151.2 (C-Ar); ^{19}F NMR (470 MHz, MeOD) δ -83.8; LRMS (ES^{+}) *m/z* 285.90 [M+H]$^{+}$.

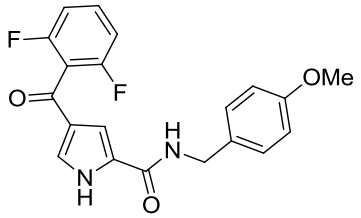
2,2,2-Trifluoroethyl 4-aminobenzenesulfonate (83)



2,2,2-Trifluoroethyl 4-nitrobenzenesulfonate (**82**) (200 mg, 0.70 mmol) was dissolved in a 4:1 MeOH:H_2O solution (5 mL) before sodium dithionite (486 mg, 2.8 mmol) was

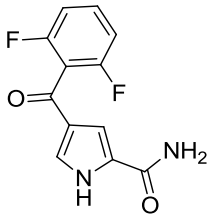
added and the mixture was placed in the microwave for 15 min at 100 ^{o}C. The solvent was removed *in vacuo* and the residue dissolved in EtOAc (20 mL) and washed with water (2 x 20 mL). The organic extract was dried over Na_2SO_4 and the solvent was removed *in vacuo* to give the title compound as a white solid (146 mg, 82%); R_f = 0.53 (50% EtOAc in Petrol); M.p.: 110-111 ^{o}C; λ_{max} (EtOH)/nm: 272; ν_{max}/cm^{-1}: 3484 (NH_2), 3379, 1625, 1598; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.23 (2H, q, *J* = 7.9 Hz CH_2), 4.27 (2H, br s, NH_2), 6.64 (2H, d, *J* = 8.9 Hz, H-Ar), 7.59 (2H, d, *J* = 8.9 Hz, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 64.4 (O*C*H_2CF_3), 114.1 (C-Ar), 122.0 (CF_3), 124.7 (C-Ar), 130.5 (C-Ar), 152.1 (C-Ar); ^{19}F NMR (470 MHz, MeOD) δ -73.80; LRMS (ES$^+$) *m/z* 256.1 [M+H]$^+$.

4-(2,6-Difluorobenzoyl)-*N*-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxamide (86)



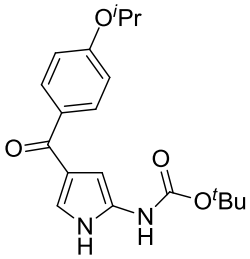
Compound **86** was synthesised according to general procedure C using, 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (100 mg, 0.40 mmol), carbonyldiimidazole (129 mg, 0.80 mmol), *p*-methoxybenzylamine (0.130 mL, 1.00 mmol) and THF (4 mL) to afford the crude product, which was purified by MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a pale yellow solid (121 mg, 82%); R_f = 0.88 (5% MeOH in EtOAc); M.p.: 179-180 ^{o}C; λ_{max} (EtOH)/nm: 283, 233; ν_{max}/cm^{-1}: 3372, 3154, 2280, 1610 (CO), 1577 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.59 (3H, s, CH_3), 4.22 (2H, d, *J* = 6.0 Hz, CH_2), 6.75 (2H, d, *J* = 8.7 Hz, *p*-subs. H-Ar), 7.07 – 7.12 (5H, m, H-5', H-3', H-3 and *p*-subs. H-Ar), 7.26 (1H, s, H-5), 7.47 (1H, dddd, *J* = 6.7, 6.8, 8.4 and 8.5 Hz, H-4'), 8.68 (1H, t, *J* = 6.0 Hz, CONH), 12.33 (1H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 41.0 (CH_2), 55.0 (OCH_3), 110.0 (C-Ar), 112.0 (C-Ar), 112.3 (C-Ar), 113.6 (C-Ar), 113.7 (C-Ar), 125.6 (C-3), 128.3, 128.6 (C-2 and C-5), 131.4 (C-4), 132.1 (C-Ar), 157.6 (C-Ar), 158.2 (C-Ar), 159.7 (CONH), 181.8 (CO) ; ^{19}F NMR (470 MHz, MeOD) δ -115.0; LRMS (ES$^+$) *m/z* 370.90 [M+H]$^+$ HRMS *m/z* calcd for $C_{20}H_{17}F_2N_2O_3$ [M+H]$^+$ 371.1204, found 371.1202.

4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxamide (87)



4-(2,6-Difluorobenzoyl)-*N*-(4-methoxybenzyl)-1*H*-pyrrole-2-carboxamide (**86**) (50 mg, 0.14 mmol) was dissolved in TFA (2 mL) and left to stir for 18 h at RT, before the solvent was removed *in vacuo*. The residue was dissolved in EtOAc (20 mL) and washed with $NaHCO_3$ (3 x 20 mL) before organic extract was dried over Na_2SO_4 and the solvent removed *in vacuo* to give the title compound as a cream solid (34 mg, 97%); R_f = 0.62 (5% MeOH in EtOAc); M.p.: 150-151 ^oC λ_{max} (EtOH)/nm: 284, 232; ν_{max}/ cm^{-1}: 3364, 3222, 2282, 1606 (CO), 1600 (CONH); ^1H NMR (500 MHz, MeOD) δ 6.99 – 7.01 (2H, m, H-3' and H-5'), 7.13 (1H, br s, H-3), 7.29 (1H, br s, H-5), 7.43 (1H, dddd, *J* = 6.5, 6.6, 8.6 and 8.7 Hz, H-4'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 109.9 (C-Ar), 110.4 (C-Ar), 113.6 (C-Ar), 120.0 (C-3), 127.9, 129.7 (C-2 and C-5), 130.2 (C-4), 131.0 (C-Ar), 155.4 (C-Ar), 160.0 ($CONH_2$), 185.0 (CO); ^{19}F NMR (470 MHz, MeOD) δ -112.3 LRMS (ES^+) *m/z* 251.10 $[M+H]^+$; HRMS *m/z* calcd for $C_{12}H_9F_2N_2O_2$ $[M+H]^+$ 251.0627, found 251.0629.

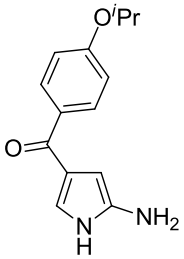
***tert*-Butyl (4-(4-isopropoxybenzoyl)-1*H*-pyrrol-2-yl)carbamate (89)**



4-(4-Isopropoxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (**73**) (137 mg, 0.50 mmol) was dissolved in ^{t}BuOH (10 mL) at 25 ^oC. NEt_3 (0.146 mL, 1.05 mmol) was added followed by the dropwise addition of diphenylphosphoryl azide (DPPA) (0.119 mL, 0.55 mmol). The reaction mixture was gently heated at 30 ^oC and left to stir overnight. The solvent was removed *in vacuo* to give the crude product, which was purified by MPLC (silica, 2-15% MeOH in EtOAc) to give the title compound as a brown oil (170 mg, 99%); R_f = 0.84 (10% MeOH in EtOAc); λ_{max} (EtOH)/nm 300, 230; ν_{max}/cm^{-1} 3332, 3158, 2975, 2167, 1741 ($CO_2{}^t$Bu), 1595 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 1.32 (6H, d, *J* =

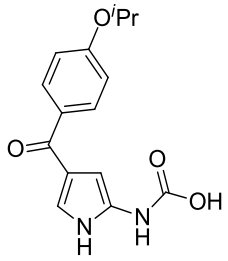
6.1 Hz, CH(CH_3)$_2$), 1.53 (9H, s, C(CH_3)$_3$), 4.76 (1H, sept., J = 6.1 Hz, CH(CH$_3$)$_2$), 7.23 (1H, br s, H-3), 7.35 (2H, d, J = 7.4 Hz, H-Ar), 7.74 (1H, br s, H-5), 7.79 (2H, d, J = 7.4 Hz, H-Ar), 12.99 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.7 (CH(*C*H$_3$)$_2$), 27.7 (C(*C*H$_3$)$_3$), 69.6 (*C*H(CH$_3$)$_2$), 80.6 (*C*(CH$_3$)$_3$), 115.0 (C-Ar), 115.1 (C-Ar), 118.0 (C-Ar), 119.9 (C-3), 128.3 (C-2 and C-5), 128.7 (C-4), 131.1 (C-Ar), 131.4 (C-Ar), 160.6 (CO$_2$^{t}Bu), 187.6 (CO); LRMS (ES$^+$) *m/z* 345.1 [M+H]$^+$.

(5-Amino-1*H*-pyrrol-3-yl)(4-isopropoxyphenyl)methanone (90)



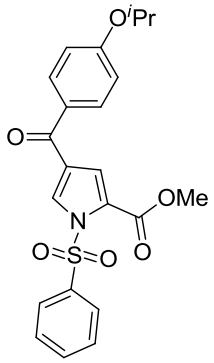
(4-(4-Isopropoxybenzoyl)-1*H*-pyrrol-2-yl)carbamic acid (**91**) (50 mg, 0.17 mmol) was dissolved in ethanol (5 mL) and heated to reflux for 30 min. The mixture was then concentrated *in vacuo* to give the title compound as a cream solid (37 mg, 90%); R_f = 0.43 (50% EtOAc in petrol); M.p. 98-99 ^{o}C; λ_{max} (EtOH)/nm 293, 228; ν_{max}/cm^{-1} 3519 (NH$_2$), 3293, 2979, 2367, 1721, 1599 (CO), 1553; ^{1}H NMR (500 MHz, CDCl$_3$) δ 1.31 (6H, d, J = 6.0 Hz CH(CH_3)$_2$), 3.82 (2H, s, NH$_2$), 4.58 (1H, sept., J = 6.0 Hz, CH(CH$_3$)$_2$), 6.87 (2H, d, J = 8.8 Hz, H-Ar), 7.27 (1H, br s, H-3), 7.48 (1H, br s, H-5), 7.77 (2H, d, J = 8.8 Hz, H-Ar), 9.56 (1H, br s, NH); ^{13}C NMR (125 MHz, CDCl$_3$) δ 22.0 (CH(*C*H$_3$)$_2$), 70.1 (*C*H(CH$_3$)$_2$), 115.1 (C-Ar), 116.7 (C-Ar), 123.5 (C-3), 126.2 (C-2 and C-5), 127.7 (C-4), 131.4 (C-Ar), 161.4 (C-Ar), 189.0 (CO); LRMS (ES$^+$) *m/z* 245.3 [M+H]$^+$.

[4-(4-Isopropoxybenzoyl)-1*H*-pyrrol-2-yl]carbamic acid (91)



tert-Butyl (4-(4-isopropoxybenzoyl)-1*H*-pyrrol-2-yl)carbamate (**89**) (266 mg, 0.77 mmol) was dissolved in TFA (6 ml) and stirred at RT for 1 h. The resulting mixture was concentrated *in vacuo* before being dissolved in DCM and washed with a saturated aqueous solution of $NaHCO_3$, dried using Na_2SO_4 and the solvent removed *in vacuo*. The title compound was obtained as a cream solid (53 mg, 28%); R_f = 0.31 (50% EtOAc in petrol); M.p. 105-106 ^oC; λ_{max} (EtOH)/nm 300, 230; ν_{max}/cm^{-1} 3296, 2979, 2551, 2160, 1721 ($NHCO_2H$), 1600 (CO) ; ^1H NMR (500 MHz, $CDCl_3$) δ 1.31 (6H, d, *J* = 6.1 Hz $CH(CH_3)_2$), 4.58 (1H, sept., *J* = 6.1 Hz, $CH(CH_3)_2$), 6.87 (2H, d, *J* = 7.0 Hz, H-Ar), 7.27 (1H, dd, *J* = 1.7 and 3.0 Hz, H-3), 7.48 (1H, dd, *J* = 1.7 and 3.2 Hz, H-5), 7.78 (2H, d, *J* = 7.0 Hz, H-Ar), 9.77 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.0 ($CH(CH_3)_2$), 77.0 ($CH(CH_3)_2$), 115.7 (C-Ar), 116.7 (C-3), 127.7 (C-2 and C-5), 127.7 (C-4), 131.5 (C-Ar), 155.7 (CO_2H), 161.5 (C-Ar), 189.1 (CO); LRMS *m/z* 289.1 $[M+H]^+$.

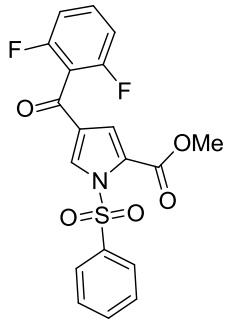
Methyl 4-(4-isopropoxybenzoyl)-1-(phenylsulfonyl)-1*H*-pyrrole-2-carboxylate (92)



Methyl 4-(4-isopropoxybenzoyl)-1*H*-pyrrole-2-carboxylate (**72**) (150 mg, 0.52 mmol) was dissolved in DCM (6 mL) before NEt_3 (152 µL, 1.1 mmol) was added followed by phenylsulfonyl chloride (139 µL, 1.1 mmol). The mixture was left to stir at RT for 3 h before being quenched with water and the product extracted with DCM (3 x 10 mL). The combined organic extracts were washed with a saturated aqueous solution of

$NaHCO_3$ (2 x 10 mL) before being dried over Na_2SO_4 and the solvent removed *in vacuo*. The crude product was purified by MPLC (silica, 2-60% EtOAc in petrol) to give the title compound as a white solid (100 mg, 65%) ;Rf = 0.77 (10% MeOH in EtOAc); M.p.: 135-136 ^{o}C; λ_{max} (EtOH)/nm: 294, 233; ν_{max}/cm^{-1}: 2977, 2366, 1730, 1598; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.40 (6H, d, *J* = 6.2 Hz, OCH(CH_3)$_2$), 3.77 (3H, s, OCH_3), 4.68 (1H, sept. *J* = 6.2 Hz, OC*H*(CH_3)$_2$), 6.97 (2H, d, *J* = 8.9 Hz, H-2' and H-6'), 7.47 (1H, d, *J* = 2.0 Hz, H-3), 7.55-7.58 (2H, m, H-Ar), 7.64-7.69 (1H, m, H-Ar), 7.87 (2H, d, *J* = 8.9 Hz, H-3' and H-5'), 8.02-8.04 (2H, m, sulfonyl H-Ar), 8.21 (1H, d, *J* = 2.0 Hz, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 22.0 (OCH(CH_3)$_2$), 52.1 (OCH_3), 70.3 (O*C*H(CH_3)$_2$), 115.3 (C-3 and C-4), 123.2 (C-2 and C-5), 128.6 (C-Ar), 129.0 (C-Ar), 131.6 (C-Ar), 132.4 (C-Ar), 134.4 (C-Ar), 169.6 (CO_2Me), 189.6 (CO); LRMS (ES$^+$) *m/z* 428.20 [M+H]$^+$; HRMS *m/z* calcd for $C_{22}H_{22}NO_6S$ [M+H]$^+$ 428.1162, found 428.1162.

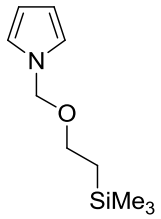
Methyl 4-(2,6-difluorobenzoyl)-1-(phenylsulfonyl)-1*H*-pyrrole-2-carboxylate (93)



Methyl 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**79**) (100 mg, 0.38 mmol) was dissolved in DCM (6 mL) before triethylamine (59 µL, 0.42 mmol) was added followed by phenylsulfonyl chloride (53 µL, 0.42 mmol). The mixture was left to stir for 3 h before being quenched with water and the product extracted with DCM (3 x 10 mL). The combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$ (2 x 10 mL) before being dried over Na_2SO_4 and the solvent removed *in vacuo*. The crude product was purified by MPLC (silica, 2-60% EtOAc in petrol) to give the title compound as a white solid (133 mg, 60%); R_f = 0.75 (10% MeOH in EtOAc); M.p.: 122-123 ^{o}C; λ_{max} (EtOH)/nm: 233; ν_{max}/cm^{-1}: 3144, 3086, 2953, 1746 (CO_2Me), 1663 (CO); ^{1}H NMR (500 MHz, MeOD) δ 3.88 (3H, s, OCH_3), 7.11-7.14 (2H, m, H-Ar), 7.18 (1H, d, *J* = 2.1 Hz, H-3), 7.56 (1H, dddd, , *J* = 6.3, 6.4, 8.5 and 8.6 Hz, H-4'), 7.63-7.67 (2H, m, H-3' and H-5'), 7.76-7.79 (1H, m, sulfonyl H-Ar), 8.07-

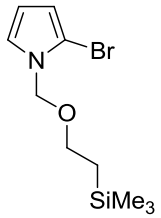
8.09 (2H, m, sulfonyl H-Ar), 8.21 (1H, d, J = 2.1 Hz, H-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 52.4(OCH_3), 112.7 (C-3 and C-4), 120.17 (C-2), 125.2 (C-5), 126.2 (C-Ar), 128.5 (C-Ar), 129.5 (C-Ar), 133.4 (C-Ar), 134.3 (C-Ar), 135.3 (C-Ar), 136.6 (C-Ar), 159.9 (CO_2Me), 181.5 (CO) LRMS (ES^+) *m/z* 406.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{14}F_2NO_3S$ $[M+H]^+$ 406.0555, found 406.0555.

1-((2-(Trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole (101)



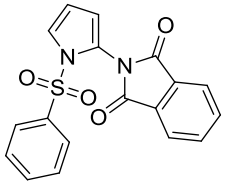
Sodium hydride (60% in mineral oil; 155 mg, 6.5 mmol) was suspended in DMF (5 mL) before pyrrole (0.4 mL, 6.0 mmol) was added at 0^{o}C. The mixture was stirred for 10 min before (2-(chloromethoxy)ethyl)trimethylsilane (1.0 mL, 6.0 mmol) was added dropwise. The mixture was allowed to warm to RT and stirred for 1 h. The mixture was then poured onto a 10% aqueous solution of $NaHCO_3$ (20 mL) and the product was extracted using Et_2O (3 x 10 mL). The combined organic extracts were washed with water followed by brine before being dried over Na_2SO_4 and the solvent removed *in vacuo.* The crude product was purified by MPLC (silica, 50% EtOAc in petrol) to give the title compound as an orange oil (0.71g, 60%); R_f = 0.63 (10% MeOH in EtOAc); λ_{max} (EtOH)/nm: 213; ν_{max}/cm^{-1}: 2953, 2896, 1554; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.02 (9H, s, $Si(CH_3)_3$), 0.85 (2H, t, J = 8.0 Hz, CH_2), 3.46 (2H, t, J = 8.0 Hz, CH_2), 5.24 (2H, s, CH_2) 6.09-6.10 (2H, m, H-2 and H-5), 6.89-6.90 (2H, m, H-3 and H-4); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.1 ($Si(CH_3)_3$), 54.9 (CH_2), 64.6 (CH_2), 77.4 (CH_2), 108.6 (C-3 and C-4), 121.2 (C-2 and C-5); LRMS (ES^+) *m/z* 198.20 $[M+H]^+$.

2-Bromo-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole (102)



1-((2-(Trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole (**101**) (50 mg, 0.25 mmol) was dissolved in THF (2 mL) at 0 ^{o}C before *N*-bromosuccinimide (46 mg, 0.26 mmol) was added slowly. The mixture was allowed to stir for 15 min before being poured onto a saturated aqueous solution of $NaHCO_3$. The product was extracted into Et_2O (2 x 10 mL) and the combined organic extracts were washed with brine before being dried over Na_2SO_4 and the solvent removed *in vacuo.* The crude product was dissolved in DCM (10 mL) and passed through a plug of silica to give the title compound as a pale yellow oil (54 mg, 99%); R_f = 0.68 (10% MeOH in EtOAc); λ_{max} (EtOH)/nm: 216; ν_{max}/cm^{-1}: 2952, 2362, 1974, 1671; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.02 (9H, s, $Si(CH_3)_3$), 0.90-0.93 (2H, m, CH_2), 3.51-3.54 (2H, m, CH_2), 5.25 (2H, s, CH_2), 6.19 (1H, d, J = 1.2 Hz, H-3), 6.20 (1H, s, H-4), 6.85-6.87 (1H, m, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.8 ($Si(CH_3)_3$), 30.3 (CH_2), 60.4 (CH_2), 65.8 (CH_2), 109.1 (C-3), 111.7 (C-4), 122.7 (C-5); LRMS (ES^{+}) *m/z* 276.2 [M+H]$^{+}$.

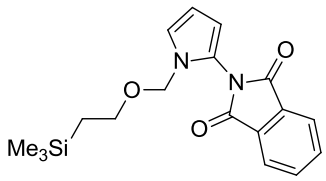
2-(1-(Phenylsulfonyl)-1*H*-pyrrol-2-yl)isoindoline-1,3-dione (111)



N-(Phenylsulfonyl) pyrrole (50 mg, 0.24 mmol) was dissolved in DCM (4 mL) before *N*-chlorophthalimide (44 mg, 0.24 mmol) and $NaHCO_3$ (39 mg, 0.46 mmol) were added. The mixture was stirred in the dark at RT overnight. Further DCM was added to the mixture before it was washed with water followed by a saturated aqueous solution of brine (10 mL respectively). The organic extract was dried over Na_2SO_4 and the solvent was removed *in vacuo* to give the crude product, which was purified by MPLC (silica, 2-60% EtOAc in petrol) to give the title compound as a beige solid (6 mg, 7%); R_f = 0.14 (10% EtOAc in petrol); M.p.: 210-211 ^{o}C (lit.[149] 212-213 ^{o}C) λ_{max} (EtOH)/nm: 263; ν_{max}/cm^{-1}: 3107, 2917, 1788 (CON), 1729 (CON) ,; ^{1}H NMR (500 MHz, $CDCl_3$) δ

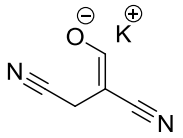
6.31 (1H, dd, *J* = 1.6 and 3.5 Hz, H-3), 6.35 (1H, dd, *J* = 3.5 and 3.8 Hz, H-4), 7.29 (1H, dd, *J* = 1.8 and 3.5 Hz, H-5), 7.38-7.42 (2H, m, sulfonyl H-Ar), 7.53-7.56 (1H, m, sulfonyl H-Ar), 7.65 (2H, dd, *J* = 1.1 and 8.5 Hz, phthalimide H-Ar), 7.74-7.78 (2H, m, sulfonyl H-Ar), 7.86-7.89 (2H, m, phthalimide H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 111.7 (C-3), 115.5 (C-4), 119.9 (C-5), 123.2 (C-Ar), 124.1 (C-Ar), 127.3 (C-Ar), 129.3 (C-Ar), 131.8 (C-Ar), 134.3 (C-2), 134.7 (C-Ar), 138.6 (C-Ar), 167.0 (NCO); LRMS (ES^+) *m/z* 353.20 $[M+H]^+$.

2-(1-((2-(Trimethylsilyl)ethoxy)methyl)-1H-pyrrol-2-yl)isoindoline-1,3-dione (114)



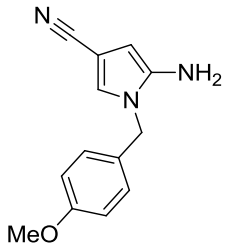
1-((2-(Trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole (**101**) (50 mg, 0.25 mmol) was dissolved in DCM (4 mL) before *N*-chlorophthalimide (44 mg, 0.24 mmol) and $NaHCO_3$ (39 mg, 0.46 mmol) were added. The mixture was stirred in the dark at RT overnight. The mixture was diluted with DCM (30 mL) before it was washed with water followed by a saturated aqueous solution of brine (10 mL respectively). The organic extracts were dried over Na_2SO_4 and the solvent removed *in vacuo* to give the crude product, which was purified by MPLC (silica, 2-60% EtOAc in petrol) giving the title compound as a brown solid (42 mg, 49%); R_f = 0.78 (10% MeOH in EtOAc); M.p.: 205-206 ^oC; λ_{max} (EtOH)/nm: 262; ν_{max}/cm^{-1}: 3609, 3277, 2950, 1787 (CON), 1728 (CON); ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.00 (9H, s, $Si(CH_3)_3$), 0.84-0.87 (2H, m, CH_2), 3.41-3.45 (2H, m CH_2), 5.25 (2H, s, CH_2), 6.33-6.38 (2H, m, H-3 and H-4), 6.93 (1H, dd, *J* = 1.9 and 3.2 Hz, H-5), 7.86-7.90 (2H, m, H-Ar), 8.01-8.05 (2H, m, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.7 ($Si(CH_3)_3$), 65.7 (CH_2), 72.5 (CH_2), 76.0 (CH_2), 108.2 (C-3), 109.5 (C-4), 122.5 (C-5), 124.0 (C-2), 131.9 (C-Ar), 134.5 (C-Ar), 167.6 (phthalimide CO); LRMS (ES^+) *m/z* 343.30 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{23}N_2O_3Si$ $[M+H]^+$ 343.1477, found 343.1477.

Potassium (*E*)-2,3-dicyanoprop-1-en-1-olate (121)



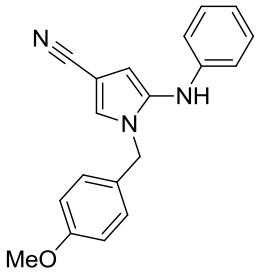
Succininitrile (2.0 g, 24.8 mmol), was dissolved in toluene (15 mL) before ^{t}BuOH (1.5 mL) and ethyl formate (2.0 mL, 24.8 mmol) were added at 0 ^{o}C. Slow addition of ^{t}BuOK (2.8 g, 24.8 mmol) caused formation of a beige precipitate. The mixture was allowed to warm to RT and left to stir for 4 h before the precipitate was filtered and washed with a 1:1 mixture of EtOH:Et_2O. After drying under vacuum, the pure product was obtained as a beige solid (3.0 g, 20.5 mmol, 84%); R_f = 0.20 (10% MeOH in EtOAc); M.p.: 199-200 ^{o}C (lit.[150] 205-210 ^{o}C); λ_{max} (EtOH)/nm 249.0; ν_{max}/cm^{-1} 2824 (CN), 1565 (CO), 1367, 1265; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.06 (2H, s, CH_2), 8.29 (1H, s, CH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 12.5 (CH_2), 61.0 (*C*CN), 120.5 (CH_2*C*N), 128.6 (C*C*N), 173.7 (CHO); LRMS (ES^{+}) *m/z* 108.2 [M+H]$^{+}$.

5-Amino-1-(4-methoxybenzyl)-1*H*-pyrrole-3-carbonitrile (122)



Potassium (*E*)-2,3-dicyanoprop-1-en-1-olate (**121**) (2.0 g, 13.6 mmol), was dissolved in EtOH (15 mL). Acetic acid (4 mL) was added followed by *p*-methoxybenzylamine (1.9 mL, 14.4 mmol) and the solution was stirred at reflux for 45 min. After cooling to RT, KOH (4.7 g, 84.0 mmol) was added in ethanol (15 mL) and the mixture was heated to reflux for a further 2.5 h. Upon cooling, the solvent was removed *in vacuo* and the residue was dissolved in EtOAc, before being washed with water. The organic extracts were separated, dried over Na_2SO_4 and the solvent removed *in vacuo* to give the crude product which was purified by MPLC (silica; 0-60% EtOAc in petrol) to give the pure product as a brown solid (2.2 g, 9.68 mmol, 70%); R_f = 0.33 (50% EtOAc in petrol); M.p. 155-156 ^oC; λ_{max} (EtOH)/nm 270.5, 226.0; ν_{max}/cm^{-1} 3315 (NH_2), 2191 (CN), 1640, 1609, 1510; ^1H NMR (500 MHz, $CDCl_3$) δ 2.98 (2H, br s, NH_2), 3.73 (3H, s, OCH_3), 4.86 (2H, s, CH_2), 5.66 (1H, d, *J* = 1.8 Hz, H-3), 6.79 (1H, d, *J* = 1.8 Hz, H-5), 6.80 (2H, d, *J* = 8.6 Hz, H-Ar), 6.97 (2H, d, *J* = 8.6 Hz, H-Ar); ^{13}C NMR (125 MHz, DMSO-d_6) δ 47.5 (CH2), 55.1 (CH3), 90.3 (C-4), 113.9 (C-3), 117.9 (CN), 122.4 (C-2), 128.8 (C-5), 139.5, 153.2, 153.6 (C-Ar); LRMS (ES^+) *m/z* 226.2 $[M-H]^-$; HRMS *m/z* calcd for $C_{13}H_{13}N_3O$ $[M-H]^-$ 226.0988, found 226.0988.

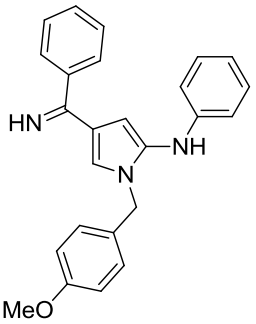
1-(4-Methoxybenzyl)-5-(phenylamino)-1*H*-pyrrole-3-carbonitrile (123)



5-Amino-1-(4-methoxybenzyl)-1*H*-pyrrole-3-carbonitrile (**122**) (320 mg, 1.41 mmol) was dissolved in dioxane (12 mL) before bromobenzene (96 µL, 0.96 mmol) and caesium carbonate (627 mg, 1.92 mmol) were added. The mixture was degassed for 15 min before $Pd_2(dba)_3$ (9 mg, 0.01 mmol) and Xantphos (11 mg, 0.02 mmol) were added

and the mixture was degassed again for a further 20 min. The reaction was heated to 100 ^{o}C for 18 h before being cooled, filtered through Celite, diluted with EtOAc and washed with water followed by brine. The organic extract was dried over Na_2SO_4 and the solvent removed *in vacuo* to give the crude product which was purified by MPLC (0-40% EtOAc in petrol) to afford the pure compound as a white solid (321 mg, 1.06 mmol, 75%); R_f = 0.52 (50% EtOAc in petrol); M.p. 83-84 ^{o}C; λ_{max} (EtOH)/nm 229.5; ν_{max}/cm^{-1}: 3358 (NH), 2200 (CN), 1603, 1590, 1513; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.73 (3H, s, OCH_3), 4.79 (2H, s, CH_2), 4.90 (2H, br s, NH_2), 6.20 (1H, dd, *J* = 0.9 and 1.9 Hz, H-3), 6.51 (2H, d, *J* = 8.8 Hz, benzyl H-Ar), 6.77-6.80 (3H, m, H-Ar), 6.94 (2H, d, *J* = 8.8 Hz, benzyl H-Ar), 6.99 (1H, d, *J* = 1.9 Hz, H-5), 7.11-7.14 (2H, m, H-Ar); ^{13}C NMR (125 MHz, DMSO-d_6) δ 48.1 (CH2), 55.0 (CH3), 89.5 (C-4), 104.4 (C-3), 113.3 (CN), 113.9, 117.0, 118.3, 126.2, 128.9 (C-Ar), 129.1 (C-5), 132.1 (C-2), 146.6, 158.7 (C-Ar); LRMS (ES^{+}) *m/z* 304.3 $[M+H]^{+}$; HRMS *m/z* calcd for $C_{19}H_{17}N_3O$ $[M-H]^{-}$ 302.1299, found 302.1299.

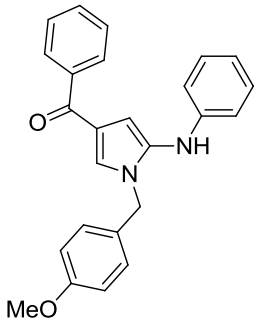
4-(Imino(phenyl)methyl)-1-(4-methoxybenzyl)-*N*-phenyl-1*H*-pyrrol-2-amine (124)



Mg turnings (24 mg, 0.98 mmol) were covered in THF (3 mL) before bromobenzene (68 µL, 0.65 mmol) was added followed by an iodine crystal to encourage turbidity and initiate reflux. After 30 min, 1-(4-Methoxybenzyl)-5-(phenylamino)-1*H*-pyrrole-3-carbonitrile (**123**) was added (100 mg, 0.33 mmol) in THF (1.0 mL). The mixture was then heated to reflux for 18 h. Upon cooling to RT HCl (1.0 M aqueous solution) was added until effervescence ceased. The product was extracted using EtOAc, dried over Na_2SO_4 and the solvent removed *in vacuo* to afford the pure product as a green solid (112 mg, 0.29 mmol 90%); R_f = 0.45 (5% MeOH in EtOAc); M.p. 131-132 ^{o}C; λ_{max} (EtOH)/nm 227; ν_{max}/cm^{-1}: 2997 (NH), 1596 (CNH), 1512; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.73 (3H, s, CH_3), 4.79 (2H, s, CH_2), 4.93 (1H, br s, NH), 6.20 (1H, d, *J* = 1.9 Hz, H-3), 6.51 (2H, d, *J* = 8.8 Hz, *p*-subs H-Ar), 6.77-6.79 (3H, m, H-Ar), 6.94 (2H, d, *J* = 8.8 Hz, *p*-subs H-Ar), 6.99 (1H, d, *J* = 1.9 Hz, H-5), 7.11-7.13 (2H, m, H-Ar), 7.26-7.29

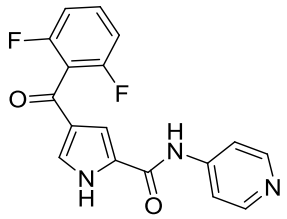
(3H, m, H-Ar), 7.35-7.38 (1H, m, H-Ar), 7.51-7.53 (1H, m H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 27.4 (CH_2), 55.3 (CH_3), 104.1 (C-3), 107.5 (C-4), 113.9, 114.4, 119.9 (C-Ar), 125.7 (C-5), 128.8 (C-2), 129.5, 143.2 (C-Ar), 158.8 (CNH); LRMS (ES^+) *m/z* 382.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{25}H_{24}N_3O$ $[M+H]^+$ 382.1914, found 382.1912.

(1-(4-Methoxybenzyl)-5-(phenylamino)-1*H*-pyrrol-3-yl)(phenyl)methanone (125)



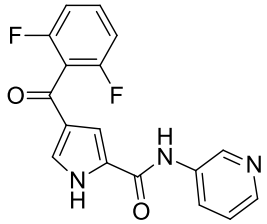
4-(Imino(phenyl)methyl)-1-(4-methoxybenzyl)-*N*-phenyl-1*H*-pyrrol-2-amine (**124**) (160 mg, 0.42 mmol) was dissolved in H_2SO_4 (1.0 M aqueous solution, 5 mL, 5.0 mmol) and heated to reflux for 2 h. After cooling, the mixture was neautralised using a 2.0 M aqueous solution of NaOH and the product was extracted using EtOAc. The organic extracts were combined, dried over Na_2SO_4 and the solvent removed *in vacuo*. The crude product was purified using MPLC (silica; 0-80% EtOAc in petrol) to give the pure product as a beige solid (136 mg, 0.35 mmol, 85%); R_f = 0.66 (5% MeOH in EtOAc); M.p. 135-136 ^oC; λ_{max} (EtOH)/nm: 342; ν_{max}/cm^{-1} 3409 (NH), 1669 (CO), 1512; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.72 (3H, s, CH3), 4.83 (2H, s, CH_2), 4.94 (1H, br s, NH), 6.48 (1H, br s, H-3), 6.56 (2H, d, *J* = 8.7 Hz, 2 x *p*-subs H-Ar), 6.76-6.79 (2H, m, H-Ar), 6.96 (2H, d, *J* = 8.7 Hz, 2 x *p*-subs H-Ar), 7.10-7.14 (2H, m, H-Ar), 7.16 (1H, d, *J* = 2.0 Hz, H-5), 7.36-7.39 (3H, m, H-Ar), 7.43-7.46 (1H, m, H-Ar), 7.45-7.47 (2H, m, H-Ar); LRMS (ES^+) *m/z* 383.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{25}H_{23}N_2O_2$ $[M+H]^+$ 383.1010, found 383.1012.

4-(2,6-Difluorobenzoyl)-*N*-(pyridin-4-yl)-1*H*-pyrrole-2-carboxamide (127)



Compound **127** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (100 mg, 0.40 mmol), carbonyldiimidazole (129 mg, 0.80 mmol), 4-aminopyridine (94 mg, 1.00 mmol) and THF (4 mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a white solid (50 mg, 38%); R_f = 0.52 (5% MeOH in EtOAc); M.p.: 290 ^oC (dec.); λ_{max} (EtOH)/nm: 292, 271; ν_{max}/cm^{-1}: 3655, 3368, 2585, 2235, 2019, 1588 (CO), 1506 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.26-7.30 (2H, m, H-3' and H-5'); 7.57 (1H, s, H-3), 7.58 (1H, s, H-5), 7.64 (1H, dddd, *J* = 6.7, 6.8, 8.4 and 8.5 Hz, H-4'), 7.75 (2H, d, *J* = 6.4 Hz, CH-pyridine), 8.47 (2H, d, *J* = 6.4 Hz, CH-N-pyridine), 10.36 (1H, s, CONH), 12.78 (1H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 112.1 (C-Ar), 112.2 (C-Ar), 112.4 (C-Ar), 113.7 (C-Ar), 122.0 (C-3), 125.9 (C-2 and C-5), 129.9 (C-4), 145.6 (C-Ar), 150.3 (C-Ar), 157.7 (C-Ar), 159.6 (C-Ar) 159.7 (CONH), 181.8 (CO); ^{19}F NMR (470 MHz, MeOD) δ -114.1; LRMS (ES^+) *m/z* 328.10 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{12}F_2N_3O_2$ $[M+H]^+$ 328.0890, found 328.0890.

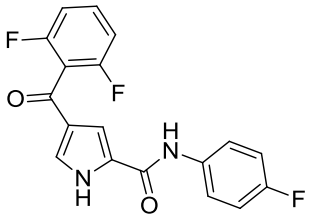
4-(2,6-Difluorobenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (128)



Compound **128** was synthesised according to general procedure C, using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), 3-aminopyridine (47 mg, 0.50 mmol) and THF (2 mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a white solid (23 mg, 35%); R_f = 0.78 (5% MeOH in EtOAc); M.p.: 160-161 ^oC; λ_{max} (EtOH)/nm: 290, 240; ν_{max}/cm^{-1}: 3243,

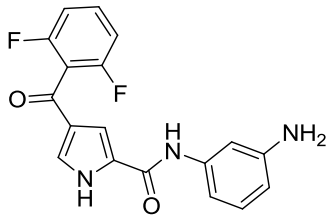
3021, 2437, 1622 (CO), 1538 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.04-7.07 (2H, m, H-3' and H-5'), 7.15 – 7.17 (1H, m, H-3), 7.30-7.32 (2H, m, CH-pyridine), 7.41 (1H, dddd, *J* = 6.8, 6.9, 8.5 and 8.6 Hz, H-4'), 7.90-7.93 (1H, m, H-5), 8.08 (1H, dd, *J* = 1.5 and 4.7 Hz, CH-pyridine), 8.67 (1H, d, *J* = 2.5 Hz CH-N-pyridine), 10.02 (1H, t, CONH), 12.50 (1H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 112.9 (C-Ar), 113.1, (C-Ar), 122.6 (C-3), 125.2 (C-2 and C-5), 128.0 (C-4), 129.5 (C-Ar), 130.6 (C-Ar), 133.2 (C-Ar), 136.4 (C-Ar), 137.5 (C-Ar), 142.4 (C-Ar), 145.1 (C-Ar), 160.9 (CONH), 184.7 (CO); ^{19}F NMR (470 MHz, MeOD) δ -115.4; LRMS (ES$^+$) *m/z* 328.30 [M+H]$^+$; HRMS *m/z* calcd for $C_{17}H_{12}F_2N_3O_2$ [M+H]$^+$ 328.0894, found 328.0895.

4-(2,6-Difluorobenzoyl)-N-(4-fluorophenyl)-1H-pyrrole-2-carboxamide (129)



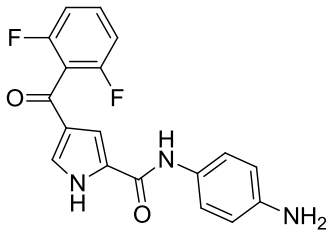
Compound **129** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), 4-fluoroaniline (48 µL, 0.50 mmol) and THF (2mL) to afford the crude product, which was purified by MPLC (silica, 2-30% MeOH in EtOAc, then NH silica, 0-10% MeOH in EtOAc) to give the title compound as a pale yellow solid (46 mg, 67%); R_f = 0.85 (10% MeOH in EtOAc); M.p.: 278-279 ^{o}C; λ_{max} (EtOH)/nm: 258, 200; ν_{max}/cm^{-1}: 3369, 3240, 3123, 2362, 2182, 1635 (CO), 1507 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.18-7.21 (2H, m, benzoyl H-3'and H-5'); 7.26-7.29 (2H, m, *p*-subs. H-Ar), 7.50 (2H, br s, H-3 and H-5), 7.63 (1H, dddd, *J* = 6.6, 6.7, 8.4 and 8.5, H-4'), 7.62-7.73 (2H, m, *p*-subs. H-Ar), 10.11 (1H, s, CONH), 12.66 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.1 (C-Ar), 112.4 (C-Ar), 115.3 (C-pyridine), 117.8 (C-Ar), 121.8 (C-3), 125.8 (C-2 and C-5), 128.4 (C-pyridine), 129.2 (C-4), 132.2 (C-Ar), 135.1 (C-pyridine), 158.3 (d, J_{CF} = 254.3 Hz, CF), 159.2 (CONH), 181.9 (CO); ^{19}F NMR (470 MHz, MeOD) δ -114.2, -121.5; LRMS (ES$^+$) *m/z* 345.20 [M+H]$^+$; HRMS *m/z* calcd for $C_{18}H_{12}F_3N_2O_2$ [M+H]$^+$ 328.0890, found 328.0890.

***N*-(3-Aminophenyl)-4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxamide (130)**



Compound **130** was synthesised according to general procedure C using, 4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), 1,3-phenylenediamine (54 mg 0.50 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc then NH silica, 0-20% MeOH in EtOAc) to give the title compound as a beige solid (45 mg, 66%); R_f = 0.60 (10% MeOH in EtOAc); M.p.: 210 ^oC (dec.); λ_{max} (EtOH)/nm: 239; ν_{max}/cm^{-1}: 3353 (NH_2), 3204, 3123, 2963, 1620 (CO), 1539 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 6.41 (1H, d, *J* = 8.3 Hz, H-Ar), 6.71 (1H, d, *J* = 8.3 Hz, H-Ar), 6.93-6.96 (2H, m, H-Ar and H-4'), 7.05 (1H, t, *J* = 8.3 Hz, H-Ar), 7.10-7.12 (2H, m, H-3 and H-5), 7.34 – 7.40 (2H, m, H-3' and H-5'), 7.53 (1H, br s, CONH), 9.74 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.1 (C-Ar), 112.5 (C-Ar), 116.3 (C-pyridine), 118.3 (C-Ar), 121.8 (C-3), 126.1 (C-2 and C-5), 129.4 (C-pyridine), 131.0 (C-4), 133.6 (C-Ar), 138.4 (C-pyridine), 156.2 (d, J_{CF} = 255.5 Hz, CF), 162.3 (CONH), 181.7 (CO); ^{19}F NMR (470 MHz, MeOD) δ -112.2; LRMS (ES^+) *m/z* 342.30 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{14}F_2N_3O_2$ $[M+H]^+$ 342.1050, found 342.1051.

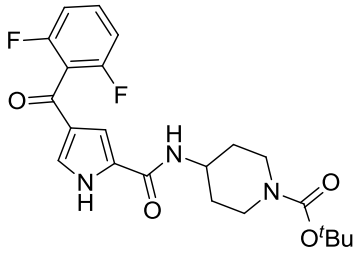
***N*-(4-Aminophenyl)-4-(2,6-difluorobenzoyl)-1H-pyrrole-2-carboxamide (131)**



Compound **131** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), 1,4-phenylenediamine (43 mg, 0.40 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc then NH silica, 0-100% EtOAc in petrol) and semi-preparitive

HPLC (C-18 silica; 5-100% acetonitrile in water (0.1% formic acid)) to give the title compound as a white solid (27 mg, 40%); R_f = 0.66; M.p.: 208 ^oC (dec.); λ_{max} (EtOH)/nm: 233; ν_{max}/cm^{-1}: 3250 (NH_2), 3120, 2237, 1789 (CO), 1624 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 6.55 (2H, d, *J* = 8.7 Hz, *p*-subs. H-Ar), 7.25-7.28 (2H, m, H-3' and H-5'), 7.31 (2H, d, *J* = 8.7 Hz, *p*-subs. H-Ar), 7.42 (1H, br s, H-3), 7.62 (1H, dddd, *J* = 6.7, 6.8, 8.7 and 8.8 Hz, H-4'), 8.18 (1H, br s, H-5), 9.70 (1H, br s, CONH), 12.50 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.4 (C-Ar), 113.8 (C-Ar), 115.9 (C-pyridine), 122.7 (C-3), 125.9 (C-2 and C-5), 130.0 (C-pyridine), 131.4 (C-4), 133.5 (C-Ar), 135.6 (C-pyridine), 159.3 (d, J_{CF} = 246.3 Hz, CF), 159.9 (CONH), 182.0 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -114.2; LRMS (ES^+) *m/z* 342.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{14}F_2N_3O_2$ $[M+H]^+$ 342.1051, found 342.1051.

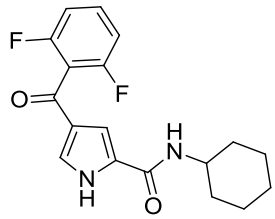
tert-Butyl 4-(4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxamido)piperidine-1-carboxylate (132)



Compound **132** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), 4-amino-1-Boc-piperidine (100 mg, 0.50 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a beige solid (50 mg, 57%); R_f = 0.71 (10% MeOH in EtOAc); M.p.: 159-160 ^oC; λ_{max} (EtOH)/nm: 286, 238; ν_{max}/cm^{-1}: 3509, 3175, 3005, 2944, 1763 ($CO_2{}^t$Bu), , 1622 (CO), 1564 (CONH); ^1H NMR (500 MHz, MeOD) δ 1.48 (9H, s, 3 x CH_3), 1.85-1.92 (4H, m, 2 x CH_2), 3.64-3.69 (1H, m, CH), 3.96-4.03 (4H, m, 2 x CH_2N), 7.10-7.14 (2H, m, H-3' and H-5'); 7.24 (1H, d, *J* = 1.50 Hz, H-3), 7.43 (1H, d, *J* = 1.50 Hz, H-5), 7.56 (1H, dddd, *J* = 6.4, 6.5, 8.6 and 8.7 Hz, H-4'); ^{13}C NMR (125 MHz, MeOD) δ 30.8 (3 x CH_3), 33.1 (2 x CH_2), 34.9 (2 x CH_2), 48.7 (NHCH), 50.7 ($CH(CH_3)_3$), 81.3 (C-Ar), 81.4 (C-Ar), 112.8 (C-Ar), 114.8 (C-3), 115.0 (C-2 and C-5), (128.3 (C-4), 156.6 (C-Ar), 159.3 (CONH), 161.8 ($CO_2{}^t$Bu), 184.5 (CO); ^{19}F NMR (470 MHz, MeOD) δ -115.4; LRMS

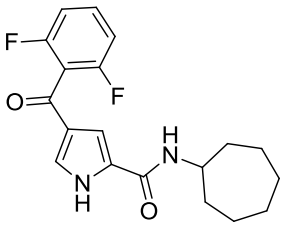
(ES$^+$) *m/z* 432.40 $[M+H]^+$; HRMS *m/z* calcd for $C_{22}H_{26}F_2N_3O_4$ $[M+H]^+$ 434.1887, found 434.1888.

_N_-Cyclohexyl-4-(2,6-difluorobenzoyl)-1_H_-pyrrole-2-carboxamide (133)



Compound **133** was synthesised according to general procedure C, using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), cyclohexylamine (57 µL, 0.50 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc then NH silica, 0-100% EtOAc in petrol) to give the title compound as a white solid (50 mg, 75%); R_f = 0.88 (10% MeOH in EtOAc); M.p.: 269-270 ^oC; λ_{max} (EtOH)/nm: 287, 236; ν_{max}/cm^{-1}: 3123, 3062, 2929, 2851, 1974, 1620 (CO), 1568 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 1.32-1.37 (2H, m, CH_2), 1.66-1.70 (2H, m, CH_2), 1.90-1.93 (2H, m, CH_2), 3.42-3.43 (2H, m, CH_2), 3.81-3.88 (1H, m, CH), 5.75 (1H, d, 1.65-1.69, *J* = 9.5 Hz), 6.91-6.94 (3H, m, H-3', H-5' and H-3), 7.26 (1H, d, *J* = 1.5 Hz, H-5), 7.34 (1H, dddd, *J* = 6.4, 6.5, 8.5 and 8.6 Hz, H-4'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.2 (CH_2), 24.8 (CH_2), 33.2 (CH_2), 48.5 (CH), 108.2 (C-Ar), 111.8 (C-Ar), 112.0 (C-Ar), 124.5 (C-3), 127.5 (C-2 and C-5), 131.5 (C-4), 171.2 (CONH), 181.9 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -112.3; LRMS (ES$^+$) *m/z* 333.30 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{19}F_2N_2O_2$ $[M+H]^+$ 333.1412, found 333.1413.

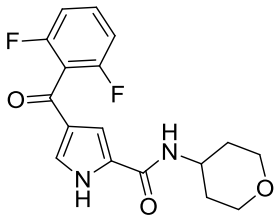
_N_-Cycloheptyl-4-(2,6-difluorobenzoyl)-1_H_-pyrrole-2-carboxamide (134)



Compound **134** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), cycloheptylamine (64 µL, 0.50 mmol) and

THF (2 mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc then NH silica, 0-100% EtOAc in petrol) to give the title compound as a white solid (51 mg, 73%); R_f = 0.87 (10% MeOH in EtOAc); M.p.: 199-200 ^oC; λ_{max} (EtOH)/nm: 287, 236; ν_{max}/cm^{-1}: 3356, 3167, 2921, 2854, 1617 (CO), 1566 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 1.45-1.57 (8H, m, 4 x CH_2), 1.84-1.94 (2H, m, CH_2), 3.40-3.58 (2H, m CH_2), 4.02-4.07 (1H, m, CH), 5.88 (1H, d, 1.32-1.37 (2H, m, CH_2), 1.66-1.70 (2H, m, CH_2), 1.90-1.93 (2H, m, CH_2), 3.42-3.43 (2H, m, CH_2), 3.81-3.88 (1H, m, CH), 5.75 (1H, d, *J* = 9.5 Hz, CONH), 6.90-6.94 (3H, m, H-3', H-5' and H-3), 7.25 (1H, br s, H-5), 7.34 (1H, dddd, *J* = 6.5, 6.6, 8.5 and 8.6 Hz, H-4'), 9.95 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.0 (CH_2), 28.1 (CH_2), 35.8 (CH_2), 51.4 (CH), 108.5 (C-Ar), 111.9 (C-Ar), 112.0 (C-Ar), 127.1 (C-Ar), 127.9 (C-3), 128.5 (C-2 and C-5), 131.4 (C-4), 178.8 (CONH), 182.5 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -112.4; LRMS (ES^+) *m/z* 347.30 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{21}F_2N_2O_2$ $[M+H]^+$ 347.1569, found 357.1571.

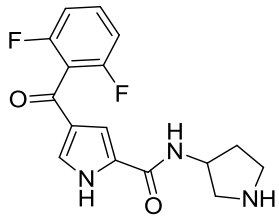
4-(2,6-Difluorobenzoyl)-*N*-(tetrahydro-2*H*-pyran-4-yl)-1*H*-pyrrole-2-carboxamide (135)



Compound **135** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), 4-aminotetrahydro-2*H*-pyran (52 µL 0.50 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a white solid (48 mg, 72%); R_f = 0.66 (10% MeOH in EtOAc); M.p.: 241-242 ^oC; λ_{max} (EtOH)/nm: 288, 236; ν_{max}/cm^{-1}: 3355, 3131, 2954, 2851, 1621 (COAr), 1537 (CONH); ^1H NMR 470 MHz, DMSO-d_6) δ 1.47-1.55 (2H, m, CH_2-THP), 1.71-1.74 (2H, m, CH_2-THP), 3.34-3.39 (2H, m, CH_2-O-THP), 3.85-3.87 (2H, m, CH_2-O-THP), 3.90-3.98 (1H, m, CH), 7.24-7.27 (3H, m, H-3', H-5' and H-3), 7.28 (1H, br s, H-5), 7.61 (1H, dddd, *J* = 6.8, 6.9, 8.5 and 8.6 Hz, H-4'), 8.20 (1H, d, *J* = 7.9 Hz, CONH), 12.47 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.6 (2 x CH_2), 36.0 (2 x CH_2), 49.0 (NHCH), 82.0 (C-Ar), 83.4 (C-Ar), 113.2 (C-Ar), 120.8 (C-3), 123.6 (C-2 and C-5), 128.4 (C-4), 155.2 (d, J_{CF}

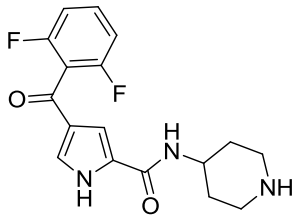
= 250.1 Hz, CF), 161.3 (CONH), 185.6 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 335.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{17}F_2N_2O_3$ $[M+H]^+$ 335.1203, found 335.1203.

4-(2,6-Difluorobenzoyl)-*N*-(pyrrolidin-3-yl)-1*H*-pyrrole-2-carboxamide (136)



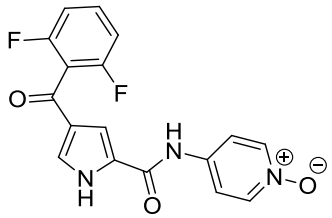
Compound **136** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), 3-aminopyrrolidine (44 µL 0.50 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a cream solid (45 mg, 70%); R_f = 0.24 (10% MeOH in EtOAc); M.p.: 206-207 ^oC; λ_{max} (EtOH)/nm: 285, 236; ν_{max}/cm^{-1}: 3333 (NH), 2918, 2117, 1997, 1622 (CO), 1588 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60-2.04 (2H, m, CH_2), 3.39-3.75 (4H, m, 2 x CH_2), 3.82-3.84 (1H, m CH), 6.95 (1H, d, *J* = 1.1 Hz, H-3), 7.23-7.27 (2H, m, H-3' and H-5'), 7.33 (1H, d, *J* = 1.1 Hz, H-5), 7.61 (1H, dddd, *J* = 6.7, 6.8, 8.5 and 8.6 Hz, H-4'); ^{13}C NMR (125 MHz, DMSO-d_6) δ 35.6 (2 x CH_2), 42.0 (CH_2), 50.1 (NHCH), 85.6 (C-Ar), 91.0 (C-Ar), 114.6 (C-Ar), 121.8 (C-3), 125.6 (C-2 and C-5), 130.1 (C-4), 159.7 (d, J_{CF} = 246.1 Hz, CF), 159.9 (CONH), 183.4 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 320.30 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{16}F_2N_3O_2$ $[M+H]^+$ 320.1209, found 320.1210.

4-(2,6-Difluorobenzoyl)-*N*-(piperidin-4-yl)-1*H*-pyrrole-2-carboxamide (137)



tert-Butyl4-(4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxamido)piperidine-1-carboxylate (**132**) (30 mg, 0.07 mmol) was dissolved in TFA (3 mL) and stirred at RT for 30 min. The solvent was removed *in vacuo* and the residue was dissolved in EtOAc before being washed with a saturated aqueous solution of $NaHCO_3$ (20 mL). The organic extract was dried over Na_2SO_4 and the solvent removed *in vacuo* to afford the crude compound as a white solid, which was purified by recrystallisation from MeOH (20 mg, 0.06 mmol , 87%); R_f = 0.50 (10% MeOH in EtOAc); M.p.: 150-151 ^oC; λ_{max} (EtOH)/nm: 286, 237; ν_{max}/cm^{-1}: 3313 (NH), 2974, 2873, 1773 (CO), 1650 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 0.88-0.92 (4H, m, 2 x CH_2-piperidine) 1.19-1.35 (5H, m, 2 x CH_2-N-piperidine and CH-piperidine), 7.49-7.53 (2H, m, H-3' and H-5'), 7.60-7.63 (1H, m, H-4'), 7.82 (1H, br s, H-3), 7.84 (1H, br s, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 38.1 (2 x CH_2), 39.0 (2 x CH_2), 48.2 (NHCH), 91.0 (C-Ar), 96.7 (C-Ar), 114.1 (C-Ar), 121.7 (C-3), 125.9 (C-2 and C-5), 129.9 (C-4), 156.1 (d, J_{CF} = 256.4 Hz, CF), 164.2 (CONH), 186.9 (CO); ^{19}F NMR (470 MHz, MeOD) δ -112.3; LRMS (ES^+) *m/z* 334.30 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{18}F_2N_3O_2$ $[M+H]^+$ 334.1364, found 334.1365.

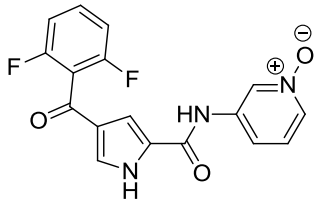
4-(4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxamido)pyridine 1-oxide (138)



4-(2,6-Difluorobenzoyl)-*N*-(pyridin-4-yl)-1*H*-pyrrole-2-carboxamide (**127**) was dissolved in DCM (2 mL) before *m*-CPBA (65%, 38 mg, 0.22 mmol) was added and the reaction was stirred at RT for 18 h. The reaction mixture was diluted with DCM and washed with an aqueous saturated solution of $NaHSO_3$. The organic extract was dried over Na_2SO_4, and the solvent removed *in vacuo* to give the crude product. Purification was achieved using MPLC (silica, 0-20% MeOH in EtOAc) to give the title compound

as a white solid (30 mg, 65%); R_f = 0.64 (5% MeOH in EtOAc); M.p.: 255 ^oC (dec.); λ_{max} (EtOH)/nm: 315; ν_{max}/cm^{-1}: 3304, 3120, 2829, 1657 (CO), 1628 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.26-7.30 (2H, m, H-3' and H-5'), 7.54 (1H, br s, H-3), 7.56 (1H, br s, H-5), 7.65 (1H, dddd, *J* = 6.7, 6.8, 8.6 and 8.7 Hz, H-4'), 7.78 (2H, d, *J* = 6.2 Hz, CH-pyridine), 8.15 (2H, d, *J* = 6.2 Hz, CH-N-pyridine), 10.50 (1H, s, CONH), 12.79 (1H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 112.0 (C-Ar), 112.2 (C-Ar), 112.5, (C-Ar), 121.0 (C-3), 125.9 (C-2 and C-5), 129.9 (C-4), 145.6 (C-pyridine), 150.3 (C-pyridine), 157.7 (C-pyridine), 159.6 (C-pyridine) 159.7 (CONH), 181.8 (CO); δ_F ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.1; LRMS (ES^+) *m/z* 344.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{12}F_2N_3O_3$ $[M+H]^+$ 344.0831, found: 344.0832.

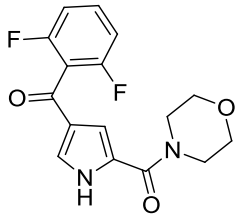
3-(4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxamido)pyridine 1-oxide (139)



4-(2,6-Difluorobenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (**128**) (30 mg, 0.09 mmol) was dissolved in DCM (2 mL) before *m*-CPBA (65%, 38 mg, 0.22 mmol) was added and the reaction was stirred at RT for 18 h. The reaction mixture was diluted with DCM and washed with an aqueous saturated solution of $NaHSO_3$. The organic extracts were dried over Na_2SO_4, and the solvent was removed *in vacuo* to give the crude product. Purification was achieved using MPLC (silica, 0-20% MeOH in EtOAc) to give the title compound as a white solid (23 mg, 77%); R_f = 0.60 (5% MeOH in EtOAc); M.p. 250 ^oC (dec); λ_{max} (EtOH)/nm 231.0; ν_{max}/cm^{-1} 2199, 2126, 1623 (CO), 1609 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.25-7.28 (2 H, m, H-3' and H-4'), 7.40 (1H, dd, *J* = 6.4 and 8.7 Hz, CH-pyridine), 7.53 (1H, br s, H-3), 7.56 (1H, br-s, H-5), 7.59 (1H, d, *J* = 6.4 Hz, CH-pyridine), 7.64 (1H, dddd, *J* = 6.4, 6.5, 8.1 and 8.2 Hz, H-Ar), 7.98 (1H, d, *J* = 6.4 Hz, CH-N-pyridine), 8.81 (1H, s, CH-N-pyridine), 10.35 (1 H, s, CONH), 12.79 (1 H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.0 (C-Ar), 112.2 (C-Ar), 112.6, (C-Ar), 123.1 (C-3), 126.0 (C-2 and C-5), 130.0 (C-4), 147.9 (C-pyridine), 150.2 (C-pyridine), 157.6 (C-pyridine), 159.6 (C-pyridine) 160.1 (CONH),

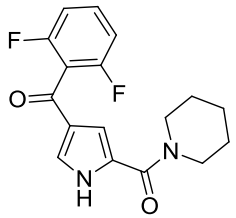
185.1 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.1; LRMS (ES$^+$) *m/z* 344.3 [M+H]$^+$; HRMS *m/z* calcd for $C_{17}H_{12}F_2N_3O_3$ [M-H]$^-$ 342.0688, found 342.0689.

(4-(2,6-Difluorobenzoyl)-1*H*-pyrrol-2-yl)(morpholino)methanone (140)



Compound **140** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), morpholine (44 µL, 0.50 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a white solid (45 mg, 70%); R_f = 0.71 (10% MeOH in EtOAc); M.p.: 210-211 ^{o}C; λ_{max} (EtOH)/nm: 284, 233; ν_{max}/cm^{-1}: 3241, 2959, 2867, 1656 (CO), 1598 (CON); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.62-3.64 (4H, m, 2 x CH_2-N-morpholine), 3.67-3.69 (4H, m, 2 x CH_2-O-morpholine), 6.90 (1H, brs, H-3), 7.23-7.26 (2H, m, H-3'and H-5'), 7.38 (1H, br s, H-5), 7.61 (1H, dddd, *J* = 6.7, 6.8, 8.6 and 8.7 Hz, H-4'), 12.43 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 40.1 (2 x C-N-morpholine), 66.1 (2x C-O-morpholine), 110.7 (C-Ar), 112.1 (C-Ar), 112.3 (C-Ar), 125.3 (C-3), 126.9 (C-2 and C-5), 129.0 (C-4), 129.0 (C-Ar), 160.5 (CON), 181.6 (CO); ^{19}F NMR (470 MHz, MeOD) δ -115.5; LRMS (ES$^+$) *m/z* 321.20 [M+H]$^+$; HRMS *m/z* calcd for $C_{17}H_{15}F_2N_2O_3$ [M+H]$^+$ 321.1050, found 321.1049.

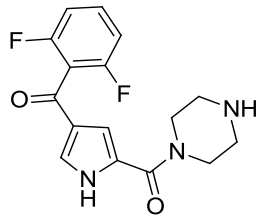
(4-(2,6-Difluorobenzoyl)-1*H*-pyrrol-2-yl)(piperidin-1-yl)methanone (141)



Compound **141** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), piperidine (49 µL, 0.50 mmol) and THF

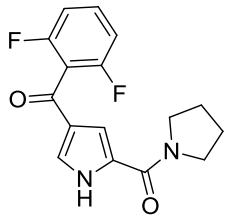
(2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc then NH silica, 0-100% EtOAc in petrol) to give the title compound as a white solid (30mg, 47%); R_f = 0.68 (10% MeOH in EtOAc); M.p.: 200-201 ^oC; λ_{max} (EtOH)/nm: 286, 236, 201; ν_{max}/cm^{-1}: 3766, 3012, 2933, 2419, 2117, 1644 (CO), 1587 (CON); ^1H NMR 470 MHz, MeOD) δ 1.65-1.69 (4H, m, 2 x CH_2-piperidine), 1.75-1.79 (2H, m, CH_2-piperidine), 3.80 (4H, br s, 2 x CH_2-N-piperidine), 6.95 (1H, d, *J* = 1.6 Hz, H-3), 7.10 – 7.15 (2H, m, H-3' and H-5'), 7.36 (1H, br s, H-5), 7.57 (1H, dddd, *J* = 6.4, 6.5, 8.5 and 8.6 Hz, H-4'); ^{13}C NMR (125 MHz, MeOD) δ 25.5 (CH_2-piperidine), 27.2 (CH_2-piperidine), 49.6 (CH_2-N-piperidine), 112.9 (C-Ar), 113.0 (C-Ar), 127.3 (C-2 and C-5), 128.5 (C-3), 130.1 (C-4), 133.2 (C-Ar), 139.8 (C-Ar), 161.8 (CON), 184.6 (CO); ^{19}F NMR (470 MHz, MeOD) δ -115.4; LRMS (ES^+) *m/z* 321.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{17}F_2N_2O_2$ $[M+H]^+$ 319.1258, found 319.1256.

(4-(2,6-Difluorobenzoyl)-1*H*-pyrrol-2-yl)(piperazin-1-yl)methanone (142)



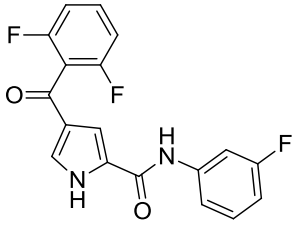
Compound **142** was synthesised according to general procedure C using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), piperazine (43 mg, 0.50 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a white solid (50 mg, 78%); R_f = 0.12 (10% MeOH in EtOAc); M.p.: 201-202 ^oC; λ_{max} (EtOH)/nm: 287, 236; ν_{max}/cm^{-1}: 3303 (NH), 2594, 2362, 2159, 2010, 1622 (CO), 1601 (CON); ^1H NMR (500 MHz, DMSO-d_6) δ 2.86-2.88 (4H, m, 2 x CH_2), 3.76 (4H, br s, 2 x CH_2), 6.89-6.92 (2H, m, H-3' and H-5'), 6.95 (1H, br s, H-3), 7.19 (1H, br s, H-5), 7.35 (1H, dddd, *J* = 6.6, 6.7, 8.5 and 8.6 Hz, H-4'), 10.67 (1H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 46.2 (4 x CH_2), 111.8 (C-Ar), 126.6, 126.8 (C-2 and C-5), 128.2 (C-4), 131.4 (C-Ar), 158.6 (C-Ar), 160.6 (C-Ar), 160.9 (CON), 182.5 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -112.4; LRMS (ES^+) *m/z* 320.30 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{16}F_2N_3O_2$ $[M+H]^+$ 320.1028, found 320.1027.

(4-(2,6-Difluorobenzoyl)-1*H*-pyrrol-2-yl)(pyrrolidin-1-yl)methanone (143)



Compound **143** was synthesised according to general procedure C using 4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxylic (**75**) (50 mg, 0.20 mmol), carbonyldiimidazole (65 mg, 0.40 mmol), pyrrolidine (42 µL 0.50 mmol) and THF (2 mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a white solid (54 mg, 90%); R_f = 0.52 (10% MeOH in EtOAc); M.p.: 228 ^oC (dec.); λ_{max} (EtOH)/nm: 286, 234; ν_{max}/cm^{-1}: 3227, 2958, 2870, 2162, 1973, 1677 (CO), 1644 (CON); ^1H NMR (500 MHz, DMSO-d_6) δ 1.82-1.85 (2H, m , CH_2-pyrrolidine), 1.94-1.97 (2H, m, CH_2-pyrrolidine), 3.48-3.51 (2H, m, CH_2-N-pyrrolidine), 3.71-3.73 (2H, m, CH_2-N-pyrrolidine), 6.97 (1H, d, *J* = 1.3 Hz, H-3), 7.22-7.24 (2H, m, H-3' and H-5') 7.33 (1H, br s, H-5), 7.62 (1H, dddd, *J* = 6.6, 6.7, 8.6 and 8.7 Hz, H-4'), 12.37 (1H, br s, NH); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 305.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{15}F_2N_2O_2$ $[M+H]^+$ 305.1098, found 305.1096.

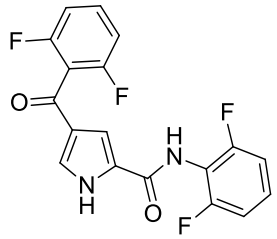
4-(2,6-Difluorobenzoyl)-*N*-(3-fluorophenyl)-1*H*-pyrrole-2-carboxamide (144)



Compound **144** was synthesised according to general procedure D, using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.2 mmol), MeCN (1 mL), 3-fluoroaniline (48 µL, 0.5 mmol) and PCl_3 (17 µL, 0.2 mmol) to afford the crude product. Purification was achieved by recrytallisation from MeOH to give the title compound as a white solid (45 mg, 65%); R_f = 0.51 (10% MeOH in EtOAc); M.p.: 285-286 ^oC; λ_{max} (EtOH)/nm: 261; ν_{max}/cm^{-1}: 3225, 2338, 2121, 1623 (CO), 1610 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 6.89-6.93 (1H, m, H-Ar), 7.25-7.28 (2H, m, H-3' and H-5'), 7.37 (1H, q, *J* = 7.8 Hz, H-Ar), 7.50-7.52 (3H, m, H-3, H- 5 and H-Ar), 7.63

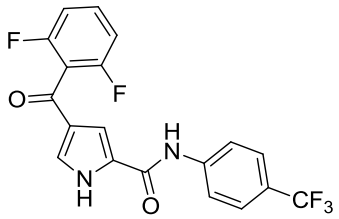
(1H, dddd, J = 6.8, 6.9, 8.4 and 8.5 Hz, H-4'), 7.71-7.74 (2H, m, H-Ar), 10.21 (1 H, s, CONH) 12.69 (1 H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.0 (C-Ar), 112.5 (C-Ar), 116.3 (C-pyridine), 118.5 (C-Ar), 120.9 (C-3), 125.6 (C-2 and C-5), 129.4 (C-pyridine), 130.1 (C-4), 133.6 (C-Ar), 136.2 (C-pyridine), 156.4 (d, J_{CF} = 255.1 Hz, CF), 159.3 (d, J_{CF} = 256.3 Hz, CF), 159.2 (CONH), 181.9 (CO) ^{19}F NMR (470 MHz, DMSO-d_6) δ -112.4, -114.2; LRMS (ES^-) *m/z* 343.10 $[M-H]^-$; HRMS *m/z* calcd for $C_{18}H_{12}F_5N_2O_2$ $[M+H]^+$ 345.0845, found 385.0845.

4-(2,6-Difluorobenzoyl)-*N*-(2,6-difluorophenyl)-1*H*-pyrrole-2-carboxamide (145)



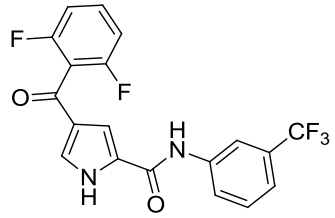
Compound **145** was synthesised according to general procedure D, using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.2 mmol), MeCN (1 mL), 2,6-difluoroaniline (54 µL, 0.5 mmol) and PCl_3 (17 µL, 0.2 mmol). The title compound was obtained after work up, without further purification, as a white solid (60 mg, 83%); R_f = 0.75 (10% MeOH in EtOAc); M.p.: 215 ^oC (dec.); λ_{max} (EtOH)/nm: 286, 240; ν_{max}/cm^{-1}: 3291, 3042, 2934, 2598, 2062, 2006, 1618 (CO), 1608 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.89-6.91 (1H, m, H-3), 7.10-7.18 (4H, m, H-Ar, H-3' and H-5'), 7.26-7.32 (1H, m H-4'), 7.36 (1H, br s, H-5), 7.48-7.54 (1H, m, H-Ar), 9.72 (1H, s, CONH), 12.62 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.5 (C-Ar), 111.6 (C-Ar), 111.7 (C-Ar), 111.8 (C-Ar) 126.7 (C-3), 126.9 (C-2 and C-5), 130.6 (C-4), 157.7 (C-Ar), 159.1, (C-Ar), 159.7 (CONH), 179.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3, -117.8; LRMS (ES^+) *m/z* 363.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{11}F_4N_2O_2$ $[M+H]^+$ 363.0748, found 363.0749.

4-(2,6-Difluorobenzoyl)-*N*-(4-(trifluoromethyl)phenyl)-1*H*-pyrrole-2-carboxamide (146)



Compound **146** was synthesised according to general procedure D, using 4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.2 mmol), MeCN (1 mL), 4-(trifluoromethyl)aniline (63 µL, 0.5 mmol) and PCl_3 (17 µL, 0.2 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 2-60% MeOH in EtOAc) followed by recrytallisation from MeOH to give the title compound as a white solid (50 mg, 64%); R_f = 0.75 (10% MeOH in EtOAc); M.p.: 299 ^oC (dec.); λ_{max} (EtOH)/nm: 297; ν_{max}/cm^{-1}: 3359, 3254, 3122, 1668, (CO) 1633 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.26-7.29 (2H, m, H-3' and H-5'), 7.53 (1H, s, H-3), 7.57 (1H, s, H-5), 7.64 (1H, dddd, *J* = 6.4, 6.5, 8.8 and 8.9 Hz, H-4'), 7.72 (2H, d, *J* = 8.9 Hz, *p*-subs. H-Ar), 7.97 (2H, d, *J* = 8.9 Hz, *p*-subs. H-Ar), 10.34 (1H, s, CONH), 12.72 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.0 (C-Ar), 112.4 (C-Ar), 113.1 (C-Ar), 119.8 (CF_3), 121.0(C-3), 124.9 (C-2 and C-5), 129.2 (C-Ar), 129.5 (C-Ar), 129.9 (C-4), 133.0 (C-Ar), 138.8 (C-Ar), 157.6 (C-Ar), 159.7 (CONH), 181.8 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -60.3, -114.1; LRMS (ES^+) *m/z* 395.40 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{12}F_5N_2O_2$ $[M+H]^+$ 395.0810, found 395.0809.

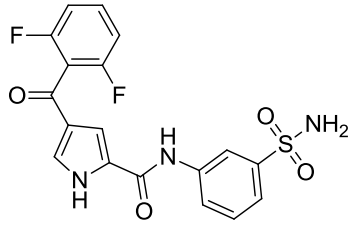
4-(2,6-Difluorobenzoyl)-*N*-(3-(trifluoromethyl)phenyl)-1*H*-pyrrole-2-carboxamide (147)



Compound **147** was synthesised according to general procedure D, using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.2 mmol), MeCN (1 mL), 3-(trifluoromethyl)aniline (63 µL, 0.5 mmol) and PCl_3 (17 µL, 0.2 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 2-60% MeOH in EtOAc) followed by recrytallisation from MeOH to give the title compound as a white solid (45 mg, 57%); R_f = 0.76 (10% MeOH in EtOAc); M.p.: 290 ^oC (dec.); λ_{max} (EtOH)/nm: 292,

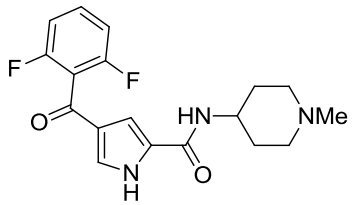
264; ν_{max}/cm^{-1}: 3362, 3223, 2296, 1624 (CO), 1541 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.26-7.30 (2H, m, H-3' and H-5'), 7.55 (2H, br s, H-3 and H-Ar), 7.60 (1H, t, *J* = 8.1 Hz, H-Ar), 7.64 (1H, dddd, *J* = 6.5, 6.6, 8.8 and 8.9 Hz, H-4'), 8.04 (1H, d, *J* = 8.1 Hz, H-Ar), 8.18 (1H, br s, H-5), 10.4 (1H, s, CONH), 12.72 (1h, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.7 (C-Ar), 112.2 (C-Ar), 112.3 (C-Ar), 119.7 (CF_3), 123.4 (C-3), 125.9 (C-2 and C-5), 129.2 (C-Ar), 129.5 (C-Ar), 129.9 (C-4), 132.3 (C-Ar). 139.7 (C-Ar), 157.6 (C-Ar), 158.7 (C-Ar), 159.7 (CONH), 181.8 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -69.3, -114.1; LRMS (ES^+) *m/z* 395.40 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{12}F_5N_2O_2$ $[M+H]^+$ 395.0811, found 395.0811.

4-(2,6-Difluorobenzoyl)-*N*-(3-sulfamoylphenyl)-1*H*-pyrrole-2-carboxamide (148)



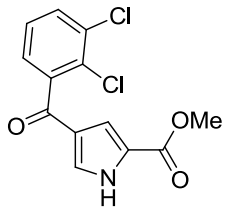
Compound **148** was synthesised according to general procedure D, using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.2 mmol), MeCN (1 mL), 3-aminobenzenesulfonamide (86 mg, 0.5 mmol) and PCl_3 (17 μL, 0.2 mmol) to afford the crude product. Purification was achieved by recrytallisation from MeOH to give the title compound as a white solid (61 mg, 75%); R_f = 0.65 (10% MeOH in EtOAc); M.p.: 309 ^{o}C (dec.); λ_{max} (EtOH)/nm: 295, 256; ν_{max}/cm^{-1}: 3345, 3228, 3215, 2343, 1621 (CO), 1532 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.21-7.24 (2 H, m, H-3' and H-5'), 7.33-7.38 (3 H, m, H-Ar), 7.49-7.50 (1 H, m, H-Ar), 7.54-7.60 (1H, m, H-4'), 7.91 (1 H, d, *J* = 3.5 Hz, H-3), 8.37 (1H, br s, H-5), 10.42 (1 H, s, CONH) 12.67 (1 H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 111.5 (C-Ar), 111.7 (C-Ar), 112.4 (C-Ar), 116.5 (C-Ar), 118.9 (C-Ar), 121.6 (C-3), 129.0 (C-2 and C-5), 129.2 (C-4), 140.3 (C-Ar), 144.5 (C-Ar), 157.7 (C-Ar), 159.6 (CONH), 183.8 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 406.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{14}F_2N_3O_4S$ $[M+H]^+$ 406.0661, found 406.0661.

4-(2,6-Difluorobenzoyl)-*N*-(1-methylpiperidin-4-yl)-1*H*-pyrrole-2-carboxamide (149)



Compound **149** was synthesised according to general procedure D using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.2 mmol), MeCN (1 mL), 4-amino-*N*-methylpiperidine (51 µL, 0.4 mmol) and PCl_3 (17 µL, 0.2 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a beige solid (50 mg, 72%); R_f = 0.65 (5% MeOH in EtOAc); M.p. 170-171 ^oC; λ_{max} (EtOH)/nm 286.0, 236.0; ν_{max}/cm^{-1} 2947 (NMe), 1748, 1617 (CO), 1529 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 1.48-1.56 (2H, m, CH_2), 1.73 (2H, d, *J* = 11.5 Hz, CH_2), 1.93 (2H, t, *J* = 11.5 Hz, CH_2N), 2.16 (3H, s, NCH_3), 2.74 (2H, d, *J* = 11.5 Hz, CH_2N), 3.64-3.71 (1H, m, CH), 7.18 (1H, br s, H-3), 7.23-7.26 (2H, m, H-3' and H-5'), 7.34 (1H, br s, H-5), 7.59 (1H, dddd, *J* = 6.3, 6.4, 8.4 and 8.5 Hz, H-4'), 8.01 (1 H, br s, CONH) 12.39 (1 H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 30.9 (2 x CH_2), 44.6 (CH_3), 46.2 (CH), 54.9 (2 x CH_2N), 112.1 (C-Ar), 112.9 (C-Ar), 113.1 (C-Ar), 122.3 (C-3), 129.8 (C-2 and C-5), 133.2 (C-4), 133.3 (C-Ar), 159.7 (CON), 162.1 (d, J_{CF} = 250.2 Hz, CF), 184.0 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 348.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{20}F_2N_3O_2$ $[M+H]^+$ 348.1521, found 348.1520.

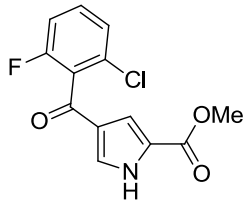
Methyl 4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylate (152)



Compound **152** was synthesised according to general procedure A using methylpyrrole-2-carboxylate (400 mg, 3.20 mmol), DCM (7 mL), $AlCl_3$ (1.07 g, 8.00 mmol) and 2,3-dichlorobenzoyl chloride (1.34 g, 6.40 mmol) to afford the crude product. Purification was achieved using MPLC (0-80% EtOAc in petrol) to give the pure compound as a white solid (681 mg, 71%); R_f = 0.60 (50% EtOAc in petrol); M.p. 183-184 ^oC; λ_{max} (EtOH)/nm 284.5, 233.0; ν_{max}/cm^{-1} 3285, 1691 (CO_2Me), 1657 (CO), 1561; ^1H NMR

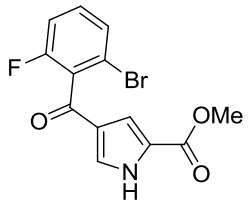
(500 MHz, DMSO-d_6) δ 3.80 (3H, s, CH_3), 7.02 (1H, dd, J = 1.6 and 2.3 Hz, H-3), 7.44-7.50 (3H, m, H-5 and H-Ar), 7.79 (1H, dd, J = 1.8 and 8.0 Hz, H-Ar), 12.85 (1H, br s, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ 51.7 (CH_3), 115.0 (C-3), 124.3 (C-2), 124.7 (C-5), 126.9 (C-4), 127.5 (C-Ar), 128.6 (C-Ar), 130.4 (C-Ar), 131.5 (C-Ar), 132.6 (C-Ar), 141.3 (C-Ar), 160.3 (CO_2Me), 187.2 (CO); LRMS (ES^+) *m/z* 296.1 $[M-H]^-$; HRMS *m/z* calcd for $C_{13}H_{10}{}^{35}Cl_2NO_3$ $[M+H]^+$ 298.0037, found 298.0037.

Methyl 4-(2-chloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (153)



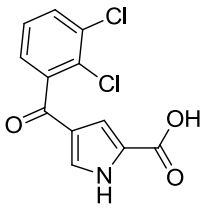
Compound **153** was synthesised according to general procedure A using methylpyrrole-2-carboxylate (3.00 g, 23.80 mmol), DCM (40 mL), $AlCl_3$ (7.93 g, 59.50 mmol) and 2-chloro-6-fluorobenzoyl chloride (6.40 mL, 47.60 mmol to afford the crude product. Purification was achieved using MPLC (0-80% EtOAc in petrol) to give the pure compound as a white solid (4.20 g, 63%); R_f = 0.48 (50% EtOAc in petrol); M.p. 183-184 ^{o}C; λ_{max} (EtOH)/nm 282, 232; ν_{max}/cm^{-1} 3224, 1732 (CO_2Me), 1639 (CO), 1563; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.00 (1H, s, H-3), 7.37-7.40 (1H, m, H-5'), 7.46 (1H, d, J = 8.2 Hz, H-3'), 7.54 (1H, dd, J = 1.5 and 3.3 Hz, H-5), 7.58 (1H, ddd, J = 6.3, 8.4 and 8.4 Hz, H-4'); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.7 (CH_3), 114.6 (C-3), 115.0 (d, J_{CF} = 21.6Hz, C-Ar), 124.5 (C-5), 125.3 (C-2), 125.8 (d, J_{CF} = 3.6 Hz, C-Ar), 127.8 (d, J_{CF} = 22.8 Hz, C-Ar), 130.1 (C-4), 130.3 (d, J_{CF} = 5.8 Hz, C-Ar), 131.9 (d, J_{CF} = 9.2 Hz, C-Ar), 158.6 (d, J_{CF} = 247.7 Hz, CF) 160.2 (CO_2Me), 183.7 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.0; LRMS (ES^+) *m/z* 282.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_{10}{}^{35}ClFNO_3$ $[M+H]^+$ 282.0328, found 282.0331.

Methyl 4-(2-bromo-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (154)



2-Bromo-6-fluorobenzoic acid (2.0 g, 9.13 mmol) was dissolved in THF (20 mL) and $SOCl_2$ (1.98 mL, 27.39 mmol) was added followed by DMF (71 µL, 0.91 mmol). The mixture was stirred at RT for 3 h before the solvent was removed *in vacuo***.** The resulting residue was dissolved in DCM (20 mL) before $AlCl_3$ (1.83 g, 13.70 mmol) was added followed by methylpyrrole-2-carboxylate (571 mg, 4.57 mmol) and the mixture was stirred at RT for 18 h. Work up was achieved according to general procedure A and the crude product was purified using MPLC (0-80% EtOAc in petrol) to give the pure compound as a white solid (1.16 g, 80%); R_f = 0.51 (50% EtOAc in petrol); M.p.: 185-186 ^oC; λ_{max} (EtOH)/nm 280; ν_{max}/cm^{-1} 3229, 1731 (CO_2Me), 1638 (CO), 1563; ^1H NMR (500 MHz, DMSO-d_6) δ 3.80 (3H, s, CH_3), 7.00 (1H, s, H-3), 7.39-7.43 (1H, m, H-5'), 7.50 (1H, ddd, J = 6.3, 8.3 and 8.3 Hz, H-4'), 7.52 (1H, s, H-5), 7.59 (1H, d, J = 8.3 Hz, H-3'), 12.90 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.6 (CH_3), 114.7 (C-3), 115.4 (d, J_{CF} = 21.6 Hz, C-Ar), 118.9 (d, J_{CF} = 5.4 Hz, C-Ar), 124.5, 125.0 (C-2 and C-5), 128.8 (d, J_{CF} = 3.3 Hz, C-Ar), 130.4 (C-4), 132.2 (d, J_{CF} = 8.2 Hz, C-Ar), 158.4 (d, J_{CF} = 248.2 Hz, CF), 160.3 (CO_2Me), 184.6 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.6; LRMS (ES^+) *m/z* 328.1 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_{10}{}^{79}BrFNO_3$ $[M+H]^+$ 325.9823, found 325.9830.

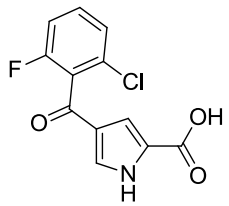
4-(2,3-Dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (155)



Compound **155** was synthesised according to general procedure B using methyl 4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylate (**152**) (600 mg, 2.01 mmol), LiOH (963 mg, 40.20 mmol) in water (26 mL) and THF (15 mL) to afford the pure product as a white solid (595 mg, 99%); R_f = 0.40 (5% MeOH in EtOAc); M.p. 209-210 ^oC; λ_{max} (EtOH)/nm: 281.5, 229.5; ν_{max}/cm^{-1} 3286, 1686 (CO_2H), 1654 (CO), 1559; ^1H NMR

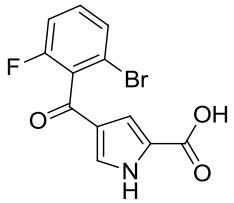
(500 MHz, DMSO-d_6) δ 6.97 (1H, br s, H-3), 7.38 (1H, dd, J = 1.6 and 3.3 Hz, H-5), 7.45 (1H, dd, J = 1.7 and 7.6 Hz, H-Ar), 7.46-7.50 (1H, m, H-Ar); 7.79 (1H, dd, J = 1.7 and 7.6 Hz, H-Ar), 12.65 (1H, s, CO_2H), 12.88 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 114.6 (C-3), 124.6 (C-5), 125.6 (C-2), 126.9 (C-4), 127.5 (C-Ar), 128.5 (C-Ar), 130.0 (C-Ar), 131.4 (C-Ar), 132.3 (C-Ar), 141.5 (C-Ar), 161.3 (CO_2H), 187.2 (CO); LRMS (ES^+) *m/z* 283.2 $[M-H]^-$; HRMS *m/z* calcd for $C_{12}H_7{}^{35}Cl_2NO_3$ $[M+H]^+$ 281.9733, Found: 281.9735.

4-(2-Chloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (156)



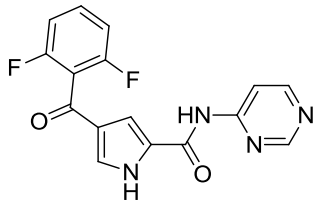
Compound **156** was synthesised according to general procedure B using methyl 4-(2-chloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**153**) (4.00 g, 14.20 mmol), LiOH (6.81 g, 284.00 mmol) in water (150 mL) and THF (100 mL) to afford the pure product as a white solid (3.75 g, 99%); R_f = 0.36 (5% MeOH in EtOAc); M.p. 209-210 ^oC; λ_{max} (EtOH)/nm 281, 233; ν_{max}/cm^{-1} 3306, 1638 (CO_2H), 1556 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.95 (1H, s, H-3), 7.35-7.40 (1H, m, H-5'), 7.44-7.46 (2H, m, H-3' and H-5), 7.57 (1H, ddd, J = 6.4, 8.4 and 8.6 Hz, H-4'), 12.69 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 114.0 (C-3), 114.9 (d, J_{CF} = 21.6Hz, C-Ar), 125.2 (C-5), 125.2 (d, J_{CF} = 3.2 Hz, C-Ar), 126.1 (C-2), 127.8 (d, J_{CF} = 6.6 Hz, C-Ar), 129.5 (C-4), 130.3 (d, J_{CF} = 6.5 Hz, C-Ar), 131.8 (d, J_{CF} = 9.3 Hz, C-Ar), 158.5 (d, J_{CF} = 247.7 Hz, CF), 161.4 (CO_2H), 183.7 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.0; LRMS (ES^+) *m/z* 268.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{12}H_6{}^{35}ClFNO_3$ $[M-H]^-$ 266.0026, found 266.0019.

4-(2-Bromo-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (157)



Compound **157** was synthesised according to general procedure B using methyl 4-(2-bromo-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**154**) (1.00 g, 3.06 mmol), LiOH (1.46 g, 61.12 mmol) in water (40 mL) and THF (25 mL) to afford the pure product as a white solid (949 mg, 99%); R_f = 0.40 (5% MeOH in EtOAc); M.p. 210-211 ^oC; λ_{max} (EtOH)/nm 281; ν_{max}/cm^{-1} 3342, 1690 (CO_2H), 1648 (CO), 1561; ^1H NMR (500 MHz, DMSO-d_6) δ 6.94 (1H, s, H-3), 7.39-7.43 (2H, m, H-5 and H-5'), 7.50 (1H, ddd, J = 6.3, 8.4 and 8.4 Hz, H-4'), 7.59 (1H, d, J = 8.4 Hz, H-Ar), 12.69 (1H, br s, NH), 12.81 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 114.1 (C-3), 115.3 (d, J_{CF} = 21.4 Hz, C-Ar), 118.9 (d, J_{CF} = 5.0 Hz, C-Ar), 124.9 (C-5), 126.0 (C-2), 128.8 (d, J_{CF} = 2.8 Hz, C-Ar), 129.6 (C-4), 129.8 (d, J_{CF} = 23.2 Hz, C-Ar), 132.1 (d, J_{CF} = 8.5 Hz, C-Ar), 158.3 (d, J_{CF} = 247.9 Hz, CF), 161.3 (CONH), 184.6 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.8; LRMS (ES^+) *m/z* 312.2 $[M-H]^-$; HRMS *m/z* calcd for $C_{12}H_6{}^{79}BrFNO_3$ $[M-H]^-$ 309.9521, found 309.9516.

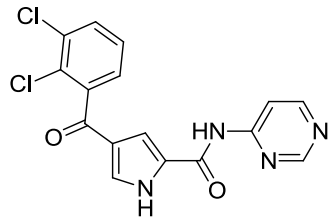
4-(2,6-Difluorobenzoyl)-*N*-(pyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (158)



Compound **158** was synthesised according to general procedure D using 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (50 mg, 0.2 mmol), MeCN (1 mL), 4-aminopyrimidine (48 mg, 0.5 mmol) and PCl_3 (17 µL, 0.2 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (50 mg, 76%); R_f = 0.68 (5% MeOH in EtOAc); M.p. 278-279 ^oC; λ_{max} (EtOH)/nm 296.0, 246.0; ν_{max}/cm^{-1}: 2976, 2863, 2118, 1641 (CO), 1553 (CONH0; ^1H NMR (500 MHz, DMSO-d_6) δ 7.26-7.29 (2H, m, H-3' and H-5'), 7.60-7.67 (2H, m, H-4' and CH-pyrimidine), 7.79 (1H, br s, H-3), 8.29 (1H, dd, J = 1.5 and 6.0 Hz, CH-pyrimidine), 7.69 (1H, d, J = 6.0 Hz, CH-pyrimidine), 8.92 (1H, br s, H-5), 11.16 (1H, br s, CONH), 12.79 (1H, br s, NH); ^{13}C NMR (125 MHz,

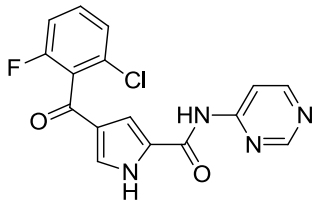
DMSO-d_6) δ 110.5 (C-Ar), 112.1 (d, J_{CF} = 21.2 Hz, C-Ar), 114.2 (C-Ar), 122.3 (C-3), 127.2 (C-2 and C-5), 132.2 (C-4), 158.7 (d, J_{CF} = 203.1 Hz, CF), 159.5 (CON), 181.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -115.4; LRMS (ES^+) *m/z* 329.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{10}F_2N_4O_2$ $[M+H]^+$ 329.0844, found: 329.0845.

4-(2,3-Dichlorobenzoyl)-*N*-(pyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (159)



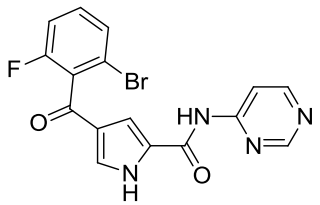
Compound **159** was synthesised according to general procedure D using 4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**152**) (75 mg, 0.25 mmol), MeCN (1.5 mL), 4-aminopyrimidine (60 mg, 0.63 mmol) and PCl_3 (22 μL, 0.25 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a yellow solid (65 mg, 0.18 mmol, 72%); R_f = 0.70 (5% MeOH in EtOAc); M.p. 199-200 ^oC; λ_{max} (EtOH)/nm 297.0, 238.0; ν_{max}/cm^{-1} 3119, 2336, 2168, 1646 (CO), 1552 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.47-7.53 (3H, m, H-Ar), 7.74 (1H, br s, H-3), 7.81 (1H, dd, *J* = 1.8 and 6.0 Hz, CH-pyrimidine), 8.18 (1H, dd, *J* = 1.8 and 6.0 Hz, CH-pyrimidine), 8.69 (1H, d, *J* = 6.0 Hz, CH-pyrimidine) 8.92 (1H, br s, H-5), 11.14 (1H, br s, CONH), 12.76 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 110.4 (C-Ar), 114.6 (C-Ar), 124.8 (C-3), 127.0 (C-2 and C-5), 128.6 (C-4), 130.2 (C-Ar), 131.4 (C-Ar), 132.3 (C-Ar), 141.6 (C-Ar), 158.2 (C-Ar), 170.3 (CON), 187.4 (CO; LRMS (ES^+) *m/z* 361.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{10}{}^{35}Cl_2N_4O_2$ $[M+H]^+$ 361.0258, found 361.0258.

4-(2-Chloro-6-fluorobenzoyl)-N-(pyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (160)



Compound **160** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), MeCN (2 mL), 4-aminopyrimidine (89 mg, 0.93 mmol) and PCl_3 (32 µL, 0.37 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (76 mg, 60%); R_f = 0.66 (5% MeOH in EtOAc); M.p. 205-206 ^oC; λ_{max} (EtOH)/nm 293.0, 241.0; ν_{max}/cm^{-1} 3123, 2961, 2159, 1976, 1636 (CO), 1555 (CONH), 1497; ^1H NMR (500 MHz, DMSO-d_6) δ 7.39-7.43 (1H, m, H-3'), 7.47-7.79 (1H, m, H-5'), 7.57-7.62 (2H, m, H-4' and CH-pyrimidine), 7.74 (1H, s, H-3), 8.18 (1H, dd, *J* = 1.2 and 5.8 Hz, CH-pyrimidine), 8.69 (1H, d, *J* = 5.8 Hz, CH-pyrimidine), 8.92 (1H, d, *J* = 1.0 Hz, H-5), 11.14 (1H, s, CONH), 12.82 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 110.5 (C-Ar), 114.1 (C-Ar), 114.9 (C-Ar), 115.0 (d, J_{CF} = 21.9 Hz, C-Ar), 125.5 (C-3), 125.8 (C-2 and C-5), 131.8 (C-4), 158.2 (d, J_{CF} = 204.1 Hz, CF), 174.0 (CON), 184.0 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.4; LRMS (ES^+) *m/z* 345.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{10}{}^{35}ClFN_4O_2$ $[M+H]^+$ 345.0553, found 345.0554.

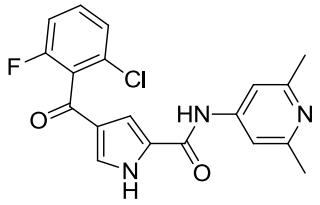
4-(2-Bromo-6-fluorobenzoyl)-*N*-(pyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (161)



Compound **161** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**157**) (150 mg, 0.48 mmol), MeCN (2 mL), 4-aminopyrimidine (114 mg, 1.20 mmol) and PCl_3 (42 µL, 0.48 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (116 mg, 62%); R_f = 0.70 (5% MeOH in EtOAc); M.p. 210-211 ^oC; λ_{max} (EtOH)/nm 294.0, 242.0; ν_{max}/cm^{-1} 3119, 2968, 1639 (CO), 1572 (CONH), 1500; ^1H NMR (500 MHz, DMSO-d_6) δ 7.42-7.45

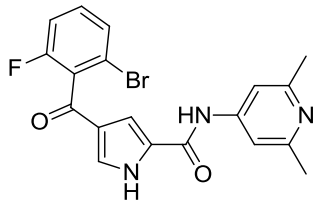
(1H, m, H-5'), 7.51 (1H, ddd, J = 6.0, 8.4 and 8.4 Hz, H-4'), 7.56 (1H, s, CH-pyrimidine), 7.60-7.62 (1H, m, H-Ar), 7.74 (1H, s, H-3), 8.18 (1H, dd, J = 1.4 and 5.8 Hz, CH-pyrimidine), 8.69 (1H, d, J = 5.8 Hz, CH-pyrimidine), 8.92 (1H, s, H-5), 11.35 (1H, s, CONH), 12.89 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 110.5, 114.2 (C-Ar), 115.2 (d, J_{CF} = 21.9 Hz, C-Ar), 119.0 (d, J_{CF} = 5.3 Hz, C-Ar), 125.1 (C-3), 127.3 (C-2 and C-5), 128.8 (C-4), 129.8, 130.0 (d, J_{CF} = 21.9 Hz, C-Ar), 132.1, 158.2 (d, J_{CF} = 247.3 Hz, CF), 174.5 (CON), 184.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 391.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{10}{}^{79}BrFN_4O_2$ $[M+H]^+$ 389.0048, found 389.0048.

4-(2-Chloro-6-fluorobenzoyl)-*N*-(2,6-dimethylpyridin-4-yl)-1*H*-pyrrole-2-carboxamide (162)



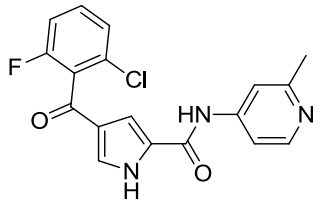
Compound **162** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), MeCN (2 mL), 4-amino-2,6-methylpyridine (226 mg, 1.86 mmol) and PCl_3 (32 µL, 0.37 mmol) with microwave irradiation at 150 ^{o}C for 45 min, to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a beige solid (69 mg, 50%); R_f = 0.65 (5% MeOH in EtOAc); M.p. 250 ^{o}C (dec.); λ_{max} (EtOH)/nm 295.5, 271.5; ν_{max}/cm^{-1} 3199, 3122, 2945, 2799, 1599 (CO), 1570 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.39 (6H, s, 2 x CH_3), 7.40-7.43 (3H, m, H-3' and 2 x CH-pyridine), 7.48-7.52 (3H, m, H-3, H-5 and H-5'), 7.60 (1H, ddd, J = 6.3, 8.4 and 8.4 Hz, H-4'), 10.16 (1H, s, CONH), 12.69 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.1 (2 x CH_3), 105.9 (2 x C-pyridine), 110.0, 112.1 (C-Ar), 115.0 (d, J_{CF} = 20.9 Hz, C-Ar), 125.3 (C-3), 125.9 (C-5), 128.2 (C-2), 129.6 (C-4), 131.8 (d, J_{CF} = 9.5 Hz, C-Ar), 146.3 (C-Ar), 157.6 (2 x C-N-pyridine), 158.5 (d, J_{CF} = 248.1 Hz, CF), 158.9 (CON), 186.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 372.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{15}{}^{35}ClFN_3O_2$ $[M+H]^+$ 372.0913 found 372.0913.

4-(2-Bromo-6-fluorobenzoyl)-*N*-(2,6-dimethylpyridin-4-yl)-1*H*-pyrrole-2-carboxamide (163)



Compound **163** was synthesised according to general procedure D using 4-(2-bromo-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**157**) (100 mg, 0.32 mmol), MeCN (2 mL), 4-amino-2,6-methylpyridine (98 mg, 0.80 mmol) and PCl_3 (28 µL, 0.32 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a cream solid (82 mg, 62%); R_f = 0.71 (5% MeOH in EtOAc); M.p. 220 ^oC (dec.); λ_{max} (EtOH)/nm 388.5, 294.5, 271.5; ν_{max}/cm^{-1} 3350, 1641 (CO), 1595 (CONH), 1556, 1522; ^1H NMR (500 MHz, DMSO-d_6) δ 2.39 (6H, s, 2 x CH_3), 7.41 (2H, s, 2 x pyridine CH), 7.44-7.46 (1H, m, H-5'), 7.48 (1H, s, H-3), 7.51-7.54 (2H, m, H-3 and H-4'), 7.62 (1H, d, *J* = 8.0 Hz, H-3'), 10.16 (1H, s, CONH), 12.68 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.1 (2 x CH_3), 110.0 (2 x C-pyridine), 112.1 (C-3), 115.4 (d, J_{CF} = 21.8 Hz, C-Ar), 119.0 (d, J_{CF} = 4.5 Hz, C-Ar), 124.9 (C-2 and C-5), 128.0 (C-4), 128.9 (d, J_{CF} = 2.9 Hz, C-Ar), 129.6 (d, J_{CF} = 21.8 Hz, C-Ar), 132.2 (d, J_{CF} = 9.2 Hz, C-Ar), 157.6 (2 x C-N-pyridine), 158.4 (d, J_{CF} = 247.7 Hz, CF), 158.9 (CON), 184.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 417.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{15}{}^{79}BrFN_3O_2$ $[M+H]^+$ 416.0405, found 416.0404.

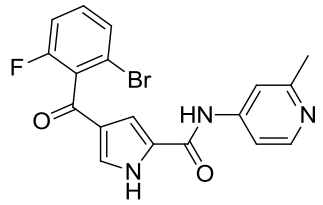
4-(2-Chloro-6-fluorobenzoyl)-*N*-(2-methylpyridin-4-yl)-1*H*-pyrrole-2-carboxamide (164)



Compound **164** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), MeCN (2 mL), 4-amino-2-methylpyridine (101 mg, 0.93 mmol) and PCl_3 (32 µL, 0.37 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a pale yellow solid (79 mg, 60%); R_f = 0.60 (5% MeOH in EtOAc); M.p. 230-231 ^oC; λ_{max} (EtOH)/nm 345.0, 294.0, 271.0; ν_{max}/cm^{-1}

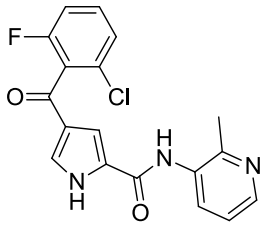
3365, 1635, 1607 (CO), 1560 (CONH), 1509, 1446; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.44 (3H, s, CH_3), 7.39-7.43 (1H, m, H-5'), 7.48 (1H, d, J = 8.1 Hz, H-3'), 7.51 (1H, s, H-3), 7.53 (1H, s, H-5), 7.56-7.62 (3H, m, H-4' and 2 x CH-pyridine), 8.33 (1H, d, J = 5.5 Hz, CH-N-pyridine) 10.25 (1H, s, CONH), 12.73 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.3 (CH_3), 111.1 (C-Ar), 112.0(C-Ar), 114.9 (d, J_{CF} = 22.4 Hz, C-Ar), 125.3 (C-3), 125.8 (C-2 and C-5), 128.1 (C-4), 129.7 (C-pyridine), 130.4 (C-Ar), 131.8 (d, J_{CF} = 8.4 Hz, C-Ar), 145.9 (C-pyridine), 149.6 (C-pyridine), 158.9 (d, J_{CF} = 241.7 Hz, CF), 173.5 (CON), 183.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 358.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{13}{}^{35}ClFN_3O_2$ $[M+H]^+$ 358.0756, found 358.0756.

4-(2-Bromo-6-fluorobenzoyl)-*N*-(2-methylpyridin-4-yl)-1*H*-pyrrole-2-carboxamide (165)



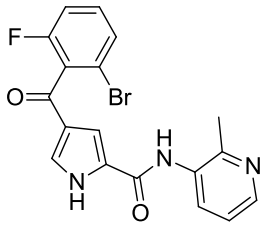
Compound **165** was synthesised according to general procedure D using 4-(2-bromo-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**157**) (100 mg, 0.32 mmol), MeCN (2 mL), 4-amino-2-methylpyridine (86 mg, 0.80 mmol) and PCl_3 (28 µL, 0.32 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as an orange solid (88 mg, 69%); R_f = 0.63 (5% MeOH in EtOAc); M.p. 205-206 ^{o}C; λ_{max} (EtOH)/nm 345.0, 295.5, 274.0; ν_{max}/cm^{-1} 3118, 2966, 1643, 1600 (CO), 1553 (CONH), 1502; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.48 (3H, s, CH_3), 7.42-7.46 (1H, m, H-5'), 7.50-7.56 (3H, m, CH-pyridine and H-4' and H-3), 7.61 (1H, d, J = 8.2 Hz, H-3'), 7.67-7.69 (2H, m, H-5 and CH-pyridine), 8.38 (1H, d, J = 5.7 Hz, CH-N-pyridine) 10.44 (1H, s, CONH), 12.77 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 23.3 (CH_3), 111.5 (C-Ar), 112.5 (C-Ar), 115.3 (d, J_{CF} = 22.5 Hz, C-Ar), 125.0 (C-3), 127.8 (C-2 and C-5), 128.9 (C-4), 130.0 (C-Ar), 132.2 (d, J_{CF} = 9.3 Hz, C-Ar), 157.5 (C-Ar), 159.0 (d, J_{CF} = 246.7 Hz, CF), 171.0 (CON), 184.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 403.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{13}{}^{79}BrFN_3O_2$ $[M+H]^+$ 402.0250, found 402.0250.

4-(2-Chloro-6-fluorobenzoyl)-*N*-(2-methylpyridin-3-yl)-1*H*-pyrrole-2-carboxamide (166)



Compound **166** was synthesised according to general procedure D using 4-(2-chloro-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**156**) (150 mg, 0.56 mmol), 3-amino-2-methylpyridine (152 mg, 1.40 mmol), PCl_3 (49 µL, 0.56 mmol) and MeCN (2 mL). The crude product was purified by MPLC (0-80% EtOAc in petrol) The product was obtained as a pale orange solid (120 mg, 60%); R_f = 0.58 (5% MeOH in EtOAc); M.p. 235-236 ^oC; λ_{max} (EtOH)/nm 240, 289; ν_{max}/cm^{-1}: 3266, 1635 (CO), 1557 (CONH), 1518; ^1H NMR (500 MHz, DMSO-d_6) δ 2.44 (3H, s, CH_3), 7.28 (1H, dd, *J* = 4.4 and 7.9 Hz, CH-pyridine), 7.38-7.48 (4H, m, H-3, CH-pyridine, H-3' and H-5'), 7.58 (1H, ddd, J = 6.3, 8.1 and 8.1 Hz, H-4'), 7.73 (1H, s, H-5), 8.93 (1H, d, *J* = 4.4 Hz, CH-N-pyridine), 9.90 (1H, s, CONH), 12.66 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.7 (CH_3), 106.1(C-pyridine), 114.1 (C-Ar), 115.2 (C-Ar), 119.0 (C-3), 125.1 (C-2 and C-5), 127.4 (C-4), 128.8 (C-pyridine), 132.2 (C-pyridine), 158.4 (d, J_{CF} = 251.0 Hz, CF), 159.4 (CONH), 184.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 358.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{13}{}^{79}ClFN_3O_2$ $[M+H]^+$ 358.0758, found 358.0758.

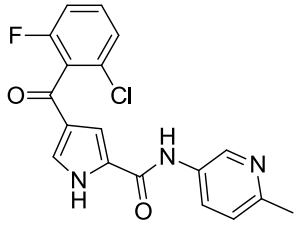
4-(2-Bromo-6-fluorobenzoyl)-*N*-(2-methylpyridin-3-yl)-1*H*-pyrrole-2-carboxamide (167)



Compound **167** was synthesised according to general procedure D using 4-(2-bromo-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**157**) (150 mg, 0.48 mmol), 3-amino-2-methylpyridine (129 mg, 1.20 mmol), PCl_3 (42 µL, 0.48 mmol) and MeCN (2 mL). The crude product was purified by MPLC (0-80% EtOAc in petrol) The product was obtained as a cream solid (104 mg, 52%); R_f = 0.62 (5% MeOH in EtOAc); M.p. 204-205 ^oC; λ_{max} (EtOH)/nm 237, 289; ν_{max}/cm^{-1}: 3171, 1637 (CO), 1520 (CONH); ^1H NMR

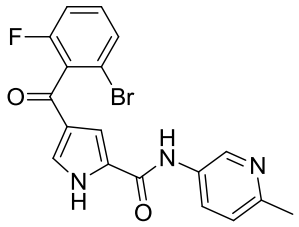
(500 MHz, DMSO-d_6) δ 2.44 (3H, s, CH_3), 7.28 (1H, dd, *J* = 4.7 and 7.9 Hz, CH-pyridine), 7.40-7.45 (3H, m, H-3, H-5 and H-5'), 7.51 (1H, ddd, *J* = 6.4, 8.7 and 8.7 Hz, H-4'), 7.61 (1H, d, *J* = 7.8 Hz, H-3'), 7.72 (1H, d, *J* = 7.9 Hz, CH-pyridine), 8.34 (1H, dd, *J* = 1.4 and 4.7 Hz, CH-N-pyridine), 9.91 (1H, s, CONH), 12.66 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.6 (CH_3), 110.2 (C-pyridine), 114.9 (C-Ar), 116.0 (C-Ar), 122.0 (C-3), 126.5 (C-2 and C-5), 128.3 (C-4), 128.9 (C-Ar), 132.2 (C-yridine), 138.9 (C-pyridine), 158.9 (d, J_{CF} = 249.6 Hz, CF), 159.5 (CON), 189.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 400.1 $[M-H]^-$; HRMS *m/z* calcd for $C_{18}H_{13}{}^{81}BrFN_3O_2$ $[M+H]^+$ 402.0246, found 402.0246.

4-(2-Chloro-6-fluorobenzoyl)-*N*-(6-methylpyridin-3-yl)-1*H*-pyrrole-2-carboxamide (168)



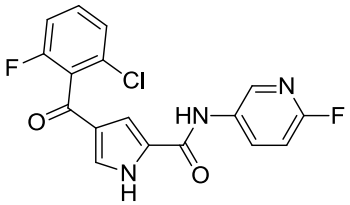
Compound **168** was synthesised according to general procedure D using 4-(2-chloro-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**156**) (150 mg, 0.56 mmol), 5-amino-2-methylpyridine (152 mg, 1.40 mmol), PCl_3 (49 µL, 0.56 mmol) and MeCN (2 mL). The crude product was purified by MPLC (0-80% EtOAc in petrol) The product was obtained as a pale orange solid (104 mg, 52%); R_f = 0.57 (5% MeOH in EtOAc); M.p. 234-235 ^oC; λ_{max} (EtOH)/nm 239, 286; ν_{max}/cm^{-1}: 3271, 1633 (CO), 1519 (CONH), 1496; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.25 (3H, s, CH_3), 7.33 (1H, d, *J* = 4.9 Hz, CH-pyridine), 7.39-7.48 (4H, m, H-3, H-5, H-3' and H-5'), 7.58 (1H, ddd, J = 6.2, 8.3 and 8.3 Hz, H-4'), 8.32 (1H, d, *J* = 4.9 Hz, CH-pyridine), 8.46 (1H, s, CH-N-pyridine), 9.95 (1H, s, CONH), 12.67 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.8 (CH_3), 106.2 (C-pyridine), 115.0 (C-Ar), 115.2 (C-Ar), 121.0 (C-3) , 125.1 (C-2 and C-5), 127.5 (C-4), 129.0 (C-pyridine), 132.2 (C-pyridine), 159.4 (d, J_{CF} = 248.6 Hz, CF), 160.1 (CON), 184.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 358.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{13}{}^{79}ClFN_3O_2$ $[M+H]^+$ 358.0758, found 358.0758.

4-(2-Bromo-6-fluorobenzoyl)-*N*-(6-methylpyridin-3-yl)-1*H*-pyrrole-2-carboxamide (169)



Compound **169** was synthesised according to general procedure D using 4-(2-bromo-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**157**) (150 mg, 0.48 mmol), 3-amino-2-methylpyridine (129 mg, 1.20 mmol), PCl_3 (42 µL, 0.48 mmol) and MeCN (2 mL). The crude product was purified by MPLC (0-80% EtOAc in petrol) The product was obtained as an orange solid (121 mg, 0.30 mmol, 63%); R_f = 0.65 (5% MeOH in EtOAc); M.p. 206-207 ^oC; λ_{max} (EtOH)/nm 226, 379; ν_{max}/cm^{-1}: 3255, 1621 (CO), 1552 (CONH), 1524; ^1H NMR (500 MHz, DMSO-d_6) δ 2.25 (3H, s, CH_3), 7.33 (1H, d, *J* = 5.1 Hz, CH-pyridine), 7.42 (1H, s, H-3), 7.43-7.45 (2H, m, H-5 and H-5'), 7.51 (1H, ddd, *J* = 6.2, 8.3 and 8.3 Hz, H-4'), 7.61 (1H, d, *J* = 7.9 Hz, H-3'), 8.32 (1H, d, *J* = 5.1 Hz, CH-pyridine), 8.46 (1H, s, CH-N-pyridine), 9.96 (1H, s, CONH), 12.66 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.7 (CH_3), 109.5 (C-pyridine), 113.2 (C-Ar), 114.1 (C-Ar), 114.9 (C-Ar), 119.9 (C-3), 125.5 (C-2 and C-5), 128.4 (C-4), 129.4 (C-Ar), 129.8 (C-pyridine), 132.3 (C-pyridine), 159.6 (d, J_{CF} = 254.6 Hz, CF), 160.4 (CONH), 186.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 400.1 $[M-H]^-$; HRMS *m/z* calcd for $C_{18}H_{13}{}^{81}BrFN_3O_2$ $[M+H]^+$ 402.0250, found 402.0250.

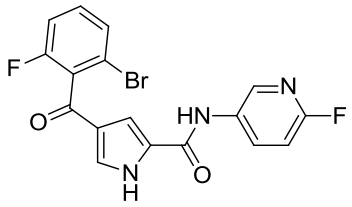
4-(2-Chloro-6-fluorobenzoyl)-*N*-(6-fluoropyridin-3-yl)-1*H*-pyrrole-2-carboxamide (170)



Compound **170** was synthesised according to general procedure D using 4-(2-chloro-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), 5-amino-2-fluoropyridine (103 mg, 0.93 mmol), PCl_3 (32 µL, 0.37 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a cream solid (66 mg, 50%); R_f = 0.42 (5% DCM in MeOH); M.p. 267-

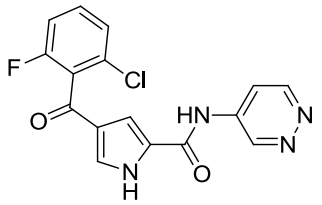
269 °C; λ_{max} (EtOH)/nm 247, 292, 379; ν_{max}/cm^{-1}: 3246, 1624, 1561 (CO), 1526 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.21 (1H, dd, J = 3.3 and 9.0 Hz, CH-pyridine), 7.39-7.43 (1H, m, H-3'), 7.46-7.49 (3H, m, H-5', H-3 and H-5), 7.59 (1H, ddd, J = 6.1, 8.4 and 8.4 Hz, H-4'), 8.26-8.29 (1H, m, CH-pyridine), 8.54 (1H, s, CH-N-pyridine), 10.30 (1H, s, CONH), 12.74 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 109.5 (C-pyridine), 113.0 (C-Ar), 120.5 (C-Ar), 120.8 (C-3), 126.1 (C-2 and C-5), 126.5 (C-pyridine), 130.4 (C-4), 135.6 (C-Ar), 140.0 (C-pyridine), 149.5 (d, J_{CF} = 245.0 Hz, CF), 153.0 (d, J_{CF} = 256.2 Hz, CF), 163.0 (CON), 189.2 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.0, -74.1; LRMS (ES$^+$) *m/z* 362.30 [M+H]$^+$; HRMS *m/z* calcd for $C_{17}H_{10}{}^{35}ClF_2N_3O_2$ [M+H]$^+$ 362.0508, found 362.0508.

4-(2-Bromo-6-fluorobenzoyl)-*N*-(6-fluoropyridin-3-yl)-1*H*-pyrrole-2-carboxamide (171)



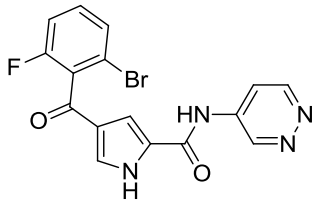
Compound **171** was synthesised according to general procedure D using 4-(2-bromo-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**157**) (100 mg, 0.37 mmol), 5-amino-2-fluoropyridine (103 mg, 0.93 mmol), PCl_3 (32 µL, 0.37 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a cream solid (58 mg, 45%); R_f = 0.46 (5% DCM in MeOH); M.p. 270-271 °C; λ_{max} (EtOH)/nm 258, 290, 379; ν_{max}/cm^{-1}: 3237, 1624 (CO), 1561 (CONH), 1526; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.21 (1H, dd, J = 3.2 and 8.9 Hz, CH-pyridine), 7.42-7.46 (3H, m, H-5', H-3 and H-5), 7.52 (1H, ddd, J = 6.0, 8.3 and 8.3 Hz, H-4'), 7.62 (1H, d, J = 8.3 Hz, H-3'), 8.26-8.30 (1H, m, CH-pyridine), 8.54 (1H, s, CH-N-pyridine), 10.33 (1H, s, CONH), 12.72 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 108.4 (C-pyridine), 114.0 (C-Ar), 120.9 (C-Ar), 121.0 (C-3), 125.9 (C-2 and C-5), 126.7 (C-pyridine), 131.0 (C-4), 136.6 (C-Ar), 143.2 (C-pyridine), 150.1 (d, J_{CF} = 245.0 Hz, CF), 153.6 (d, J_{CF} = 256.2 Hz, CF), 162.1 (CON), 191.0 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.8, -74.3; LRMS (ES$^+$) *m/z* 406.30 [M+H]$^+$; HRMS *m/z* calcd for $C_{17}H_{10}{}^{79}BrF_2N_3O_2$ [M+H]$^+$ 405.9999, found 405.9999.

4-(2-Chloro-6-fluorobenzoyl)-*N*-(pyridazin-4-yl)-1*H*-pyrrole-2-carboxamide (172)



Compound **172** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), MeCN (2 mL), 4-aminopyridazine (88 mg, 0.93 mmol) and PCl_3 (32 µL, 0.37 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a beige solid (82 mg, 65%); R_f = 0.51 (5% MeOH in EtOAc); M.p. 200-202 ^oC; λ_{max} (EtOH)/nm 345.0, 296.0; ν_{max}/cm^{-1} 3088, 1636, 1551 (CO), 1519 (CONH), 1445; ^1H NMR (500 MHz, DMSO-d_6) δ 7.39-7.42 (1H, m, H-5'), 7.48 (1H, d, *J* = 8.3 Hz, H-3'), 7.54 (1H, s, H-3), 7.57 (1H, s, H-5), 7.60 (1H, ddd, *J* = 6.3, 8.3 and 8.3 Hz, H-4'), 8.05 (1H, dd, *J* = 2.7 and 5.9 Hz, CH-pyridazine), 9.06 (1H, dd, *J* = 1.0 and 5.9 Hz, CH-N-pyridazine) 9.47 (1H, dd, *J* = 1.0 and 2.7 Hz, CH-N-pyridazine), 10.58 (1H, s, CONH), 12.87 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.8 (C-pyridazine), 114.0 (C-Ar), 114.9 (d, J_{CF} = 21.8 Hz, C-Ar), 115.1 (C-Ar), 120.4 (C-3), 125.9 (C-2 and C-5), 131.9 (C-4), 138.1 (d, J_{CF} = 9.0 Hz, C-Ar), 143.9 (C-N-pyridazine), 151.4 (d, J_{CF} = 243.8 Hz, CF), 177.7 (CON), 186.8 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 345.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{10}{}^{35}ClFN_4O_2$ $[M+H]^+$ 345.0553, found 345.0554.

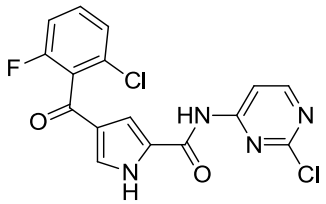
4-(2-Bromo-6-fluorobenzoyl)-*N*-(pyridazin-4-yl)-1*H*-pyrrole-2-carboxamide (173)



Compound **173** was synthesised according to general procedure D using 4-(2-bromo-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**157**) (100 mg, 0.32 mmol), MeCN (2 mL), 4-aminopyridazine (76 mg, 0.80 mmol) and PCl_3 (28 µL, 0.37 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a orange solid (76 mg, 61%); R_f = 0.56 (5% MeOH in EtOAc); M.p. 199-200 ^oC; λ_{max} (EtOH)/nm 298.5; ν_{max}/cm^{-1} 3119, 1988, 1637 (CO),

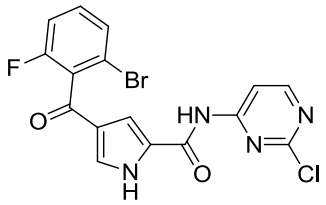
1600 (CONH), 1552, 1520; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.43-7.46 (1H, m, H-5'), 7.50-7.55 (3H, m, H-3, H-5 and H-4'), 7.62 (1H, d, J = 7.8 Hz, H-3'), 8.05 (1H, dd, J = 2.7 and 6.0 Hz, CH-pyridazine), 9.06 (1H, d, J = 6.0Hz, CH-N-pyridazine) 9.48 (1H, dd, J = 1.0 and 2.7 Hz, CH-N-pyridazine), 10.58 (1H, s, CONH), 12.86 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.8 (C-pyridazine), 114.0(C-Ar), 115.5 (d, J_{CF} = 21.9 Hz, C-Ar), 124.4 (C-3), 126.8 (C-2 and C-5), 128.9 (C-4), 138.1 (d, J_{CF} = 8.6 Hz, C-Ar), 140.7 (C-N-pyridazine), 143.6 (C-Ar), 151.4 (d, J_{CF} = 248.8 Hz, CF), 173.6 (CON), 186.6 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES$^+$) *m/z* 391.2 [M+H]$^+$; HRMS *m/z* calcd for $C_{16}H_{10}{}^{35}ClFN_4O_2$ [M+H]$^+$ 389.0035, found 389.0034.

4-(2-Chloro-6-fluorobenzoyl)-*N*-(2-chloropyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (174)



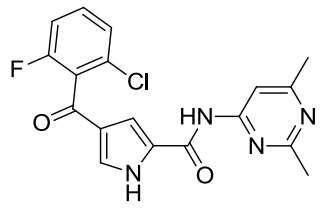
Compound **174** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), MeCN (2 mL), 4-amino-2-chloropyrimidine (120 mg, 0.93 mmol) and PCl_3 (32 µL, 0.37 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (77 mg, 0.20 mmol, 55%); R_f = 0.65 (5% MeOH in EtOAc); M.p. 215-216 ^{o}C; λ_{max} (EtOH)/nm 301.0, 243.0; ν_{max}/cm^{-1} 3112, 2978, 1645 (CO), 1554 (CONH), 1498; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.39-7.43 (1H, m, H-5'), 7.48 (1H, d, J = 7.8 Hz, H-3'), 7.57-7.62 (2H, m, H-3 and H-4') 7.77 (1H, s, H-5), 8.18 (1H, d, J = 5.8 Hz, CH-pyrimidine), 8.63 (1H, d, J = 5.8 Hz, CH-N-pyrimidine), 11.49 (1H, s, CONH), 12.85 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 109.1 (C-pyrimidine), 113.0 (C-Ar), 114.8 (d, J_{CF} = 22.4Hz, C-Ar), 115.0 (C-Ar), 125.8 (C-3), 126.9 (C-2 and C-5), 130.1 (C-4), 149.2 (C-N-pyrimidine), 160.2 (C-Ar), 161.0 (d, J_{CF} = 243.6 Hz, CF), 169.0 (C-pyrimidine), 176.1 (CON), 180.4 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES$^+$) *m/z* 379.2 [M+H]$^+$; HRMS *m/z* calcd for $C_{16}H_9{}^{35}Cl_2FN_4O_2$ [M+H]$^+$ 379.0152, found 379.0152.

4-(2-Bromo-6-fluorobenzoyl)-*N*-(2-chloropyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (175)



Compound **175** was synthesised according to general procedure D using 4-(2-bromo-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**157**) (100 mg, 0.32 mmol), MeCN (2 mL), 4-amino-2-chloropyrimidine (104 mg, 0.80 mmol) and PCl_3 (28 µL, 0.32 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (78 mg, 58%); Rf = 0.69 (5% MeOH in EtOAc); M.p. 218-219 ^{o}C; λ_{max} (EtOH)/nm 298.5, 241.5; ν_{max}/cm^{-1} 3278, 1646, 1555, 1498; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.44 (1H, dd, *J* = 1.0 and 9.0 Hz, H-5'), 7.52 (1H, ddd, *J* = 6.2, 8.2 and 8.2 Hz, H-4'), 7.60-7.63 (2H, m, H-3 and H-3') 7.76 (1H, s, H-5), 8.18 (1H, d, *J* = 5.8 Hz, CH-pyrimidine), 8.62 (1H, d, *J* = 5.8 Hz, CH-N-pyrimidine), 11.50 (1H, s, CONH), 12.84 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 109.1 (C-pyrimidine), 114.8 (C-Ar), 115.2, (C-Ar), 115.8 (d, J_{CF} = 21.0 Hz, C-Ar), 120.8 (C-3), 125.2 (C-2 and C-5), 128.8 (C-4), 143.2 (C-N-pyrimidine), 159.4 (C-Ar), 161.0 (d, J_{CF} = 235.4 Hz, CF), 162.0 (C-pyrimidine), 170.4 (CON), 180.4 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^{+}) *m/z* 425.1 [M+H]$^{+}$; HRMS *m/z* calcd for $C_{16}H_9{}^{79}Br^{35}ClFN_4O_2$ [M+H]$^{+}$ 422.9647, found 422.9647.

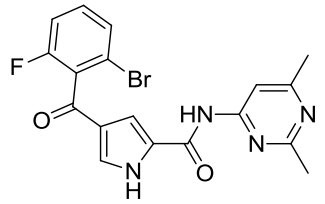
4-(2-Chloro-6-fluorobenzoyl)-*N*-(2,6-dimethylpyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (176)



Compound **176** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**156**) (150 mg, 0.56 mmol), MeCN (2 mL), 4-amino-2,6-dimethylpyrimidine (172 mg, 1.40 mmol) and PCl_3 (49 µL, 0.56 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as an orange solid (100 mg, 48%); R_f = 0.54 (5% MeOH in EtOAc); M.p. 220-221 ^{o}C; λ_{max} (EtOH)/nm 243.0, 293.5; ν_{max}/cm^{-1} 3130, 1653 (CO), 1553 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.45 (3H, s, CH_3),

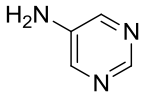
2.54 (3H, s, NCH_3N), 7.41 (1H, dd, J = 1.0 and 9.0 Hz, H-5'), 7.48 (1H, d, J = 8.1 Hz, H-3'), 7.57-7.61 (2H, m, H-4' and H-3), 7.75 (1H, s, H-5), 7.96 (CH-pyrimidine), 11.20 (CONH), 12.78 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.7 (CH_3), 59.7 (NCH_3N), 106.2 (C-pyrimidine), 114.3 (C-Ar), 114.8 (d, J_{CF} = 21.8 Hz, C-Ar), 115.0 (C-Ar), 125.4 (C-Ar), 127.2 (C-3), 129.9 (C-2 and C-5), 130.4 (C-4), 131.8 (d, J_{CF} = 9.0 Hz, C-Ar), 157.6 (d, J_{CF} = 248.7 Hz, CF), 143.4 (C-N-pyrimidine), 159.5 (CON), 184.0 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 373.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{15}{}^{35}ClFN_4O_2$ $[M+H]^+$ 373.0862, found 373.0861.

4-(2-Bromo-6-fluorobenzoyl)-*N*-(2,6-dimethylpyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (177)



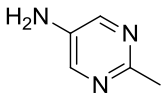
Compound **177** was synthesised according to general procedure D using 4-(2-bromo-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**157**) (150 mg, 0.48 mmol), MeCN (2 mL), 4-amino-2,6-dimethylpyrimidine (147 mg, 1.20 mmol) and PCl_3 (42 µL, 0.48 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a cream solid (110 mg, 55%); R_f = 0.67 (5% MeOH in EtOAc); M.p. 215-216 °C; λ_{max} (EtOH)/nm 242.5, 295.5; ν_{max}/cm^{-1} 3127, 1652, 1601 (CO), 1553 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.42 (3H, s, CH_3), 2.50 (3H, s, NCH_3N), 7.41 (1H, dd, J = 1.0 and 9.0 Hz, H-5'), 7.51 (1H, ddd, J = 6.3, 8.1 and 8.1 Hz, H-4'), 7.55 (1H, s, H-3), 7.61 (1H, d, J = 8.1 Hz, H-3'), 7.73 (1H, s, H-5), 7.90 (CH-pyrimidine), 11.04 (CONH), 12.73 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 20.7 (CH_3), 59.7 (NCH_3N), 106.1 (C-pyrimidine), 114.1 (C-Ar), 115.2 (d, J_{CF} = 21.8 Hz, C-Ar), 119.0 (d, J_{CF} = 4.6 Hz, CF), 125.1 (C-Ar), 127.4 (C-3), 128.8 (C-2 and C-5), 132.2 (C-4), 149.2 (C-N-pyrimidine), 159.4 (CON), 184.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 415.2 $[M-H]^-$; HRMS *m/z* calcd for $C_{18}H_{15}{}^{81}BrFN_4O_2$ $[M+H]^+$ 417.0357, found 417.0359.

Pyrimidin-5-amine (180)



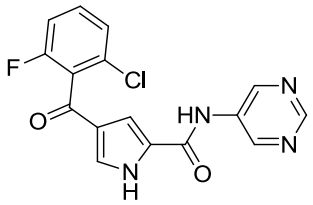
5-Amino-4,6-dichloropyrimidine (500 mg, 3.05 mmol) was dissolved in MeOH (30 mL) before NH_4HCOO (1.92 g, 30.5 mmol) was added followed by Pd/C (50 mg, 0.1% w/w). The resulting mixture was heated to reflux for 1 h. Upon cooling, the reaction was filtered through Celite and the solvent removed *in vacuo*. The crude product was purified by MPLC (silica; 0-20% MeOH in DCM) to give the product as a yellow solid (232 mg, 80%); R_f = 0.32 (10% MeOH in DCM); M.p. 168-169 ^oC (lit. 165-166 ^oC); λ_{max} (EtOH)/nm 314, 245; ν_{max}/cm^{-1}: 3178 (NH_2), 1662, 1554; ^1H NMR (500 MHz, DMSO-d_6) δ 5.54 (2H, br s, NH_2), 8.09 (2H, s, H-4 and H-6), 8.35 (1H, s, H-2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 141.4 (C-4 and C-6), 143.0 (C-5), 146.7 (C-2); LRMS (ES^+) *m/z* 96.1 $[M+H]^+$.

2-Methylpyrimidin-5-amine (181)



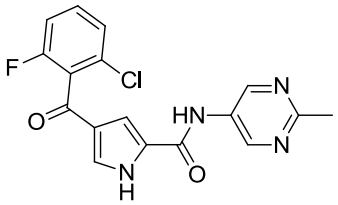
5-Amino-4,6-dichloro-2-methylpyrimidine (3.0 g, 16.9 mmol) was dissolved in MeOH (100 mL) before ammonium formate (10.6 g, 169 mmol) was added followed by Pd/C (300 mg, 10% w/w). The reaction was heated to reflux for 1 h before cooling and being filtered through celite. The solvent was removed in vacuo and the crude mixture was purified by MPLC (0-20% MeOH in DCM) to give the title compound as a beige oil (1.48 g, 80%); R_f = 0.35 (10% MeOH in DCM); λ_{max} (EtOH)/nm 244, 317; ν_{max}/cm^{-1}: 3016 (NH_2), 2118, 1635, 1555; ^1H NMR (500 MHz, DMSO-d_6) δ 2.39 (3H, s, CH_3), 5.28 (2H, br s, NH_2), 8.01 (2H, s, C-pyrimidine), 8.43 (1H, s, C-pyrimidine);^{13}C NMR (125 MHz, DMSO-d_6) δ 22.0 (CH_3), 140.1 (C-pyrimidine), 145.2 (C-N-pyrimidine), 157.0 (N-C-N-pyrimidine); LRMS (ES^+) *m/z* 110.1 $[M+H]^+$.

4-(2-Chloro-6-fluorobenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (182)



Compound **181** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), MeCN (2 mL), 5-aminopyrimidine (**180**) (88 mg, 0.93 mmol) and PCl_3 (32 µL, 0.37 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as an orange solid (100 mg, 79%); R_f = 0.52 (5% MeOH in EtOAc); M.p. 227 ^oC (dec.); λ_{max} (EtOH)/nm 262.0, 292.0; ν_{max}/cm^{-1} 2960, 2862, 1968, 1637 (CO), 1529 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.42 (1H, dd, J = 1.0 and 9.0 Hz, H-5'), 7.48 -7.50 (2H, m, H-3' and H-3), 7.55 (1H, s, H-5), 7.60 (1H, ddd, J = 6.3, 8.3 and 8.3 Hz, H-4'), 8.92 (1H, s, N-CH-N-pyrimidine), 9.13 (2H, s, 2 x CH-N-pyrimidine), 10.44 (1H, s, CONH), 12.93 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.1 (C-pyrimidine), 114.9 (C-Ar), 115.1 (d, J_{CF} = 23.4 Hz, C-Ar), 125.3 (C-Ar), 125.9 (C-3), 127.6 (C-2 and C-5), 129.7 (C-4), 130.4 (d, J_{CF} = 22.8 Hz, C-Ar), 131.9 (d, J_{CF} = 8.6 Hz, C-Ar), 134.3 (C-N-pyrimidine), 147.8 (C-Ar), 153.2 (d, J_{CF} = 245.2 Hz, CF), 158.8 (CON), 183.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 344.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{11}{}^{35}ClFN_4O_2$ $[M+H]^+$ 345.0549, found 345.0550.

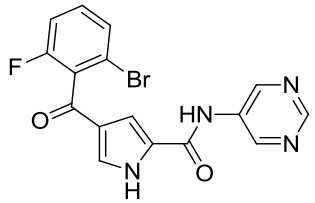
4-(2-Chloro-6-fluorobenzoyl)-*N*-(2-methylpyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (183)



Compound **183** was synthesised according to general procedure D using 4-(2-chloro-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), 2-Methylpyrimidin-5-amine (**181**) (102 mg, 0.93 mmol), PCl_3 (32 µL, 0.37 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a yellow solid (53 mg, 0.14 mmol, 40%); R_f = 0.45 (5% DCM in MeOH); M.p. 270 ^oC (dec.); λ_{max} (EtOH)/nm 268, 290, 379; ν_{max}/cm^{-1}: 3320,

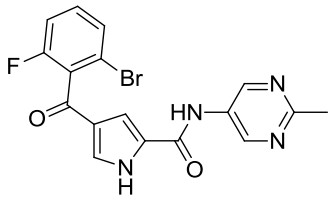
1637 (CO), 1516 (CONH); ^{1}H NMR (500 MHz, MeOD) δ 2.68 (3H, s, CH_3), 7.25-7.28 (1H, m, H-5'), 7.40 (1H, d, *J* = 8.5 Hz, H-3'), 7.44 (1H, d, *J* = 1.5 Hz, H-3), 7.49 (1H, d, *J* = 1.5 Hz, H-5), 7.53 (1H, ddd, *J* = 6.1, 8.5 and 8.5 Hz, H-4'), 9.07 (2H, s, CH-pyrimidine); ^{13}C NMR (125 MHz, MeOD) δ 25.0 (CH_3), 108.4 (C-pyrimidine), 115.0 (C-Ar), 121.9 (C-Ar), 122.0 (C-3), 126.5 (C-2 and C-5), 127.2 (C-pyrimidine), 131.0 (C-4), 135.6 (C-Ar), 143.2 (C-N-pyridine), 156.7 (N-C-N-pyrimidine), 154.6 (d, J_{CF} = 253.2 Hz, CF), 159.8 (CON), 187.0 (CO);^{19}F NMR (470 MHz, DMSO-d_6) δ -115.3; LRMS (ES^+) *m/z* 359.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{12}{}^{35}ClFN_4O_2$ $[M+H]^+$ 359.0710, found 359.0710.

4-(2-Bromo-6-fluorobenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (184)



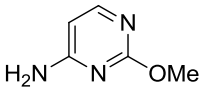
Compound **181** was synthesised according to general procedure D using 4-(2-bromo-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**157**) (100 mg, 0.32 mmol), MeCN (2 mL), 5-aminopyrimidine (**180**) (76 mg, 0.80 mmol) and PCl_3 (28 µL, 0.32 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as an orange solid (75 mg, 60%); R_f = 0.56 (5% MeOH in EtOAc); M.p. 220-221 ^{o}C; λ_{max} (EtOH)/nm 293.0; ν_{max}/cm^{-1} 3339, 1721, 1628 (CO), 1528 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.43-7.46 (2H, H-5' and H-3), 7.50 -7.54 (2H, m, H-4' and H-5), 7.60 (1H, d, *J* = 7.9 Hz, H-3'), 8.92 (1H, s, N-CH-N-pyrimidine), 9.13 (2H, s, 2 x CH-N-pyrimidine), 10.45 (1H, s, CONH), 12.83 (1H, s, NH); δ_C ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.2 (C-pyrimidine), 115.3 (d, J_{CF} = 21.8 Hz, C-Ar), 119.0 (d, J_{CF} = 4.5 Hz, C-Ar), 125.0 (C-Ar), 127.6 (C-3), 128.9 (C-2 and C-5), 129.7 (C-4), 132.2 (d, J_{CF} = 8.4 Hz, C-Ar), 134.3 (d, J_{CF} = 21.8 Hz, C-Ar), 147.8 (C-N-pyrimidine), 153.2 (N-C-N-pyrimidine), 157.5 (C-Ar), 158.8 (d, J_{CF} = 236.8 Hz, CF) 159.4 (CON), 184.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 391.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{11}{}^{79}BrFN_4O_2$ $[M+H]^+$ 389.0047, found 389.0044.

4-(2-Bromo-6-fluorobenzoyl)-*N*-(2-methylpyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (185)



Compound **185** was synthesised according to general procedure D using 4-(2-bromo-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**157**) (100 mg, 0.32 mmol), 2-Methylpyrimidin-5-amine (**181**) (87 mg, 0.80 mmol), PCl_3 (28 µL, 0.32 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a yellow solid (51 mg, 0.13 mmol, 40%); R_f = 0.48 (5% DCM in MeOH); M.p. 280 ^oC (dec.); λ_{max} (EtOH)/nm 294, 379; ν_{max}/cm^{-1}: 3326, 1632 (CO), 1600 (CONH), 1536, 1443, 1242; ^1H NMR (500 MHz, MeOD) δ 2.68 (3H, s, CH_3), 7.28-7.32 (1H, m, H-5'), 7.43-7.49 (3H, m, H-3', H-3 and H-5), 7.54-7.58 (1H, m, H-4'), 9.06 (2H, s, CH-pyrimidine); ^{13}C NMR (125 MHz, MeOD) δ 30.0 (CH_3), 109.9 (C-pyrimidine), 116.0 (C-Ar), 120.8 (C-Ar), 121.5 (C-3), 125.6 (C-2 and C-5), 128.2 (C-pyrimidine), 130.1 (C-4), 136.2 (C-Ar), 145.6 (C-N-pyridine), 155.5 (N-C-N-pyrimidine), 153.1 (d, J_{CF} = 245.3 Hz, CF), 164.0 (CON), 190.0 (CO);^{19}F NMR (470 MHz, DMSO-d_6) δ -115.3; LRMS (ES^+) *m/z* 403.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{12}{}^{79}BrFN_4O_2$ $[M+H]^+$ 403.0203, found 403.0202.

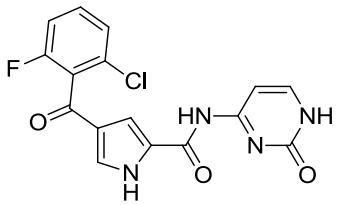
2-Methoxypyrimidin-4-amine (187)



Sodium (78 mg, 3.40 mmol) was dissolved in MeOH (8 mL) and this was allowed to stir at RT for 15 min before 4-amino-2-chloropyrimidine (400 mg, 3.10 mmol) in MeOH (4 mL) was added. The reaction was heated to 50 ^oC for 4 h before being cooled and water added. The product was extracted into EtOAc and the combined organic layers were dried over Na_2SO_4. The solvent was removed *in vacuo* and the crude product purified by MPLC (silica; 0-10% MeOH in DCM) to give the pure product as a white solid (349 mg, 90%) R_f = 0.25 (5% MeOH in DCM); M.p. 167-168 ^oC (lit. 168-169 ^oC); λ_{max} (EtOH)/nm 272, 228; ν_{max}/cm^{-1} 3157 (NH_2), 1652, 1594, 1557; ^1H NMR (500 MHz, DMSO-d_6) δ 3.15 (3H, s, CH_3), 6.13 (1H, d, J = 5.70 Hz, CH-pyrimidine), , 6.91 (2H, br s, NH_2), 7.92 (1H, d, J = 5.70 Hz, CH-N-pyrimidine); ^{13}C NMR (125

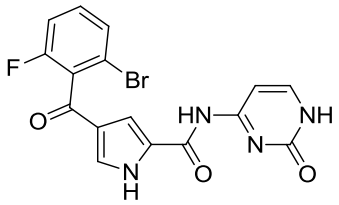
MHz, DMSO-d_6) δ 53.5 (CH_3), 99.3 (C-5), 156.7 (C-6), 165.3 (C-2); LRMS (ES^+) *m/z* 126.1 $[M+H]^+$.

4-(2-Chloro-6-fluorobenzoyl)-*N*-(2-oxo-1,2-dihydropyrimidin-4-yl)-1*H*-pyrrole-2-carboxamide (188)



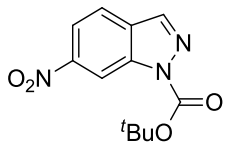
Compound **188** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**156**) (100 mg, 0.37 mmol), MeCN (2 mL), 4-amino-2-methoxypyrimidine (**187**) (116 mg, 0.93 mmol) and PCl_3 (32 µL, 0.37 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a cream solid (40 mg, 30%); R_f = 0.45 (5% MeOH in EtOAc); M.p. 199-200 ^oC; λ_{max} (EtOH)/nm 298.0; ν_{max}/cm^{-1} 3332, 1676 (CO), 1637 (CONH), 1553 (NCONH), 1513, 1449; ^1H NMR (500 MHz, DMSO-d_6) δ 7.22 (1H, br s, H-3), 7.38-7.42 (1H, m, H-5'), 7.47 (1H, d, *J* = 8.1 Hz, H-3'), 7.56-7.61 (2H, m, pyrimidone CH and H-4'), 7.70 (1H, br s, H-5), 7.85 (1H, d, *J* = 6.0 Hz, pyrimidone CH), 11.04 (1H, br s, CONH), 11.60 (1H, br s, pyrimidone NH), 12.77 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 95.2 (C-pyrimidone), 114.5 (C-Ar), 114.8 (C-Ar), 115.0 (d, J_{CF} = 20.8 Hz, C-Ar), 125.5 (C-Ar), 125.8 (C-3), 130.3 (C-2 and C-5), 131.8 (C-4), 157.6 (C-pyrimidone-C and CO-pyrimidone), 159.7 (C-pyrimidone and CON), 184.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.3; LRMS (ES^+) *m/z* 361.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{11}{}^{35}ClFN_4O_3$ $[M+H]^+$ 361.0500, found 361.0500.

4-(2-Bromo-6-fluorobenzoyl)-N-(2-oxo-1,2-dihydropyrimidin-4-yl)-1H-pyrrole-2-carboxamide (189)



Compound **189** was synthesised according to general procedure D using 4-(2-bromo-6-fluoro-benzoyl)-1*H*-pyrrole-2-carboxylic acid (**157**) (100 mg, 0.32 mmol), MeCN (2 mL), 4-amino-2-methoxypyrimidine (**187**) (100 mg, 0.80 mmol) and PCl_3 (28 µL, 0.32 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a cream solid (60 mg, 45%); R_f = 0.47 (5% MeOH in EtOAc); M.p. 195-196 ^oC; λ_{max} (EtOH)/nm 262.0, 295.0, 305.0; ν_{max}/cm^{-1} 3348, 1677 (CO), 1637 (CONH), 1554 (NCONH), 1516; ^1H NMR (500 MHz, DMSO-d_6) δ 7.23 (1H, br s, H-3), 7.41-7.44 (1H, m, H-5'), 7.50 (1H, ddd, *J* = 6.3, 8.2 and 8.2 Hz, H-4'), 7.55 (1H, br s, pyrimidone CH), 7.60 (1H, d, *J* = 6.3 Hz, H-3'), 7.72 (1H, br s, H-5), 7.86 (1H, d, *J* = 6.3 Hz, pyrimidone CH), 11.02 (1H, br s, CONH), 11.60 (1H, br s, pyrimidone NH), 12.79 (1H, br s, NH); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 405.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{11}{}^{79}BrFN_4O_3$ $[M+H]^+$ 404.9993, found 404.9996.

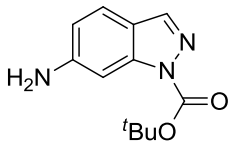
***tert*-Butyl 6-nitro-1*H*-indazole-1-carboxylate (191)**



6-Nitroindazole (1.00 g, 6.13 mmol) was suspended in DCM (25 mL) before triethylamine (0.855 mL, 6.13 mmol) was added followed by Boc_2O (1.34 g, 6.13 mmol). The mixture was left to stir at RT for 18 h before being quenched with water (20 mL). The product was extracted using DCM (3 x 30 mL), the combined organic fractions dried over Na_2SO_4 and the solvent removed *in vacuo.* The crude product was purified by MPLC (0-10% EtOAc in petrol) to give the desired product as a yellow solid (1.48 g, 92%); M.p. 132-133 ^oC (lit.[151] 134-136 ^oC) ; λ_{max} (EtOH)/nm 277, 325; ν_{max}/cm^{-1}: 3067, 2122, 1737 ($CO_2{}^tBu$), 1532 (NO_2); ^1H NMR (500 MHz, DMSO-d_6) δ 1.69 (9H, s, 3 x CH_3), 8.15-8.22 (2H, m, H-4 and H-5), 8.65 (1H, br s, H-7), 8.88 (1H, br s, H-3);^{13}C NMR (125 MHz, DMSO-d_6) δ 28.1 ($C(CH_3)_3$), 77.1 ($C(CH_3)_3$), 111.1 (C-

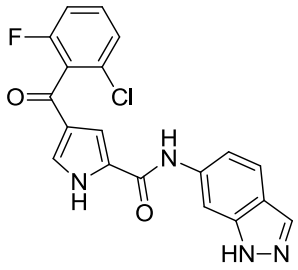
Ar), 118.6 (C-Ar), 121.8 (C-Ar), 129.1 (C-Ar), 138.8 (C-Ar), 138.9 (C-Ar), 148.1 (C-Ar), 180.7 ($CO_2{}^tBu$); LRMS (ES^+) *m/z* 264.2 $[M+H]^+$.

tert-Butyl 6-amino-1*H*-indazole-1-carboxylate (192)



tert-Butyl 6-nitro-1*H*-indazole-1-carboxylate (**191**) (500 mg, 1.90 mmol) was dissolved in THF (38 mL) and the solution was flowed through the H-cube on full H_2 mode at a rate of 1 mL/min continuously for 2.5 h. The solvent was removed *in vacuo* and the crude mixture was purified by MPLC (0-50% EtOAc in petrol) to give the product as a pale yellow solid (399 mg, 90%); M.p. 170-173 ^oC (lit.[151] 171-172 ^oC); λ_{max} (EtOH)/nm 277, 328; ν_{max}/cm^{-1}: 3065 (NH_2), 2096, 1742 ($CO_2{}^tBu$), 1565; ^1H NMR (500 MHz, DMSO-d_6) δ 1.65 (9H, s, 3 x CH_3), 5.20 (2H, br s, NH_2), 8.10-8.19 (2H, m, H-4 and H-5), 8.71 (1H, br s, H-7), 8.89 (1H, br s, H-3);^{13}C NMR (125 MHz, DMSO-d_6) δ 28.1 ($C(CH_3)_3$), 77.1 ($C(CH_3)_3$), 111.1 (C-Ar), 118.6 (C-Ar), 121.8 (C-Ar), 129.1 (C-Ar), 138.8 (C-Ar), 138.9 (C-Ar), 148.1 (C-Ar), 180.7 ($CO_2{}^tBu$); LRMS (ES^+) *m/z* 234.2 $[M+H]^+$.

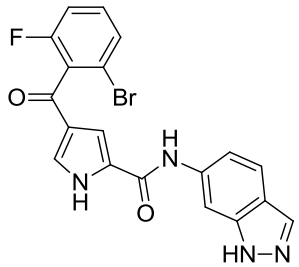
4-(2-Chloro-6-fluorobenzoyl)-*N*-(1*H*-indazol-6-yl)-1*H*-pyrrole-2-carboxamide (195)



Compound **195** was synthesised according to general procedure C using 4-(2-chloro-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**156**) (150 mg, 0.56 mmol), CDI (182 mg, 1.12 mmol), *tert*-Butyl 6-amino-1*H*-indazole-1-carboxylate (**192**) (327 mg, 1.40 mmol) and THF (4 mL). The crude product was purified by MPLC (0-8% MeOH in DCM). After initial purification, a crude mixture remained and this was dissolved in TFA (2 mL) and allowed to stir at RT for 1.5 h. The solvent was removed *in vacuo* and the residue re-dissolved in EtOAc (10 mL) and washed with a saturated aqueous solution of

$NaHCO_3$ (3 x 10 mL). The organic fraction was dried over Na_2SO_4 and the solvent removed *in vacuo*. The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the pure product as a pale yellow solid (32 mg, 16% yield); R_f = 0.40 (5% DCM in MeOH); M.p. 215-216 ^oC; λ_{max} (EtOH)/nm 250, 305; ν_{max}/cm^{-1}: 3322, 3124, 1630 (CO), 1558 (CONH), 1511; ^1H NMR (500 MHz, DMSO-d_6) δ 7.34 (1H, d, *J* = 8.0 Hz, H-3'), 7.39-7.44 (2H, m, H-3 and H-5'), 7.48 (1H, d, *J* = 9.0 Hz, Indazole-H-Ar), 7.59 (1H, ddd, *J* = 5.8, 8.3 and 8.3 Hz, H-4'), 7.70 (1H, d, *J* = 9.0 Hz, Indazole-H-Ar), 7.99 (1H, s, Indazole-H-Ar), 8.20 (1H, s, Indazole-H-Ar), 10.14 (1H, s, CONH), 12.65 (1H, s, NH), 12.94 (1H, s, Indazole-NH); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.7; LRMS (ES^+) *m/z* 383.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{12}{}^{35}ClFN_4O_2$ $[M+H]^+$ 383.0709, found 383.0709.

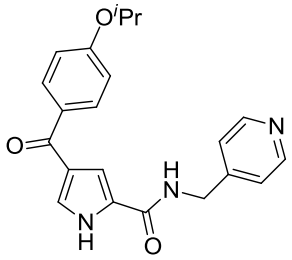
4-(2-Bromo-6-fluorobenzoyl)-*N*-(1*H*-indazol-6-yl)-1*H*-pyrrole-2-carboxamide (196)



Compound **196** was synthesised according to general procedure C using 4-(2-bromo-6-fluorobenzoyl)-1H-pyrrole-2-carboxylic acid (**157**) (150 mg, 0.48 mmol), CDI (155 mg, 0.96 mmol), *tert*-Butyl 6-amino-1*H*-indazole-1-carboxylate (**192**) (280 mg, 1.20 mmol) and THF (4 mL). The crude product was purified by MPLC (0-8% MeOH in DCM). After initial purification, a crude mixture remained and this was dissolved in TFA (2 mL) and allowed to stir at RT for 1.5 h. The solvent was removed *in vacuo* and the residue re-dissolved in EtOAc (10 mL) and washed with a saturated aqueous solution of $NaHCO_3$ (3 x 10 mL). The organic fraction was dried over Na_2SO_4 and the solvent removed *in vacuo*. The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the pure product as a pale yellow solid (32 mg, 16% yield); R_f = 0.40 (5% DCM in MeOH); M.p. 215-216 ^oC; λ_{max} (EtOH)/nm 250, 305; ν_{max}/cm^{-1}: 3271, 3116, 1631 (CO), 1597 (CONH), 1562, 1442; ^1H NMR (500 MHz, DMSO-d_6) δ 7.35 (1H, d, *J* = 8.0 Hz, H-3'), 7.42-7.46 (2H, m, H-3 and H-5'), 7.49-7.54 (2H, m, H-5 and H-4'), 7.62 (1H, d, *J* = 8.9 Hz, Indazole-H-Ar), 7.69 (1H, d, *J* = 8.9 Hz, Indazole-H-Ar), 7.99 (1H, s, Indazole-H-Ar), 8.19 (1H, s, Indazole-H-Ar), 10.16 (1H, s, CONH), 12.64 (1H, s, NH),

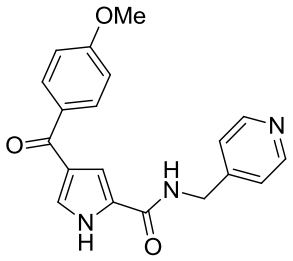
12.94 (1H, s, Indazole-NH); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.6; LRMS (ES^+) *m/z* 425.10 $[M-H]^-$; HRMS *m/z* calcd for $C_{19}H_{12}{}^{81}BrFN_4O_2$ $[M+H]^+$ 427.0202, found 427.0202.

4-(4-Isopropoxybenzoyl)-*N*-(pyridin-4-ylmethyl)-1*H*-pyrrole-2-carboxamide (199)



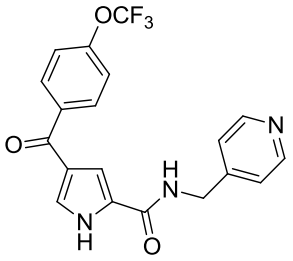
Compound **199** was synthesised according to general procedure C, using 4-(4-isopropoxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (**73**) (150 mg, 0.55 mmol), carbonyldiimidazole (178 mg, 1.10 mmol), 4-picolylamine (139 µL, 1.38 mmol) and THF (3 mL). The crude residue was purified by MPLC (silica, 2-30% MeOH in EtOAc) followed by recrystallisation from EtOAc to give the title compound as a yellow solid (153 mg, 77%); R_f = 0.16 (50% MeOH in EtOAc); M.p. 242-243 ^oC (dec.); λ_{max} (EtOH)/nm 294; ν_{max}/cm^{-1}: 3210, 2606, 2162, 2028, 1632 (CO), 1533 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.31 (6H, d, *J* = 6.0 Hz, CH(C*H*$_3$)$_2$), 4.48 (2H, br d, *J* = 6.0 Hz, CH_2), 4.82 (1H, sept., *J* = 6.0 Hz, C*H*$(CH_3)_2$), 7.05 (2H, d, *J* = 8.8 Hz, H-Ar), 7.31 (2H, d, *J* = 6.0 Hz, CH-pyridine), 7.40 (1H, d, *J* = 1.5 Hz, H-3), 7.44 (1H, d, *J* = 1.5 Hz, H-5), 7.80 (2H, d, *J* = 8.8 Hz, H-Ar), 8.51 (2H, d, *J* = 6.0 Hz, CH-N-pyridine), 9.04 (1H, t, *J* = 6.0 Hz, CONH), 12.34 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.7 (CH(*C*H$_3$)$_2$), 41.1 (CH_2), 69.5 (*C*H$(CH_3)_2$), 111.7(C-Ar), 115.0 (C-Ar), 122.1 (C-3), 122.7 (C-pyridine), 124.2 (C-2 and C-5), 127.2 (C-4), 130.9 (C-Ar), 131.1 (C-Ar), 135.1 (C-Ar), 148.6 (C-pyridine), 149.5 (C-pyridine), 149.5 (C-pyridine), 160.5 (CONH), 187.6 (CO); LRMS (ES^+) *m/z* 364.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{21}H_{22}N_3O_3$ $[M+H]^+$ 364.1660, found 364.1661.

4-(4-Methoxybenzoyl)-*N*-(pyridin-4-ylmethyl)-1*H*-pyrrole-2-carboxamide (200)



Compound **200** was synthesised according to general procedure C, using 4-(4-methoxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (**205**) (200 mg, 0.81 mmol), carbonyldiimidazole (263 mg, 1.62 mmol), 4-picolylamine (205 μL, 2.01 mmol) and THF (4 mL). The crude residue was purified by MPLC (silica, 2-30% MeOH in EtOAc) followed by recrystallisation from MeOH to give the title compound as a cream solid (200 mg, 74%); R_f = 0.38 (10% MeOH in EtOAc); M.p. 221-222 ^{o}C; λ_{max} (EtOH)/nm 292, 241; ν_{max}/cm^{-1} 3315, 3036, 2956, 2840, 1730 (CO), 1598 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.63 (3H, s, CH_3), 4.25 (2H, br d, *J* = 6.0 Hz, CH_2), 6.85 (2H, d, *J* = 8.8 Hz, H-Ar), 7.08 (2H, d, *J* = 6.0 Hz, CH-pyridine), 7.17 (1H, d, *J* = 1.3 Hz, H-3), 7.21 (1H, d, *J* = 1.3 Hz, H-5), 7.60 (2H, d, *J* = 8.8 Hz, H-Ar), 8.29 (2H, d, *J* = 6.0 Hz, CH-N-pyridine), 8.74 (1H, t, *J* = 6.0 Hz, CONH), 12.09 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ, 55.4 (CH_3), 59.7 (CH_2), 111.6 (C-Ar), 113.7 (C-Ar), 122.1 (C-3), 124.1 (C-pyridine), 127.2 (C-2 and C-5), 130.8 (C-4), 148.6 (C-pyridine), 149.5 (C-pyridine), 160.4 (C-Ar), 162.1 (CONH), 187.9 (CO); LRMS (ES$^+$) *m/z* 336.2 [M+H]$^+$; HRMS *m/z* calcd for $C_{19}H_{18}N_3O_3$ [M+H]$^+$ 336.1334, found 336.1336.

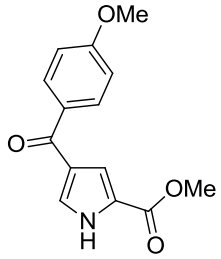
***N*-(Pyridin-4-ylmethyl)-4-(4-(trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxamide (201)**



Compound **201** was synthesised according to general procedure C, using 4-(4-(trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxylic acid (**206**) (100 mg, 0.33 mmol), carbonyldiimidazole (107 mg, 0.66 mmol), 4-picolylamine (83 μL, 0.83 mmol) and THF (3 mL). The crude residue was purified by MPLC (silica, 2-30% MeOH in EtOAc)

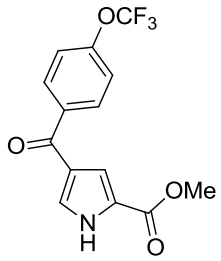
and the title compound obtained as a cream solid (120 mg, 94%); R_f = 0.41 (5% MeOH in EtOAc); M.p. 204 ^oC (dec.); λ_{max} (EtOH)/nm 292, 242; ν_{max}/cm^{-1} 3466, 3360, 3207, 2555, 2199, 2012, 1699 (CO), 1618 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 0.25 (2H, br d, *J* = 6.0 Hz, CH_2), 7.05 (2H, d, *J* = 6.0, CH-pyridine), 7.15-7.16 (1 H, m, H-3), 7.26 (1H, d, *J* = 1.5 Hz, H-5), 7.28 (2H, d, *J* = 8.0 Hz, H-Ar), 7.68 (2H, d, *J* = 8.0 Hz, H-Ar), 8.27 (2H, d, *J* = 6.0 Hz, CH-N-pyridine), 8.74 (1H, t, *J* = 6.0 Hz, CONH), 12.20 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 59.7 (CH_2) , 111.5 (C-Ar), 120.7 (C-3), 122.1 (C-pyridine), 124.1 (C-2 and C-5), 128.1 (CF_3), 130.8 (C-4), 138.1 (C-Ar), 148.5 (C-pyridine), 149.5 (C-pyridine), 150.5 (C-Ar), 160.3 (CONH), 187.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.6; LRMS (ES^+) *m/z* 390.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{15}F_3N_3O_3$ $[M+H]^+$ 390.1067, found 390.1067.

Methyl 4-(4-methoxybenzoyl)-1*H*-pyrrole-2-carboxylate (203)



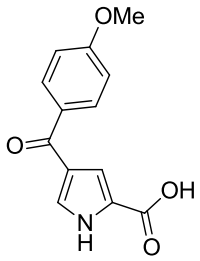
Compound **203** was synthesised according to general procedure A, using methyl pyrrole-2-carboxylate, **69** (400 mg, 3.20 mmol), DCM (7 mL), 4-methoxybenzoyl chloride (1.09 g, 6.40 mmol) and $AlCl_3$ (1.07 g, 8.0 mmol). The crude residue was purified by MPLC (silica, 2-100% EtOAc in petrol) followed by a final wash with $NaHCO_3$ to remove remaining starting material. The title compound was obtained as a yellow solid (641 mg, 77%); R_f = 0.42 (50% EtOAc in Petrol); M.p.: 150-151 ^oC (dec.); λ_{max} (EtOH)/nm: 291, 227; ν_{max}/cm^{-1}: 3301, 2567, 2366, 2144, 1697 (CO_2Me), 1590 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 3.82 (3H, s, $COOCH_3$), 3.86 (3H, s, OCH_3), 7.09 (2H, d, *J* = 8.9 Hz H-Ar), 7.50 (1H, dd, *J* = 1.7 and 3.2 Hz, H-3), 7.58 (1H, dd, *J* = 1.7 and 3.3 Hz, H-5), 7.83 (2H, d, *J* = 8.9 Hz, H-Ar), 12.70 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.5 (CH_3), 55.4 (CH_3), 113.8 (C-Ar), 115.9 (C-Ar), 123.2 (C-3), 124.6 (C-2 and C-5), 128.9 (C-4), 130.9 (C-Ar), 131.1 (C-Ar), 160.5 (C-Ar), 162.3 (CO_2Me), 187.6 (CO); LRMS (ES^+) *m/z* 259.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{14}H_{14}NO_4$ $[M+H]^+$ 260.0920, found 260.0917.

Methyl 4-(4-(trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxylate (204)



Compound **204** was synthesised using general procedure A using methyl pyrrole-2-carboxylate, **69** (200 mg, 1.60 mmol), DCM (5 mL), 4-(trifluoromethoxy)benzoyl chloride (0.50 mL, 3.20 mmol) and $AlCl_3$ (533 mg, 4.00 mmol). The crude residue was purified by MPLC (silica, 2-100% EtOAc in petrol) and the title compound obtained as a cream solid (393 mg, 78%); R_f = 0.51 (10% EtOAc in petrol); M.p.: 133-134 ^oC (dec.); λ_{max} (EtOH)/nm: 289, 240; ν_{max}/cm^{-1}: 3219, 2958, 2368, 1695 (CO_2Me), 1623 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 4.05 (3H, s, CH_3), 7.41 (1H, dd, *J* = 1.7 and 3.9 Hz, H-3), 7.77 (2H, d, *J* = 8.0 Hz, H-Ar), 7.87 (1H, dd, *J* = 1.7 and 3.9 Hz, H-5), 8.17 (2H, d, *J* = 8.0 Hz, H-Ar), 13.05 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.6 (CH_3), 115.8, 118.9 (C-Ar), 121.04 (C-Ar), 123.7 (C-3), 124.1 (C-2 and C-5), 129.7 (C-4), 130.9 (CF_3) , 137.6 (C-Ar), 150.6 (C-Ar), 160.5 (CO_2Me), 187.6 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.6; LRMS (ES^+) *m/z* 314.1 $[M+H]^+$; HRMS *m/z* calcd for $C_{14}H_{12}F_3NO_4$ $[M+H]^+$ 314.0635, found 314.0641.

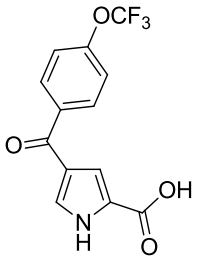
4-(4-Methoxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (205)



Compound **205** was synthesised using general procedure B, using methyl 4-(4-methoxybenzoyl)-1*H*-pyrrole-2-carboxylate (**203**) (350 mg, 1.34 mmol), LiOH (0.64 g, 26.8 mmol) in water (18 mL) and THF (14 mL) to give the title compound as a cream solid (277 mg, 84%); R_f = 0.15 (10% MeOH in EtOAc); M.p.: 171-172 ^oC; λ_{max} (EtOH)/nm: 292, 227; ν_{max}/cm^{-1}: 3256, 2912, 1678 (CO_2H), 1600 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 3.91 (3H, s, CH_3), 7.13 (2H, d, *J* = 8.9 Hz H-Ar), 7.15 (1H, dd, *J* = 1.7 and 3.1 Hz, H-3), 7.57 (1H dd, *J* = 1.7 and 3.3 Hz, H-5), 7.87 (2H, d, *J* = 8.9 Hz, H-

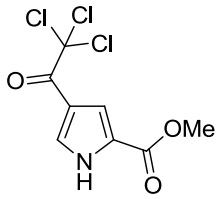
Ar), 12.56 (1H, br s, OH), 12.85 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.4 (CH_3), 113.8 (C-Ar), 115.5 (C-4), 124.5 (C-2 and C-5), 128.4 (C-4), 130.9 (C-Ar), 131.3 (C-Ar), 161.6 (C-Ar), 162.2 (CO2H), 187.7 (CO); LRMS (ES^+) *m/z* 246.0 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_{10}NO_4$ $[M-H]^-$ 244.0615, found 244.0620.

4-(4-(Trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxylic acid (206)



Compound **206** was synthesised according to general procedure B, using methyl 4-(4-(trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxylate (**204**) (200 mg, 0.64 mmol), LiOH (305 mg, 1.72 mmol) in water (9 mL) and THF (8 mL) to give the title compound as a cream solid (150 mg, 78%); R_f = 0.61 (5% MeOH in EtOAc); M.p.: 155-156 ^oC (dec.); λ_{max} (EtOH)/nm: 235; ν_{max}/cm^{-1}: 3277, 2898, 2572, 2362, 1685 (CO_2H), 1554 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.17 (1H, dd, *J* = 1.7 and 3.3 Hz, H-3), 7.62 (2H, d, *J* = 7.9 Hz, H-Ar), 7.62 (1H, dd, *J* = 1.7 and 3.3 Hz, H-5), 7.98 (2H, d, *J* = 7.9 Hz, H-Ar), 12.68 (1H, br s, OH), 12.93 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 115.4 (C-Ar), 120.7 (C-Ar), 123.9 (C-3), 125.0 (C-2 and C-5), 129.3 (C-4), 130.9 (CF_3), 137.7 (C-Ar), 161.5 (CO_2H), 187.7 (CO); δ_F (470 MHz, DMSO-d_6)/ppm: -56.6; LRMS (ES^+) *m/z* 300.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_9F_3NO_4$ $[M+H]^+$ 298.0333, found 298.0337.

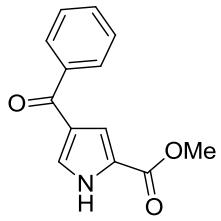
Methyl 4-(2,2,2-trichloroacetyl)-1*H*-pyrrole-2-carboxylate (209)



Compound **209** was synthesised according to general procedure A, using methyl pyrrole-2-carboxylate (**69**) (400 mg, 3.2 mmol), DCM (7 mL), trichloroacetyl chloride (0.718 mL, 6.4 mmol) and $AlCl_3$ (1.07 g, 8.0 mmol). The crude residue was purified by

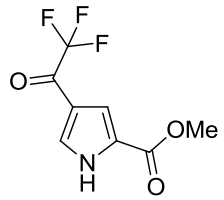
MPLC (silica, 2-100% EtOAc in petrol) and the title compound obtained as a peach solid (770 mg, 89%); R_f = 0.66 (50% EtOAc in Petrol); M.p. 116-117 ^oC (lit.[152] 118-119 ^oC); λ_{max} (EtOH)/nm 289, 236; ν_{max}/cm^{-1} 3271, 2608, 1695 (CO_2Me), 1680 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 3.63 (3H, s, CH_3), 7.15 (1H, dd, *J* = 1.8 and 3.2 Hz, H-3), 7.76 (1H, dd, *J* = 1.8 and 3.5 Hz, H-5), 12.92 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.6 (CH_3), 114.9 (CCl_3), 116.9 (C-3), 123.9 (C-2 and C-5), 131.9 (C-4), 160.0 (CO_2Me), 181.1 (CO); LRMS (ES^+) *m/z* 270.9 $[M+H]^+$.

Methyl 4-benzoyl-1*H*-pyrrole-2-carboxylate (211)



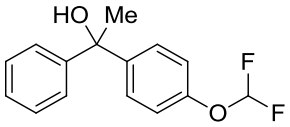
Methyl 4-(2,2,2-trifluoroacetyl)-1*H*-pyrrole-2-carboxylate (**213**) (50 mg, 0.23 mmol) was dissolved in THF (1.0 mL) at -78 ^oC before phenylmagnesium bromide (1.0 M in THF) (0.32 mL, 0.32 mmol) was added cautiously to give a yellow solution, which was stirred at -78 ^oC for 3 h before being allowed to warm to RT and stirred for a further 2 h. The reaction was then quenched with a 0.01M solution of HCl and the mixture was extracted with Et_2O (3 x 30 mL). The combined organic layers were washed with brine (70 mL), dried over Na_2SO_4 and the solvent removed *in vacuo*. The crude residue was purified by recrystallisation from EtOAc to afford the title compound as a white solid (20 mg, 38%); R_f = 0.71 (50% EtOAc in petrol); M.p. 149-150 ^oC (lit.[153] 118-119 ^oC) λ_{max} (EtOH)/nm 288; ν_{max}/cm^{-1} 3354, 2521, 2161, 2029, 1701 (CO_2Me), 1684 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 3.71 (3H, s, CH_3), 6.72 (1H, br s, H-3), 6.84 (1H, br s, H-5), 7.23-7.25 (3H, m, H-Ar), 7.45 (2H, d, *J* = 8.2 Hz, H-Ar); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.9 (CH_3), 115.9 (C-3), 124.0 (C-2 and C-5), 128.5 (C-4), 128.8 (C-Ar), 128.8 (C-Ar), 129.3 (C-Ar), 138.9 (C-Ar), 157.4 (CO_2Me), 189.8 (CO); LRMS (ES^+) *m/z* 229.1 $[M+H]^+$.

Methyl 4-(2,2,2-trifluoroacetyl)-1*H*-pyrrole-2-carboxylate (213)



Compound **213** was synthesised according to general procedure A, using methyl pyrrole-2-carboxylate (**69**) (400 mg, 3.20 mmol), DCM (7 mL), trifluoracetic anhydride (0.890 mL, 6.4 mmol) and $AlCl_3$ (1.07 g, 8.00 mmol). The crude residue was purified by MPLC (silica, 2-100% EtOAc in petrol) and the title compound obtained as a white solid (531 mg, 2.4 mmol, 75%); Rf = 0.38 (10% EtOAc in petrol); M.p. 112-113 ^oC; λ_{max} (EtOH)/nm 283, 233; ν_{max}/cm^{-1} 3252, 2360, 1687 (CO_2Me), 1561 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 3.71 (3H, s, CH_3), 7.14 (1H, br s, H-3), 7.86 (1H, br s, H-5), 13.16 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.9 (CH_3), 115.3 (C-3), 117.5 (CF_3), 117.6 (C-2), 125.2 (C-5), 131.5 (C-4), 160.0 (CO_2Me), 174.3 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) -72.9; LRMS (ES^+) *m/z* 221.0 $[M+H]^+$.

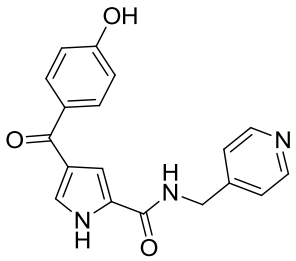
1-(4-(Difluoromethoxy)phenyl)-1-phenylethanol (216)



Mg turnings (33 mg, 1.40 mmol) were covered with diethyl ether (4 mL) at RT and 1-(bromo-4-difluoromethoxy)benzene (0.123 mL, 0.90 mmol) was added carefully with stirring . A crystal of iodine was added until boiling was observed, and the reaction was left to stir for 15 min before acetophenone (97 µL, 0.83 mmol) was added and the reaction left for 5 h. The mixture was quenched with 0.01 M HCl solution and extracted with Et_2O (3 x 30 mL). The combined organic extracts were washed with brine before being dried over Na_2SO_4 and the solvent removed *in vacuo* to give the title compound as an orange oil (175 mg, 80%); R_f = 0.46 (10% MeOH in EtOAc); λ_{max} (EtOH)/nm: 201; ν_{max}/cm^{-1}: 3365 (OH), 2959, 2159, 1963, 1654; ^1H NMR (500 MHz, MeOD) δ 2.37 (3H, s, CH_3), 6.79 (1H, t, *J* = 73.6 Hz, CHF_2), 7.05 (2H, d, *J* = 8.5 Hz, H-Ar), 7.44-7.48 (5H, m, H-Ar), 7.96 (2H, d, *J* = 8.5 Hz, H-Ar); ^{13}C NMR (125 MHz, MeOD) δ 26.9 (CH_3), 62.0 ($(Ar)_2C(CH_3)OH$), 115.4 (C-Ar), 117.5 (C-Ar), 119.2 (C-Ar), 122.3 (C-Ar),

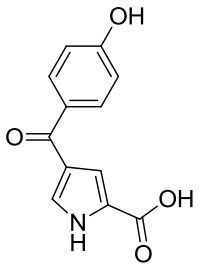
128.8 (C-Ar), 133.4 (C-Ar), 134.5 (C-Ar), 138.3 (C-Ar), 151.9 ($OCHF_2$); ^{19}F NMR (470 MHz, DMSO-d_6) δ -83.0; LRMS (ES^+) *m/z* 265.30 $[M+H]^+$.

4-(4-Hydroxybenzoyl)-*N*-(pyridin-4-ylmethyl)-1*H*-pyrrole-2-carboxamide (217)



Compound **217** was synthesised according to general procedure C using 4-(4-hydroxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (**218**) (50 mg, 0.22 mmol), carbonyldiimidazole (71 mg, 0.44 mmol), 4-picolylamine (56 µL, 0.56 mmol) and THF (2mL) to afford the crude product, which was purified using MPLC (silica, 2-30% MeOH in EtOAc) to give the title compound as a white solid (23 mg, 32%); R_f = 0.25 (10% MeOH in EtOAc); M.p.: 201-202 ^oC; λ_{max} (EtOH)/nm: 235; ν_{max}/cm^{-1}: 3298, 3001, 2945, 2830, 1710 (CO), 1593 (CONH); ^1H NMR (500 MHz, MeOD) δ 4.50 (2H, s, CH_2), 6.79 (2H, d, *J* = 7.8 Hz, *p*-subs. H-Ar), 7.24 (1H, d, *J* = 1.57 Hz, H-3), 7.31 (2H, d, *J* = 5.1 Hz, CH-pyridine), 87.42 (1H, d, *J* = 1.57 Hz, H-5), 7.69 (2H, d, *J* = 7.8 Hz, *p*-subs. H-Ar), 8.39 (2H, br s, CH-N-pyridine); ^{13}C NMR (125 MHz, MeOD) δ 45.2 (CH_2), 114.1 (C-Ar), 123.2 (C-3), 125.7 (C-pyridine), 125.9 (C-2 and C-5), 131.6 (C-4), 132.0 (C-Ar), 145.6 (C-pyridine), 146.2 (C-pyridine), 151.3 (C-pyridine), 159.2 (C-Ar), 164.5 (CONH), 191.6 (CO); LRMS (ES^+) *m/z* 322.30 $[M+H]^+$; HRMS *m/z* cald for $C_{18}H_{13}F_2N_2O_2$ $[M+H]^+$ 322.1189, found 322.1191.

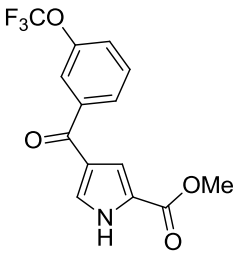
4-(4-Hydroxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (218)



4-(4-Methoxybenzoyl)-1*H*-pyrrole-2-carboxylic acid (**205**) (100 mg, 0.41 mmol) was dissolved in BBr_3 (1.0M in DCM, 5 mL) at 0 ^oC. The reaction was allowed to warm to

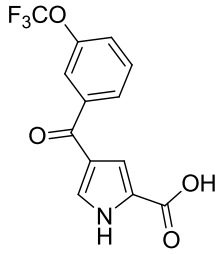
RT overnight. The mixture was quenched with water and neutralised using a 2M aqueous solution of NaOH. The product was extracted with EtOAc (20 mL) and the organic extract dried over Na_2SO_4 and the solvent removed *in vacuo*. Purification was achieved using MPLC (silica, 2-20% MeOH in DCM) to give the pure product as a pale yellow solid (75 mg, 79%); R_f = 0.10 (10% MeOH in EtOAc); M.p.: 160-161 ^oC; λ_{max} (EtOH)/nm: 234; ν_{max}/cm^{-1}: 3406 (OH), 2371, 2135, 1710 (CO_2H), 1614 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 6.69 (1H, d, *J* = 1.7 Hz, H-3), 6.79 (2H, d, *J* = 8.5 Hz, H-Ar), 7.14 (1H, d, *J* = 1.7 Hz, H-5), 7.65 (2H, d, *J* = 8.5 Hz, H-Ar); ^{13}C NMR (125 MHz, DMSO-d_6) δ 117.3 (C-Ar), 123.9 (C-3), 125.0 (C-2), 128.6 (C-5), 131.0 (C-4), 130.8 (C-Ar), 131.6 (C-Ar), 132.9 (C-Ar), 159.0 (C-Ar), 164.6 (CO_2H), 184.0 (CO); LRMS (ES^+) *m/z* 230.20 $[M-H]^-$; HRMS *m/z* calcd for $C_{12}H_8NO_4$ $[M-H]^-$ 230.0453, found 230.0459.

Methyl 4-(3-(trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxylate (220)



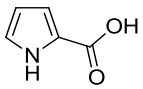
Compound **220** was synthesised according to general procedure A, using methyl pyrrole-2-carboxylate (**69**) (200 mg, 1.60 mmol), DCM (4 mL), 3-trifluoromethoxybenzoyl chloride (0.52 mL, 3.20 mmol) and $AlCl_3$ (533 mg, 4.00 mmol) to give the crude compound, which was purified by MPLC (silica, 2-100% EtOAc in petrol) to give the title compound as a cream solid (394 mg, 79%); R_f = 0.29 (50% EtOAc in Petrol); M.p.: 99-101 ^oC (dec.); λ_{max} (EtOH)/nm: 285, 238; ν_{max}/cm^{-1}: 3355, 3184, 3064, 2157, 2028, 1713 (CO_2Me), 1637 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 3.87 (3H, s, CH_3), 7.21 (1H, dd, *J* = 1.8 and 3.2 Hz, H-3), 7.67-7.72 (4H, m, H-5 and H-Ar), 7.88 (1H, d, *J* = 7.5 Hz, H-Ar), 12.88 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.5 (CH_3), 115.8 (C-Ar), 121.1 (C-Ar), 123.9 (C-Ar), 124.2 (C-3), 127.6 (C-2 and C-5), 130.8 (C-4), 133.2 (CF_3), 140.8 (C-Ar), 148.3 (C-Ar), 160.4 (C-Ar), 165.9 (CO_2Me), 187.3 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.8; LRMS (ES^-) *m/z* 314.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{14}H_{11}F_3NO_4$ $[M+H]^+$ 314.0635, found 314.0637.

4-(3-(Trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxylic acid (221)



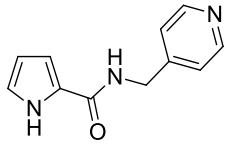
Compound **221** was synthesised according to general procedure B, using methyl 4-(3-(trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxylate (**220**) (200 mg, 0.64 mmol), LiOH (305 mg, 1.72 mmol) in water (9 mL) and THF (8 mL) to give the title compound as a cream solid (163 mg, 85%); R_f = 0.28 (50% MeOH in EtOAc); M.p.: 145-146 ^oC; λ_{max} (EtOH)/nm: 288, 235; ν_{max}/cm^{-1}: 3226, 2923, 2559, 1683 (CO_2H), 1626 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 7.12 (1H, dd, *J* = 1.7 and 3.2 Hz, H-3), 7.57, (1H, dd, *J* = 1.7 and 3.4 Hz, H-5), 7.64 - 7.67 (3H, m, H-Ar), 7.84 (1H, d, *J* = 7.5 Hz, H-Ar), 12.65 (1H, br s, OH), 12.91 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 115.3 (C-Ar), 119.1 (C-Ar), 120.5 (C-Ar), 121.1 (C-Ar), 123.8 (C-3), 125.2 (C-2), 127.6 (C-5), 129.3 (C-4), 130.8 (CF_3), 141.0 (C-Ar), 148.3 (C-Ar), 161.4 (CO_2H), 187.4 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.8; LRMS (ES^-) *m/z* 298.1 $[M-H]^-$ HRMS *m/z* calcd for $C_{13}H_7F_3NO_4$ $[M-H]^-$ 298.0333, found 298.0324.

1*H*-Pyrrole-2-carboxylic acid (222)



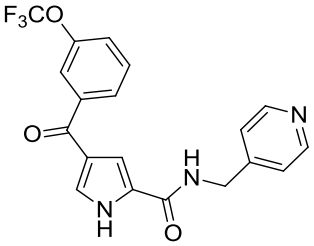
Compound **222** was synthesised according to general procedure B, using methyl pyrrole-2-carboxylate (**69**) (400 mg, 3.1 mmol), LiOH (1.48 g, 62 mmol) in water (40 mL) and THF (14 mL) to give the title compound as a cream solid (348 mg, 98%); R_f = 0.31 (5% MeOH in EtOAc); M.p 197-198 ^oC (lit.[154] 190 ^oC); λ_{max} (EtOH)/nm 262; ν_{max}/cm^{-1} 3348, 2989, 2907, 2624, 1649 (CO_2H), 1437; ^1H NMR (500 MHz, DMSO-d_6) δ 6.16-6.18 (1H, m, H-4), 6.74 (1H, ddd, *J* = 3.6, 2.5 and 1.5 Hz, H-3), 6.98-7.00 (1H, m, H-5); ^{13}C NMR (125 MHz, DMSO-d_6) δ 70.2 (C-4), 110.6 (C-3), 116.7 (C-2), 124.5 (C-5), 164.5 (CO_2H); LRMS (ES^+) *m/z* 112.0 $[M+H]^+$.

***N*-(Pyridin-4-ylmethyl)-1*H*-pyrrole-2-carboxamide (223)**



Compound **223** was synthesised according to general procedure C, using 1*H*-pyrrole-2-carboxylic acid (250 mg, 2.25 mmol), carbonyldiimidazole (730 mg, 4.50 mmol), 4-picolylamine (0.569 mL, 5.63 mmol) and THF (6 mL). The crude residue was purified by MPLC (silica, 2-30% MeOH in EtOAc) and the title compound obtained as a cream solid (403 mg, 89%); R_f = 0.31 (5% MeOH in EtOAc); M.p 165-166 ^oC (lit.[154] 170-171 ^oC); λ_{max} (EtOH)/nm 264; ν_{max}/cm^{-1} 3216, 3072, 2922, 1561 (CONH), 1407; ^1H NMR (500 MHz, DMSO-d_6) δ 4.25 (2H, d, *J* = 6.0 Hz, CH_2), 5.91 (1H, t, *J* = 3.5 Hz H-4), 6.64, (1H, d, *J* = 3.5, H-3), 6.57 (1H, br s, H-5) 7.08 (2H, d, *J* = 5.6 Hz, CH-pyridine), 8.29 (2H, d, *J* = 5.6 Hz, CH-N-pyridine), 8.42 (1H, t, *J* = 6.0 Hz, CONH), 11.30 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 42.1 (CH_2), 109.3 (C-4), 110.1 (C-3), 122.1 (C-pyridine), 125.3 (C-2 and C-5), 147.6 (C-pyridine), 150.1 (C-pyridine), 161.2(CONH) LRMS (ES^+) *m/z* 202.1 $[M+H]^+$.

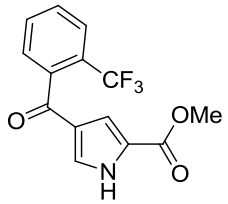
***N*-(Pyridin-4-ylmethyl)-4-(3-(trifluoromethoxy)benzoyl)-1*H*-pyrrole-2-carboxamide (224)**



Compound **224** was synthesised according to general procedure A, using *N*-benzyl-1*H*-pyrrole-2-carboxamide (**223**) (100 mg, 0.50 mmol), 3-(trifluoromethoxy)benzoyl chloride (0.162 mL, 1.00 mmol), DCM (4 mL), and $AlCl_3$ (167 mg, 1.25 mmol). The crude residue was purified by reversed phase MPLC (C18 silica, 1-50% MeOH (1% formic acid) in H_2O (1% formic acid)) and the title compound obtained as a dark red solid (163 mg, 84%); R_f = 0.89 (5% MeOH in EtOAc); M.p 203 ^oC (dec.); λ_{max} (EtOH)/nm 241; ν_{max}/cm^{-1} 3232, 2924, 2362, 2188,1650 (CO), 1560 (CONH); ^1H NMR (500 MHz, MeOD) δ 4.59 (2 H, br d, *J* = 5.9 Hz, CH_2), 7.24 (1 H, br s, H-3), 7.40-7.41 (2 H, m, H-5 and H-Ar), 7.49-7.52 (1 H, m, H-Ar), 7.54 (1 H, s, H-Ar), 7.62 (2H, d, *J* =

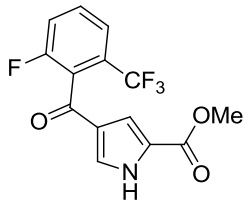
6.1 Hz, CH-pyridine), 7.70 (1H, d, *J* = 8.0 Hz, H-Ar), 8.51 (2H, d, *J* = 6.1 Hz, CH-N-pyridine), 8.93 (1H, t, *J* = 5.9 Hz, CONH); ^{13}C NMR (125 MHz, MeOD) δ 43.3 (CH_2), 113.1 (C-Ar), 122.1 (C-3), 125.4 (C-pyridine), 125.7 (C-2 and C-5), 128.7 (CF_3), 130.1 (C-4), 131.6 (C-Ar), 142.6 (C-pyridine), 145.7 (C-pyridine), 150.6 (C-pyridine), 158.2 (C-Ar), 163.1 (CONH), 190.7 (CO); ^{19}F NMR (470 MHz, MeOD) δ -56.8; LRMS (ES^+) *m/z* 390.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{15}F_3N_3O_3$ $[M+H]^+$ 390.1054, found 390.1053.

Methyl 4-(2-(trifluoromethyl)benzoyl)-1H-pyrrole-2-carboxylate (228)



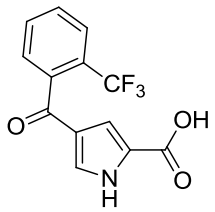
Compound **228** was synthesised according to general procedure A using methylpyrrole-2-carboxylate (**69**) (400 mg, 3.20 mmol), DCM (7 mL), $AlCl_3$ (1.07 g, 8.00 mmol) and 2-(trifluoromethyl)benzoyl chloride (0.94 mL, 6.40 mmol) to afford the crude product. Purification was achieved using MPLC (0-80% EtOAc in petrol) to give the pure compound as a pale yellow solid (800 mg, 84%); R_f = 0.48 (50% EtOAc in petrol); M.p. 143-144 ^{o}C; λ_{max} (EtOH)/nm 279.0, 231.5; ν_{max}/cm^{-1} 3282, 1710 (CO_2Me), 1642 (CO), 1556,; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.80 (3H, s, CH_3), 6.98 (1H, s, H-3), 7.39 (1H, s, H-5), 7.59 (1H, d, *J* = 7.3 Hz, H-Ar), 7.73-7.80 (2H, m, H-Ar), 7.87 (1H, d, *J* = 7.3 Hz, H-Ar), 12.82 (1H, s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 51.6 (CH_3), 115.1 (C-3), 124.1 (CF_3), 126.6 (C-2 and C-5), 128.1 (C-4), 129.9 (C-Ar), 130.1 (C-Ar), 132.3 (C-Ar), 160.3 (CO_2Me), 188.8 (CO); LRMS (ES^+) *m/z* 296.1 $[M-H]^-$; ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.8; HRMS *m/z* calcd for $C_{14}H_{11}F_3NO_3$ $[M-H]^-$ 296.0539, found 296.0538.

Methyl 4-(2-fluoro-6-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylate (229)



Compound **229** was synthesised according to general procedure A using methylpyrrole-2-carboxylate (**69**) (400 mg, 3.20 mmol), DCM (7 mL), $AlCl_3$ (1.07 g, 8.00 mmol) and 2-fluoro-6-(trifluoromethyl)benzoyl chloride (0.99 mL, 6.40 mmol) to afford the crude product. Purification was achieved using MPLC (0-80% EtOAc in petrol) to give the pure compound as a pale yellow solid (604 mg, 60%); R_f = 0.56 (50% EtOAc in petrol); M.p. 148-149 ^oC; λ_{max} (EtOH)/nm 275.0, 231.5; ν_{max}/cm^{-1} 3274, 1699 (CO_2Me), 1657 (CO), 1563,; ^1H NMR (500 MHz, $CDCl_3$) δ 3.80 (3H, s, CH_3), 7.07 (1H, s, H-3), 7.27-7.31 (1H, m, H-Ar), 7.36 (1H, dd, *J* = 1.8 and 3.3 Hz, H-5), 7.47-7.51 (2H, m, H-Ar), 9.44 (1H, s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.0 (CH_3), 115.4 (C-3), 119.5 (CF_3), 122.3 (C-2 and C-3), 124.5 (C-4), 127.3 (C-Ar), 131.0 (C-Ar), 131.1 (C-Ar), 158.3 (d, J_{CF} = 246.8, CF), 161.0 (CO_2Me), 181.3 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.2, -58.2,; LRMS (ES^+) *m/z* 316.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{14}H_8F_4NO_3$ $[M-H]^-$ 316.0596, found 316.0596.

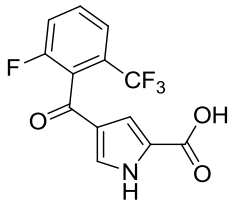
4-(2-(Trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylic acid (230)



Compound **230** was synthesised according to general procedure B using methyl 4-(2-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylate (**69**) (450 mg, 1.43 mmol), LiOH (685 mg, 28.60 mmol) in water (18 mL) and THF (12 mL) to afford the pure product as a white solid (430 mg, 99%); R_f = 0.40 (5% MeOH in EtOAc); M.p. 210-211 ^oC; λ_{max} (EtOH)/nm 274.0, 231.0; ν_{max}/cm^{-1} 3350, 1650 (CO_2H), 1564 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 6.93-6.94 (1H, m, H-3), 7.31 (1H, dd, *J* = 1.5 and 3.4 Hz, H-5), 7.58 (1H, d, *J* = 7.6 Hz, H-Ar), 7.72-7.79 (2H, m, H-Ar), 7.88 (1H, d, *J* = 7.6 Hz, H-Ar), 12.62 (1H, s, CO_2H), 12.88 (1H, br s, NH); ^{13}C NMR (500 MHz, DMSO-d_6) δ 114.7 (C-3), 125.2 (C-2), 125.4 (C-5), 125.8 (C-Ar), 126.0 (CF_3), 126.6 (C-Ar), 128.1 (C-Ar),

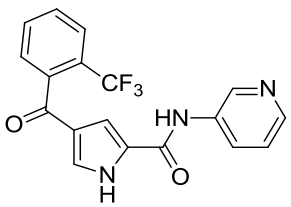
129.5 (C-4), 130.1, 132.3, 138.8 (C-Ar), 161.4 (CO_2H), 188.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -58.0; LRMS (ES^+) *m/z* 282.1 $[M-H]^-$; HRMS *m/z* calcd for $C_{13}H_8F_3NO_3$ $[M-H]^-$ 282.0384, found 282.0387.

4-(2-Fluoro-6-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylic acid (231)



Compound **231** was synthesised according to general procedure B using methyl 4-(2-fluoro-6-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylate (**69**) (450 mg, 1.43 mmol), LiOH (685 mg, 28.60 mmol) in water (18 mL) and THF (12 mL) to afford the pure product as a white solid (430 mg, 99%); R_f = 0.35 (5% MeOH in EtOAc); M.p. 210-211 ^oC; λ_{max} (EtOH)/nm 274.0, 231.0; ν_{max}/cm^{-1} 3350, 1650 (CO_2H), 1564 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 6.95 (1H, s, H-3), 7.46 (1H, s, H-5), 7.69-7.73 (2H, m, H-3' and H-5'), 7.77 (1H, ddd, *J* = 5.4, 8.0 and 8.0 Hz, H-4'), 12.69 (1H, s, NH), 12.99 (1H, s, COOH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 114.0 (C-3), 120.4 (d, J_{CF} = 22.2 Hz, C-Ar), 121.9, 122.7 (C-2 and C-5), 123.0 (q, J_{CF} = 273.5 Hz, CF_3), 125.8 (C-Ar), 126.9 (d, J_{CF} = 24.2 Hz, C-Ar), 127.6 (d, J_{CF} = 31.0 Hz, C-Ar), 129.5 (C-4), 132.0 (d, J_{CF} = 9.4 Hz, C-Ar), 158.3 (d, J_{CF} = 240.6 Hz, CF-Ar), 161.3 (CO_2H), 184.1 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.7, -57.0; LRMS (ES^+) *m/z* 302.20 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_7F_4NO_3$ $[M-H]^-$ 300.0289, found 300.0280.

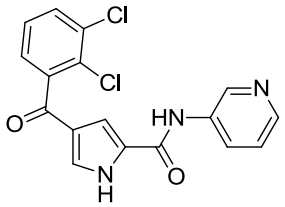
***N*-(Pyridin-3-yl)-4-(2-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxamide (232)**



Compound **232** was synthesised according to general procedure D using 4-(2-trifluoromethylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**230**) (50 mg, 0.18 mmol), MeCN (1 mL), 3-aminopyridine (42 mg, 0.44 mmol) and PCl_3 (16 µL, 0.18 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH

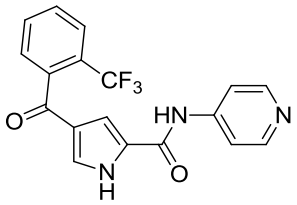
in EtOAc) to give the pure compound as a white solid (25 mg, 38%); R_f = 0.57 (5% MeOH in EtOAc); M.p. 197-198 ^oC; λ_{max} (EtOH)/nm 235, 291; ν_{max}/cm^{-1}: 2362, 2050, 2000, 1635 (CO), 1533 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.34 (1 H, s, H-3), 7.40-7.43 (1 H, m, CH-pyridine), 7.50 (1H, s, CH-pyridine), 7.62 (1 H, d, *J* = 7.5 Hz, H-6'), 7.75-7.83 (2 H, m, H-4' and H-5'), 7.90 (1H, d, *J* = 7.5 Hz, H-3'), 8.15-8.17 (1H, m, CH-N-pyridine), 8.31 (1H, d, *J* = 5.9 Hz, CH-N-pyridine), 10.25 (1 H, s, CONH), 12.63 (1 H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 114.2 (2 x C-pyridine), 120.1 (C-3), 126.9 (C-2 and C-5), 130.2 (C-4), 131.4 (C-Ar), 131.6 (C-Ar), 132.9 (C-Ar), 135.6 (C-Ar), 137.7 (C-Ar), 143.4 (C-Ar), 145.6 (2 x C-N-pyridine), 148.1(C-pyridine), 161.5 (CONH), 189.1 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.8; LRMS (ES^+) *m/z* 358.2 $[M-H]^-$; HRMS *m/z* calcd for $C_{18}H_{12}F_3N_3O_2$ $[M+H]^+$ 360.0954, found: 360.0959.

4-(2,3-Dichlorobenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (233)



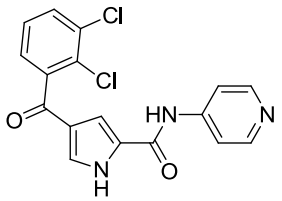
Compound **233** was synthesised according to general procedure D using 4-(2,3-Dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**155**) (50 mg, 0.17 mmol), MeCN (1 mL), 3-aminopyridine (39 mg, 0.42 mmol) and PCl_3 (15 µL, 0.17 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a beige solid (45 mg, 75%); R_f = 0.61 (5% MeOH in EtOAc); M.p. 199-200 ^oC; λ_{max} (EtOH)/nm 292; ν_{max}/cm^{-1} 3356, 2365, 2163, 2026, 1641 (CO), 1526 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.37 (1 H, dd, *J* = 4.9 and 8.2 Hz, H-Ar), 7.42 (1H, s, H-3), 7.47-7.53 (3 H, m, 2 x H-Ar and H-5), 7.79 (1 H, dd, *J* = 1.4 and 7.5 Hz, CH-pyridine), 8.13-8.16 (1H, m, CH-pyridine), 8.29 (1 H, dd, *J* = 1.4 and 4.7 Hz, CH-N-pyridine), 8.90 (1 H, d, *J* = 2.4 Hz, CH-N-pyridine), 10.22 (1 H, s, CONH), 12.67 (1 H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 114.1 (2 x C-pyridine), 123.4 (C-3), 125.1, 126.0 (C-2 and C-5), 129.9 (C-4), 130.1 (C-Ar), 131.5 (C-Ar), 132.8 (C-Ar), 135.9 (C-Ar), 138.1 (C-Ar), 143.6 (C-Ar), 145.0 (2 x C-N-pyridine), 146.2 (C-pyridine), 159.8 (CONH), 187.6 (CO); LRMS (ES^+) *m/z* 359.0 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{10}{}^{35}Cl_2N_3O_2$ $[M-H]^-$ 358.0161, found: 358.0161.

***N*-(Pyridin-4-yl)-4-(2-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxamide (234)**



Compound **234** was synthesised according to general procedure D using 4-(2-trifluoromethylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**230**) (50 mg, 0.18 mmol), MeCN (1 mL), 4-aminopyridine (42 µL, 0.44 mmol) and PCl_3 (16 µL, 0.18 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (35 mg, 0.10 mmol, 58%); R_f = 0.60 (5% MeOH in EtOAc); M.p. 175-176 ^oC; λ_{max} (EtOH)/nm 271, 292; ν_{max}/cm^{-1}: 3343, 3115, 2977, 1732 (CO), 1624 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.35 (1 H, s, H-3), 7.53 (1 H, s, H- 5), 7.62 (1H, d, *J* = 7.8 Hz, H-Ar), 7.74 (2 H, d, *J* = 6.5 Hz, CH-pyridine), 7.76-7.82 (2H, m, H-Ar), 7.91 (1H, d, *J* = 7.8 Hz, H-Ar) 8.46 (2 H, d, *J* = 6.5 Hz, CH-N-pyridine), 10.33 (1 H, s, CONH) 12.67 (1 H, br s, NH); ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.8; LRMS (ES^+) *m/z* 360.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{12}F_3N_3O_2$ $[M+H]^+$ 360.0954, found: 360.0959.

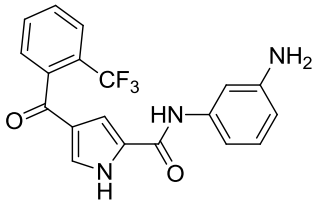
4-(2,3-Dichlorobenzoyl)-*N*-(pyridin-4-yl)-1*H*-pyrrole-2-carboxamide (235)



Compound **235** was synthesised according to general procedure D using 4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**155**) (50 mg, 0.17 mmol), MeCN (1 mL), 4-aminopyridine (39 mg, 0.42 mmol) and PCl_3 (15 µL, 0.17 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (40 mg, 67%); R_f = 0.60 (5% MeOH in EtOAc); M.p. 251-252 ^oC; λ_{max} (EtOH)/nm 272, 293; ν_{max}/cm^{-1}: 2588, 2342, 2160, 2018, 1636 (CO), 1591 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.45-7.56 (4 H, m, 2 x H-Ar, H-3 and H-5), 7.74 (2H, d, *J* = 5.9 Hz, CH-pyridine), 7.80 (2 H, dd, *J* = 2.4 and 7.8 Hz, H-Ar), 8.46 (2 H, d, *J* = 5.9 Hz, CH-N-pyridine), 10.33 (1 H, s, CONH) 12.71 (1 H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 113.2 (2 x C-pyridine), 125.3

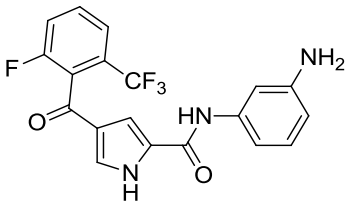
(C-3), 126.7, 127.9 (C-2 and C-5), 129.2 (C-4), 129.9 (C-Ar), 131.1 (C-Ar), 132.7 (C-Ar), 134.8 (C-Ar), 137.5 (C-Ar), 142.4 (C-Ar), 143.2 (2 x C-N-pyridine), 145.2 (C-pyridine), 160.9 (CONH), 190.6 (CO); LRMS (ES^+) *m/z* 359.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{12}{}^{35}Cl_2N_3O_2$ $[M+H]^+$ 359.0215, found: 359.0214.

_N_-(3-Aminophenyl)-4-(2-(trifluoromethyl)benzoyl)-1_H_-pyrrole-2-carboxamide (236)



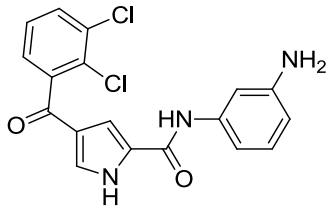
Compound **236** was synthesised according to general procedure D using 4-(2-trifluoromethylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**230**) (50 mg, 0.18 mmol), MeCN (1 mL), 1,3-phenylenediamine (48 mg, 0.44 mmol) and PCl_3 (16 µL, 0.18 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (37 mg, 55%); R_f = 0.50 (5% MeOH in EtOAc); M.p. 205 ^oC (dec.); λ_{max} (EtOH)/nm 236; ν_{max}/cm^{-1}: 3337 (NH_2), 2360, 1611 (CO), 1557 (CONH); ^1H NMR (500 MHz, MeOD) δ 6.52 (1H, d, *J* = 7.7 Hz, H-Ar), 6.94 (1H, d, *J* = 7.7 Hz, H-Ar), 7.06-7.09 (1H, m, H-Ar), 7.13-7.14 (1H, m, H-Ar), 7.33 (1H, d, *J* = 1.5 Hz, H-3), 7.39 (1H, d, *J* = 1.5 Hz, H-5), 7.57 (1H, d, *J* = 7.1 Hz, H-6'), 7.71-7.78 (2H, m, H-4' and H-5'), 7.85 (1H, d, *J* = 7.1 Hz, H-3'); ^{13}C NMR (125 MHz, MeOD) δ 109.2, 112.0, 112.5, 112.8, 127.2, 127.7 (C-Ar), 127.8 (C-3), 129.5 (C-2 and C-5), 130.3 (C-4), 130.5 (C-Ar), 131.1 (C-Ar), 133.0 (C-Ar), 140.2 (C-Ar), 149.3 (C-Ar), 160.8 (CON), 192.4 (CO); ^{19}F NMR (470 MHz, MeOD) δ -59.4; LRMS (ES^+) *m/z* 374.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{14}F_3N_3O_2Na$ $[M+Na]^+$ 396.0928, found 396.0928.

***N*-(3-Aminophenyl)-4-(2-fluoro-6-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxamide (237)**



Compound **237** was synthesised according to general procedure D using 4-(2-fluoro,6-trifluoromethylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**231**) (50 mg, 0.17 mmol), MeCN (1 mL), 1,3-phenylenediamine (45 mg, 0.42 mmol) and PCl_3 (15 µL, 0.17 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a beige solid (30 mg, 45%); R_f = 0.45 (5% MeOH in EtOAc); M.p. 251-252 ^oC; λ_{max} (EtOH)/nm 238; ν_{max}/cm^{-1} 2364 (NH_2), 2336, 1615 (CO), 1559 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 6.31 (1H, d, *J* = 8.3 Hz, H-Ar), 6.84 (1H, d, *J* = 8.3 Hz, H-Ar), 6.93-6.97 (1H, m, H-4'), 7.00-7.01 (1H, m, H-Ar), 7.38 (1H, br s, H-3), 7.46 (1H, br s, H-5), 7.71-7.82 (3H, m, H-3', H-5' and H-Ar), 9.75 (1H, br s, CONH), 12.52 (1H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 109.2 (C-Ar), 112.0 (C-Ar), 112.9 (C-Ar), 120.9 (C-3), 121.0 (d, J_{CF} = 22.8 Hz, C-Ar), 123.5 (C-Ar), 127.9 (C-2 and C-5), 128.9 (d, J_{CF} = 22.8 Hz, C-Ar), 130.0 (C-Ar), 130.3 (C-Ar), 130.6 (C-Ar), 132.8 (d, J_{CF} = 8.9 Hz, C-Ar), 140.2 (C-Ar), 149.3 (C-Ar), 160.3 (d, J_{CF} = 243.5 Hz, CF-Ar), 160.7 (CON), 187.2 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.4, -57.0; LRMS (ES^+) *m/z* 392.4 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{13}F_4N_3O_2$ $[M+H]^+$ 392.1018, found 392.1019.

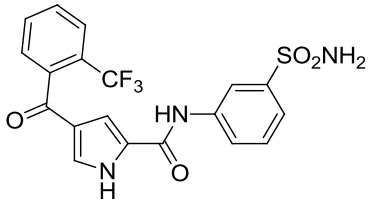
***N*-(3-Aminophenyl)-4-(2,3-dichlorobenzoyl)-1H-pyrrole-2-carboxamide (238)**



Compound **238** was synthesised according to general procedure D using 4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**155**) (50 mg, 0.17 mmol), MeCN (1 mL), 1,3-phenylenediamine (45 mg, 0.42 mmol) and PCl_3 (15 µL, 0.17 mmol). The reaction was performed at 100 ^oC. Purification of the crude product was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a beige solid (35 mg, 52%); R_f = 0.58 (5% MeOH in EtOAc); M.p. 251-252 ^oC; λ_{max} (EtOH)/nm 216;

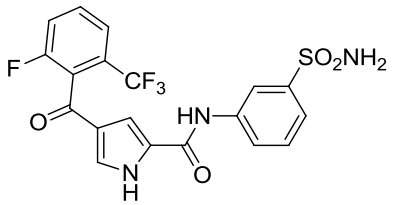
ν_{max}/cm^{-1}: 3333 (NH_2), 3123, 2160, 2023, 1596 (CO), 1557 (CONH); ^{1}H NMR (500 MHz, MeOD) δ 6.52 (1H, d, *J* = 7.5 Hz, H-Ar), 6.94 (1H, d, *J* = 7.5 Hz, H-Ar), 7.06-7.09 (1H, m, H-Ar), 7.13-7.14 (1H, m, H-Ar), 7.39-7.41 (2H, m, H-6' and H-3), 7.42 (1H, d, *J* = 2.2 Hz, H-5), 7.44-7.47 (1H, m, H-4'), 7.70 (1H, dd, *J* = 1.6 and 8.0 Hz, H-5'), 8.55 (1H, br s, CONH); ^{13}C NMR (500 MHz, MeOD) δ 109.2 (C-Ar), 112.0 (C-Ar), 112.5 (C-Ar), 112.8 (C-Ar), 128.0 (C-3), 129.2 (C-2 and C-5), 129.9 (C-4), 130.3 (C-Ar), 130.6 (C-Ar), 132.6 (C-Ar), 134.8 (C-Ar), 140.2 (C-Ar), 149.6 (C-Ar), 160.8 (CON), 180.0 (CO); LRMS (ES$^+$) *m/z* 372.2 [M-H]$^-$; HRMS *m/z* calcd for $C_{18}H_{13}{}^{35}Cl_2N_3O_2$ [M+H]$^+$ 374.0461, found 374.0461.

_N_-(3-Sulfamoylphenyl)-4-(2-(trifluoromethyl)benzoyl)-1_H_-pyrrole-2-carboxamide (239)



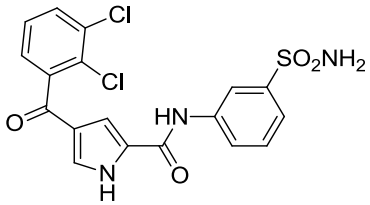
Compound **239** was synthesised according to general procedure D using 4-(2-trifluoromethylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**230**) (50 mg, 0.18 mmol), MeCN (1 mL), 3-aminobenzenesulfonamide (75 mg, 0.44 mmol) and PCl_3 (16 µL, 0.18 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the title compound as a white solid (40 mg, 51%); R_f = 0.55 (5% MeOH in EtOAc); M.p. 301-302 ^{o}C; λ_{max} (EtOH)/nm 292; ν_{max}/cm^{-1}: 3400, 3013, 2948, 2775, 2112, 1777, 1706 (CO), 1670 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.33 (1 H, s, H-3), 7.37 (2H, br s, SO_2NH_2), 7.53-7.55 (3H, m, H-3', H-5' and H-6'), 7.62 (1H, d, *J* = 7.5 Hz, H-Ar), 7.74-7.77 (2H, m, H-Ar), 7.90 (1H, d, *J* = 7.5 Hz, H-Ar), 7.96-7.99 (1H, m, H-4'), 8.28 (1 H, s, H- 5), 10.32 (1 H, s, CONH) 12.55 (1 H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.1 (C-Ar), 117.0 (C-Ar), 120.4 (C-3), 125.4 (C-2 and C-5), 130.1 (C-4), 139.2 (C-Ar), 139.8 (C-Ar), 157.4 (CON), 177.1 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.8; LRMS (ES$^+$) *m/z* 436.0 [M-H]$^-$; HRMS *m/z* calcd for $C_{19}H_{14}F_3N_3O_4SNa$ [M+Na]$^+$ 455.0990, found 455.0991.

4-(2-Fluoro-6-(trifluoromethyl)benzoyl)-*N*-(3-sulfamoylphenyl)-1*H*-pyrrole-2-carboxamide (240)



Compound **240** was synthesised according to general procedure D using 4-(2-fluoro,6-trifluoromethylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**231**) (50 mg, 0.17 mmol), MeCN (1 mL), 3-aminobenzenesulfonamide (72 mg, 0.42 mmol) and PCl_3 (17 µL, 0.17 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a pale yellow solid (57 mg, 75%); R_f = 0.53 (5% MeOH in EtOAc); M.p. 263-264 ^oC; λ_{max} (EtOH)/nm 291; ν_{max}/cm^{-1}: 3201, 2667, 2339, 1650 (CO), 1559 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 7.37 (2 H, s, SO_2NH_2), 7.47 (1 H, s, H-3), 7.52 (1 H, s,H- 5), 7.52 (2H, d, *J* = 7.6 Hz, H-Ar), 7.72-7.82 (3H, m, H-3', H-4' and H-5'), 7.96-7.98 (1H, m, H-Ar), 8.26 (1H, s, H-Ar), 10.31 (1 H, s, CONH) 12.65 (1 H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 111.4 (C-Ar), 116.9 (C-Ar), 122.7 (C-3), 125.8 (C-2 and C-5), 129.4(C-4), 139.2 (C-Ar), 144.6 (C-Ar), 157.4 (CON), 177.1 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -56.9, -114.6; LRMS (ES^+) *m/z* 456.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{13}F_4N_3O_4S$ $[M-H]^-$ 454.0483, found 454.0483.

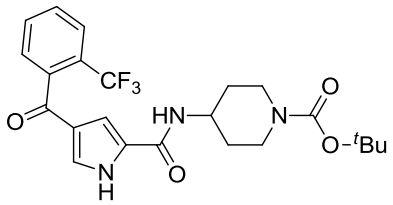
4-(2,3-Dichlorobenzoyl)-*N*-(3-sulfamoylphenyl)-1*H*-pyrrole-2-carboxamide (241)



Compound **241** was synthesised according to general procedure D using 4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**155**) (50 mg, 0.17 mmol), MeCN (1 mL), 3-aminobenzenesulfonamide (72 mg, 0.42 mmol) and PCl_3 (15 µL, 0.17 mmol) to afford the crude product. Purification was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a beige solid (43 mg, 57%); R_f = 0.62 (5% MeOH in EtOAc); M.p. 261-262 ^oC; λ_{max} (EtOH)/nm: 236; ν_{max}/cm^{-1} 3346, 2853, 1622 (CO), 1558 (CONH), 1532; ^1H NMR (500 MHz, DMSO-d_6) δ 7.40 (2H, br s, SO_2NH_2), 7.43 (1H, s, H-3), 7.47-7.55 (5H, m, 2 x H-Ar, H-4', H-5' and H-6'), 7.81 (1H, dd, *J* =

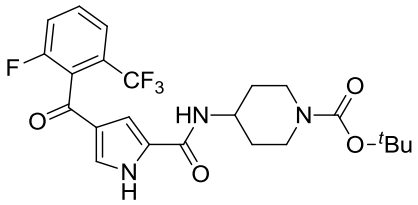
2.0 and 7.6 Hz, H-Ar), 7.98-8.00 (1H, m, H-Ar), 8.27 (1H, s, H-Ar), 10.35 (1H, s, CONH), 12.69 (1H, s, NH); ^{13}C NMR (125 MHz, MeOD) δ 112.8 (C-Ar), 113.0 (C-Ar), 115.5 (C-Ar), 119.2 (C-Ar), 122.4 (C-3), 124.8 (C-2 and C-5), 130.6 (C-4), 132.7 (C-Ar), 145.5 (C-Ar), 150.1 (CON), 176.6 (CO); LRMS (ES^+) *m/z* 438.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{13}{}^{35}Cl_2N_3O_4S$ $[M+H]^+$ 435.9923, found 435.9923.

tert-Butyl-4-(4-(2-(trifluoromethyl)benzoyl)-1H-pyrrole-2-carboxamido)piperidine-1-carboxylate (242)



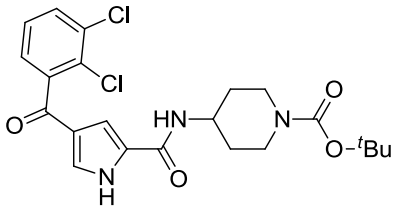
Compound **242** was synthesised according to general procedure C using 4-(2-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylic acid (**230**) (100 mg, 0.35 mmol), THF (4 mL), 4-amino-1-boc-piperidine (176 mg, 0.88 mmol) and CDI (113 mg, 0.70 mmol). Purification of the crude product was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a yellow solid (134 mg, 82%); R_f = 0.61 (5% MeOH in EtOAc); M.p. 195-196 ^oC; λ_{max} (EtOH)/nm 236.0, 282.5; ν_{max}/cm^{-1} 3183, 1620 ($CO_2{}^t$Bu), 1568 (CO), 1534 (CONH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.25-1.33 (2H, m, CH_2), 1.40 (9H, s, 3 x CH_3), 1.84-1.88 (2H, m, CH_2), 2.74-2.82 (2H, m, CH_2N), 3.94-4.05 (3H, m, CH_2N and CH), 7.03 (1H, dd, *J* = 1.3 and 2.4 Hz, H-3); 7.09 (1H, dd, *J* = 1.3 and 3.2 Hz, H-5), 7.39 (1H, d, *J* = 7.1 Hz, H-6'), 7.52-7.57 (2H, m, H-4' and H-5'), 7.69 (1H, d, *J* = 7.1 Hz, H-3'), 10.02 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.4 (3 x CH_3), 32.0 (2 x CH_2), 47.0 (2 x CH_2N), 79.8 (CH), 109.3 (C-Ar), 122.5 (C-3), 126.6 (C-Ar), 126.7, 126.8 (C-5 and C-5), 127.9 (C-Ar), 128.2 (C-Ar), 129.7 (C-Ar), 131.4 (C-4), 139.0 (C-Ar), 154.6 ($CO_2{}^t$Bu), 159.8 (CON), 189.9 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -59.0; LRMS (ES^+) *m/z* 482.40 $[M-H]^-$; HRMS *m/z* calcd for $C_{23}H_{27}F_3N_3O_4$ $[M+H]^+$ 483.2210, found 483.2210.

***tert*-Butyl-4-(4-(2-fluoro-6-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxamido)piperidine-1-carboxylate (243)**



Compound **243** was synthesised according to general procedure C using 4-(2-fluoro, 6-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxylic acid (**231**) (100 mg, 0.33 mmol), THF (4 mL), 4-amino-1-boc-piperidine (165 mg, 0.83 mmol) and CDI (108 mg, 0.66 mmol). Purification of the crude product was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a peach solid (119 mg, 75%); R_f = 0.60 (5% MeOH in EtOAc); M.p. 190-191 ^oC; λ_{max} (EtOH)/nm 236.0, 274.5; ν_{max}/cm^{-1}: 3242, 1623 ($CO_2{}^tBu$), 1568 (CO), 1537 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 1.27-1.36 (2H, m, CH_2), 1.39 (9H, s, 3 x CH3), 1.86-1.90 (2H, m, CH2), 2.80-2.83 (2H, m, CH_2N), 3.96-4.04 (3H, m, CH_2N and CH), 6.96-6.97 (1H, m, H-3); 7.15 (1H, dd, *J* = 1.3 and 3.2 Hz, H-5), 7.28-7.31 (2H, m, H-3' and H-5'), 7.48-7.53 (1H, m, H-4'), 9.92 (1H, br s, NH); ^{13}C NMR (500 MHz, MeOD) δ 28.4 (3 x CH_3), 32.1 (2 x CH_2), 47.1 (2 x CH_2N), 79.8 (CH), 108.6, 119.6 (d, J_{CF} = 22.9 Hz, C-Ar), 119.7 (C-3), 122.3 (C-Ar), 127.1 (C-2 and C-5), 127.7, 128.0 (C-Ar), 131.1 (C-4), 131.5 (d, J_{CF} = 8.1 Hz, C-Ar), 154.7 (d, J_{CF} = 243.1 Hz, CF), 159.6 ($CO_2{}^tBu$), 160.0 (CON), 184.9 (CO); ^{19}F NMR (470 MHz, MeOD) δ -113.2, -58.1; LRMS (ES^+) *m/z* 482.4 $[M-H]^-$; HRMS *m/z* calcd for $C_{23}H_{26}F_4N_3O_4$ $[M+H]^+$ 484.1235, found 484.1230.

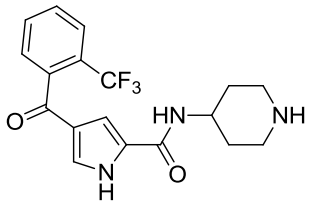
***tert*-Butyl 4-(4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxamido)piperidine-1-carboxylate (244)**



Compound **244** was synthesised according to general procedure C using 4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**155**) (100 mg, 0.35 mmol), THF (4 mL), 4-amino-1-boc-piperidine (175 mg, 0.88 mmol) and CDI (114 mg, 0.70 mmol). Purification of the crude product was achieved using MPLC (silica, 0-10% MeOH in EtOAc) to give the pure compound as a white solid (131 mg, 80%); R_f = 0.65 (5% MeOH in EtOAc); M.p. 185-186 ^oC; λ_{max} (EtOH)/nm 236.5, 287.0; ν_{max}/cm^{-1}: 3158,

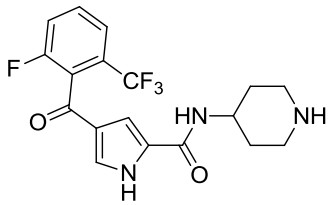
1686 ($CO_2{}^tBu$), 1621 (CO), 1571 (CONH), 1537; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.41 (9H, s, 3 x CH_3), 1.67-1.77 (4H, m, 2 x CH_2), 3.50-3.56 (1H, m, CH), 3.67-3.84 (4H, m, 2 x CH_2N), 7.17 (1H, d, *J* = 1.3 Hz, H-3); 7.27 (1H, d, *J* = 1.3 Hz, H-5), 7.42 (1H, dd, *J* = 1.5 and 8.0 Hz, H-Ar), 7.47-7.45 (1H, m, H-Ar), 7.48 (1H, dd, *J* = 1.5 and 8.0 Hz, H-Ar), 8.10 (1H, d, *J* = 7.7 Hz, CONH), 12.02 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.4 (3 x CH_3), 32.1 (2 x CH_2), 47.1 (2 x CH_2N), 79.8 (CH), 109.0 (C-Ar), 123.9 (C-3), 126.4 (C-2 and C-5), 127.4 (C-Ar), 127.9 (C-Ar), 131.5 (C-4), 141.5 (C-Ar), 159.5 ($CO_2{}^tBu$), 163.7 (CON), 184.2 (CO); LRMS (ES^+) *m/z* 464.3 $[M-H]^-$; HRMS *m/z* calcd for $C_{22}H_{26}{}^{35}Cl_2N_3O_4$ $[M+H]^+$ 466.1295, found 466.1294.

_N_-(Piperidin-4-yl)-4-(2-(trifluoromethyl)benzoyl)-1_H_-pyrrole-2-carboxamide (245)



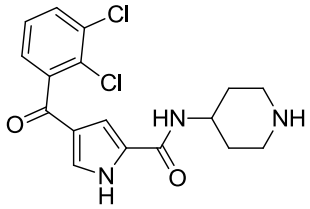
Compound **245** was synthesised according to general procedure E using *tert*-Butyl 4-(4-(2-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxamido)piperidine-1-carboxylate (**230**) (40 mg, 0.09 mmol), TFA (1.0 mL), Et_3SiH (34 µL, 0.10 mmol) and DCM (2 mL). The pure product was obtained as a white solid (30 mg, 97%); R_f = 0.48 (5% MeOH in EtOAc); M.p. 233-134 ^oC; λ_{max} (EtOH)/nm 236, 341; ν_{max}/cm^{-1} 3311 (NH), 2939, 2833, 2671, 1626 (CO), 1617 (CONH); ^{1}H NMR (500 MHz, MeOD) δ 1.30-1.38 (2H, m, CH_2), 1.69 (2H, d, *J* = 10.3 Hz, CH_2), 2.46-2.48 (2H, m, CH_2N), 2.10 (2H, d, *J* = 10.3 Hz, CH_2N), 3.72-3.79 (1H, m, CH), 7.15 (2H, br s, H-3 and H-5), 7.55 (1H, d, *J* = 7.3 Hz, H-6'), 7.70-7.78 (2H, m, H-4' and H-5'), 7.85 (1H, d, *J* = 7.3 Hz, H-3'), 8.03 (1H, br s, CONH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 33.0 (2 x CH_2), 49.2 (2 x CH_2N), 79.9 (CH), 110.5 (C-Ar), 123.4 (C-3), 128.0 (C-Ar), 126.8 (C-5 and C-5), 128.1 (C-Ar), 128.5 (C-Ar), 129.9 (C-Ar), 131.0 (C-4), 140.1 (C-Ar), 164.1 (CON), 184.2 (CO); ^{19}F NMR (470 MHz, MeOD) δ -56.8; LRMS (ES^+) *m/z* 366.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{19}F_3N_3O_2$ $[M+H]^+$ 366.1423, found 366.1424.

4-(2-Fluoro-6-(trifluoromethyl)benzoyl)-*N*-(piperidin-4-yl)-1*H*-pyrrole-2-carboxamide (246)



Compound **246** was synthesised according to general procedure E using *tert*-Butyl 4-(4-(2-fluoro-6-(trifluoromethyl)benzoyl)-1*H*-pyrrole-2-carboxamido)piperidine-1-carboxylate (**231**) (40 mg, 0.08 mmol), TFA (1.0 mL), Et_3SiH (34 µL, 0.20 mmol) and DCM (2 mL). the pure product was obtained as a white solid (20 mg, 65%); R_f = 0.45 (5% MeOH in EtOAc); M.p. 225-227 ^oC; λ_{max} (EtOH)/nm 236; ν_{max}/cm^{-1}: 2815 (NH), 2106, 1720 (CO), 1615 (CONH); ^1H NMR (500 MHz, MeOD) δ 1.34-1.42 (2H, m, CH_2), 1.79 (2H, d, *J* = 11.9 Hz, CH_2), 2.52-2.57 (2H, m, CH_2N), 2.95 (2H, d, *J* = 11.9 Hz, CH_2N), 3.77-3.83 (1H, m, CH), 7.05 (1 H, s, H-3), 7.21 (1 H, s,H- 5), 7.39-7.42 (1H, m, H-4'), 7.52-7.61 (2H, m, H-3' and H-4'); ^{13}C NMR (125 MHz, MeOD) δ 33.3 (2 x CH_2), 44.7 (2 x CH_2N), 58.4 (CH), 112.3 (C-Ar), 120.8 (C-3), 123.5 (C-2 and C-5), 127.8 (C-4), 131.3 (C-Ar), 132.6 (C-Ar), 159.5 (C-Ar), 161.4 (C-Ar), 162.6 (CONH), 186.9 (CO); ^{19}F NMR (470 MHz, MeOD) δ -59.6, -115.8; LRMS (ES^+) *m/z* 384.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{17}F_4N_3O_2$ $[M+H]^+$ 392.1018, found 392.1019.

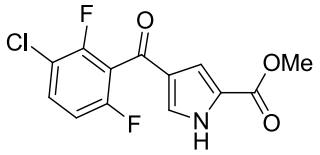
4-(2,3-Dichlorobenzoyl)-*N*-(piperidin-4-yl)-1*H*-pyrrole-2-carboxamide (247)



Compound **247** was synthesised according to general procedure E using *tert*-Butyl 4-(4-(2,3-dichlorobenzoyl)-1*H*-pyrrole-2-carboxamido)piperidine-1-carboxylate (**155**) (20 mg, 0.04 mmol), TFA (0.50 mL), Et_3SiH (17 µL, 0.10 mmol) and DCM (1 mL). the pure product was obtained as a white solid (12 mg, 0.03 mmol, 80%); R_f = 0.55 (5% MeOH in EtOAc); M.p. 251 ^oC (dec.); λ_{max} (EtOH)/nm: 236, 288; ν_{max}/cm^{-1} 3411 (NH), 3310, 2114, 1632 (CO), 1601 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 1.28-1.38 (2H, m, CH_2), 1.69 (2H, d, *J* = 12.3 Hz, CH_2), 2.45-2.48 (2H, m, CH_2N), 2.95 (2H, d, *J* = 12.3 Hz, CH_2N), 3.71-3.81 (1H, m, CH), 7.18 (1H, br s, H-3), 7.25 (1H, br s, H-5), 7.42 (1H, d, *J* = 7.3 Hz, H-Ar), 7.47-7.50 (2H, m, H-Ar), 7.77 (1H, d, *J* = 7.3 Hz, H-Ar), 8.06 (1H, br s, CONH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 36.0 (2 x CH_2), 45.6 (2

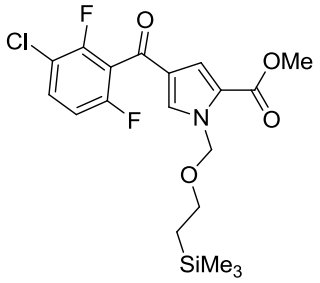
x CH_2N), 59.9 (CH), 113.0 (C-Ar), 121.2 (C-3), 123.6 (C-2 and C-5), 129.2 (C-4), 131.4 (C-Ar), 133.5 (C-Ar), 158.4 (C-Ar), 162.1 (C-Ar), 164.2 (CONH), 184.7 (CO); LRMS (ES^+) *m/z* 367.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{18}{}^{35}Cl_2N_3O_2$ $[M+H]^+$ 366.0775, found: 366.0775.

Methyl 4-(3-chloro-2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylate (268)



Compound **268** was synthesised according to general procedure A using methyl pyrrole-2-carboxylate (**69**) (2.00 g, 16.0 mmol), 3-chloro-2,6-difluorobenzoyl chloride (6.74 g, 32.0 mmol), $AlCl_3$ (5.33 g, 40.0 mmol) and DCM (40 mL). The crude mixture was purified by MPLC (0-50% EtOAc in petrol) to give the pure product as a white solid (4.50 g, 94%); R_f = 0.45 (5% MeOH in EtOAc); M.p. 187-188 ^oC; λ_{max} (EtOH)/nm 234, 283; ν_{max}/cm^{-1}: 3256,1694 (CO_2Me), 1650 (CO), 1614, 1560; ^1H NMR (500 MHz, $CDCl_3$) δ 3.82 (3H, s, CH_3), 6.88-6.92 (1H, m, H-5'), 7.15 (1H, br s, H-3), 7.41 (1H, dddd, *J* = 6.8, 6.9, 8.5 and 8.6 ,H-4'), 7.43 (1H, br s, H-5), 9.74 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.1 (CH_3), 112.6 (C-Ar), 112.8 (C-Ar), 115.6 (C-Ar), 124.6 (C-3), 128.2 (C-2 and C-5), 131.8 (C-4), 131.9 (C-Ar), 155.1 (d, J_{CF} = 245.3 Hz, CF), 162.2 (d, J_{CF} = 219.7 Hz, C-Ar), 166.0 (CO_2Me), 191.0 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -113.4, -112.6; LRMS (ES^+) *m/z* 300.6 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_9{}^{35}ClF_2NO_3$ $[M+H]^+$ 300.0240, found 300.0242.

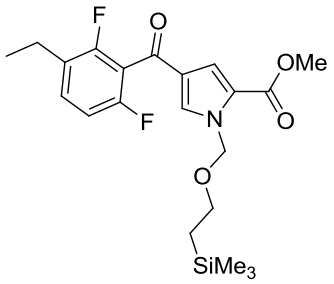
Methyl 4-(3-chloro-2,6-difluorobenzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (269)



Methyl 4-(3-chloro-2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**268**) (500 mg, 1.67 mmol) was dissolved in THF (5 mL) at 0 ^oC before potassium *tert*-butoxide (225 mg, 2.00 mmol) was added and the mixture was stirred for 10 min. SEM chloride (0.354 mL, 2.00 mmol) was added and the mixture was allowed to warm to RT overnight. The product was extracted with ethyl acetate and washed with water followed by brine. The

combined organic extracts were dried over Na_2SO_4 and the solvent was removed *in vacuo.* The product was purified using MPLC (0-50% ethyl acetate in petrol) to give the title product as a colourless oil (0.68 g, 95%); R_f = 0.55 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 234, 279; ν_{max}/cm^{-1}: 2952, 1716 (CO_2Me), 1660 (CO), 1578, 1542; ^1H NMR (500 MHz, $CDCl_3$) δ 0.00 (1H, s, $Si(CH_3)_3$), 0.93-0.96 (2H, m, CH_2), 3.57-3.61 (2H, m, CH_2), 3.86 (3H, s, CH_3), 5.73 (2H, s, CH_2O), 6.98-7.01 (1H, m, H-5'), 7.33 (1H, br s, H-3), 7.50 (1H, dddd, *J* = 6.7, 6.8, 8.6 and 8.7 ,H-4'), 7.57 (1H, br s, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.8 ($Si(CH_3)_3$), 51.7 (2 x CH_2), 67.0 (CH_3), 76.8 (CH_2O), 112.6 (C-Ar), 112.7 (C-Ar), 119.0 (C-Ar), 124.2 (C-3), 124.6 (C-2 and C-5), 131.7 (C-4), 131.8 (C-Ar), 132.7 (C-Ar), 154.8 (d, J_{CF} = 246.0 Hz, CF), 157.2 (d, J_{CF} = 218.4 Hz, C-Ar), 160.8 (CO_2Me), 180.9 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -113.5, -112.8; LRMS (ES^+) *m/z* 430.8 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{23}{}^{35}ClF_2NO_4Si$ $[M+H]^+$ 430.1051, found 430.1051.

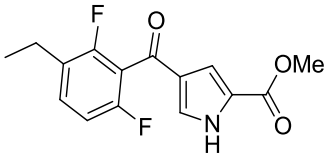
Methyl 4-(3-ethyl-2,6-difluorobenzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (270)



Methyl 4-(3-chloro-2,6-difluorobenzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (**269**) (150 mg, 0.35 mmol) was dissolved in dioxane (3 mL), along with ethyl boronic acid (78 mg, 1.05 mmol) and Cs_2CO_3 (171 mg, 0.53 mmol). The mixture was degassed for 20 min before dichloro [1,1' bis(di-tert-butylphosphino)]ferrocene palladium (II) (22 mg, 0.035 mmol) was added and it was degassed for a further 20 min. The reaction was heated under microwave irradiation for 90 min at 150 ^oC before being filtered through Celite and the solvent removed *in vacuo.* The crude product was purified by MPLC (0-20% EtOAc in petrol) to give the compound as a pale yellow oil (88 mg, 60%); R_f = 0.54 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 235, 279; ν_{max}/cm^{-1}: 2953, 1719 (CO_2Me), 1660 (CO), 1543; ^1H NMR (500 MHz, $CDCl_3$) δ 0.00 (1H, s, $Si(CH_3)_3$), 0.93-0.96 (2H, m, CH_2), 1.26 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 2.70 (2H, q, *J* = 7.5 Hz, CH_2CH_3), 3.57-3.60 (2H, m, CH_2), 3.87 (3H, s, CH_3), 5.73 (2H, s, CH_2O), 6.91-6.95 (1H, m, H-5'), 7.26-7.30 (1H, m, H-4'), 7.36 (1H,

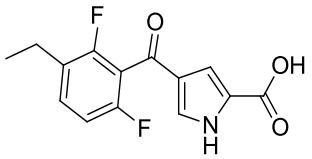
br s, H-3), 7.56 (1H, br s, H-5); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3 (CH_2CH_3), 17.8 ($Si(CH_3)_3$), 51.7 (2 x CH_2), 52.0 (CH_2CH_3), 66.0 (CH_3), 75.0 (CH_2O), 111.3 (C-Ar), 111.8 (C-Ar), 119.2 (C-Ar), 123.9 (C-3), 125.1 (C-2 and C-5), 127.5 (C-4), 131.2 (C-Ar), 132.8 (C-Ar), 156.7 (d, J_{CF} = 247.5 Hz, CF), 161.0 (CO_2Me), 168.1 (d, J_{CF} = 220.4 Hz, C-Ar), 182.8 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -117.5, -119.6; LRMS (ES^+) *m/z* 424.4 $[M+H]^+$; HRMS *m/z* calcd for $C_{21}H_{28}F_2NO_4Si$ $[M+H]^+$ 424.1750, found 424.1752.

Methyl 4-(3-ethyl-2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylate (271)



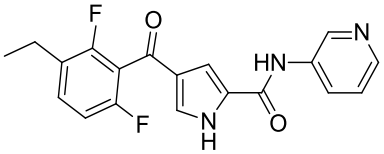
Methyl 4-(3-ethyl-2,6-difluorobenzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (**270**) (145 mg, 0.34 mmol) was dissolved in THF (1 mL) before TBAF (0.7 mL, 1.0 M in THF) was added and the reaction was heated to 65 ^{o}C for 18 h. Upon cooling to RT the solvent was removed *in vacuo*. The crude product was purified by MPLC (0-8 % MeOH in DCM) to give the pure product as a colourless oil (65 mg, 65%); R_f = 0.42 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 236, 278; ν_{max}/cm^{-1}: 2952, 1720 (CO_2Me), 1668 (CO), 1541; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.27 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 2.71 (2H, q, *J* = 7.4 Hz, CH_2CH_3), 3.87 (3H, s, CH_3), 6.91-6.95 (1H, m, H-5'), 7.26-7.30 (1H, dddd, *J* = 6.6, 6.7, 8.6 and 8.7 ,H-4'), 7.35 (1H, br s, H-3), 7.58 (1H, br s, H-5), 9.65 (1H, s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.6 (CH_2CH_3), 49.0 (CH_2CH_3), 65.0 (CH_3), 112.3 (C-Ar), 112.6 (C-Ar), 120.1 (C-Ar), 123.6 (C-3), 126.6 (C-2 and C-5), 129.5 (C-4), 131.2 (C-Ar), 132.7 (C-Ar), 153.1 (d, J_{CF} = 245.1 Hz, CF), 163.0 (CO_2Me), 167.2 (d, J_{CF} = 216.8 Hz, C-Ar), 186.0 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -117.6, -119.1; LRMS (ES^+) *m/z* 294.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{15}H_{14}F_2NO_3$ $[M+H]^+$ 294.1852, found 294.1856.

4-(3-Ethyl-2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (272)



Compound **272** was synthesised according to general procedure B using, Methyl 4-(3-ethyl-2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**271**) (60 mg, 0.20 mmol), THF (3 mL), LiOH (96 mg) in H_2O (3 mL). The pure product was obtained as a white solid (55 mg, 98%); R_f = 0.35 (5% MeOH in EtOAc); M.p. λ_{max} (EtOH)/nm 235, 280; ν_{max}/cm^{-1}: 2969, 1715 (CO_2H), 1659 (CO), 1536; ^1H NMR (500 MHz, DMSO-d_6) δ 1.27 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 2.74 (2H, q, *J* = 7.5 Hz, CH_2CH_3), 7.07 (1H, s, H-3), 7.23-7.27 (1H, m, H-5'), 7.55-7.61 (2H, m, H-5 and H-4'), 12.76 (1H, s, NH), 13.03 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 15.0 (CH_2CH_3), 48.1 (CH_2CH_3), 111.6 (C-Ar), 112.0 (C-Ar), 121.1 (C-Ar), 123.4 (C-3), 126.2 (C-2 and C-5), 129.5 (C-4), 132.2 (C-Ar), 132.7 (C-Ar), 154.1 (d, J_{CF} = 248.1 Hz, CF), 166.2 (d, J_{CF} = 214.4 Hz, C-Ar), 168.0 (CO_2H), 185.6 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -117.6, -119.7; LRMS (ES^+) *m/z* 278.2 $[M-H]^-$; HRMS *m/z* calcd for $C_{14}H_{10}F_2NO_3$ $[M-H]^-$ 278.0634, found 278.0626.

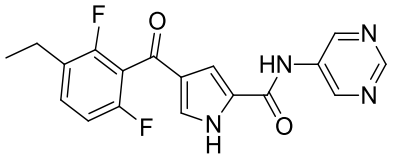
(3-Ethyl-2,6-difluorophenyl)(5-((pyridin-3-ylamino)methyl)-1*H*-pyrrol-3-yl)methanone (273)



Compound **273** was synthesised according to general procedure D using 4-(3-Ethyl-2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**272**) (70 mg, 0.25 mmol), 3-aminopyridine (59 mg, 0.63 mmol), PCl_3 (22 µL, 0.25 mmol) and MeCN (1 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) followed by (C-18 silica, 1:1 MeCN (0.1% formic acid):H_20 (0.1% formic acid) followed by semi-prep. HPLC (C-18 silica; 5-100% acetonitrile in water (0.1% formic acid)) to give the title compound as a white solid (15 mg, 13%); R_f = 0.43 (5% DCM in MeOH); M.p. 255-266 ^oC; λ_{max} (EtOH)/nm 253, 294; ν_{max}/cm^{-1}: 3233, 3121, 2970, 2923, 1625 (CO), 1536 (CONH); ^1H NMR (500 MHz, MeOD) δ 1.15 (3H, t, *J* = 7.6 Hz, CH_2CH_3), 2.62 (2H, q, *J* = 7.6 Hz, CH_2CH_3), 6.92-6.96 (1H, m, H-Ar), 7.31-7.35 (2H, m, H-Ar and CH-

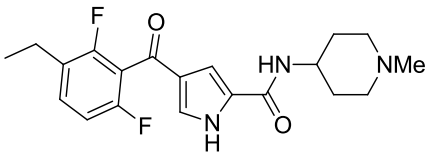
pyridine), 7.37 (1H, s, H-3), 7.39 (1H, s, H-5), 8.13 (1H, d, *J* = 6.6 Hz, CH-pyridine), 8.18 (1H, br s, CH-N-pyridine), 8.80 (1H, br s, CH-N-pyridine); ^{13}C NMR (125 MHz, MeOD) δ14.8 (CH_2CH_3), 22.7 (CH_2CH_3), 112.5 (d, *J* = 21.8 Hz, C-Ar), 112.7 (C-Ar), 119.0 (C-3), 125.3 (C-5), 128.0 (C-2), 128.8 (d, *J* = 16.7 Hz, C-Ar), 129.4 (C-4), 129.6 (C-pyridine), 132.8 (dd, *J* = 7.2 and 9.6 Hz, C-Ar), 142.3 (C-pyridine), 145.1 (C-pyridine), 158.3 (d, J_{CF} = 247.3 Hz, C-F), 158.9 (d, J_{CF} = 243.9 Hz, C-F), 160.9 (CON), 185.2 (CO); ^{19}F NMR (470 MHz, MeOD) δ -119.1, -120.8; LRMS (ES^+) *m/z* 356.4 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{15}F_2N_4O_2$ $[M+H]^+$ 356.1053, found 356.1056.

4-(3-Ethyl-2,6-difluorobenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (274)



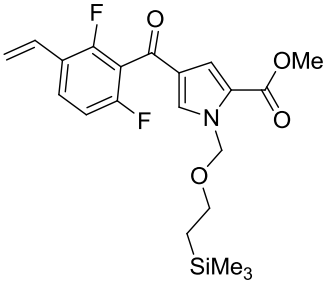
Compound **274** was synthesised according to general procedure D using 4-(3-Ethyl-2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**272**) (70 mg, 0.25 mmol), 5-aminopyrimidine (59 mg, 0.63 mmol), PCl_3 (22 µL, 0.25 mmol) and MeCN (1 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) followed by (C-18 silica, 1:1 MeCN (0.1% formic acid):H_20 (0.1% formic acid) followed by semi-prep. HPLC (C-18 silica; 5-100% acetonitrile in water (0.1% formic acid)) to give the title compound as a white solid (25 mg, 28%); R_f = 0.43 (5% DCM in MeOH); M.p. 259-260 ^oC; λ_{max} (EtOH)/nm 267, 291; ν_{max}/cm^{-1}: 3332, 3252, 2971, 1665, 1626 (CO), 1589 (CONH), 1524; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.29 (3H, t, *J* = 7.7 Hz, CH_2CH_3), 2.75 (2H, q, *J* = 7.7 Hz, CH_2CH_3), 7.27-7.30 (1H, m, H-Ar), 7.57-7.64 (3H, m, H-3, H-5 and H-Ar), 9.00 (1H, s, CH-pyrimidine), 9.22 (2H, s, 2 x CH-pyrimidine), 10.52 (1H, br s, CONH), 12.89 (1H, br s, NH); ^{19}F NMR (470 MHz, $CDCl_3$) δ -117.6, -119.7; LRMS (ES^+) *m/z* 372.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{15}F_2N_4O_2$ $[M+H]^+$ 357.1158, found 357.1158.

4-(3-Ethyl-2,6-difluorobenzoyl)-*N*-(1-methylpiperidin-4-yl)-1*H*-pyrrole-2-carboxamide (275)



Compound **275** was synthesised according to general procedure D using 4-(3-Ethyl-2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**272**) (90 mg, 0.32 mmol), 4-amino-1-methylpiperidine (76 mg, 0.80 mmol), PCl_3 (28 µL, 0.32 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) followed by (C-18 silica, 1:1 MeCN (0.1% formic acid):H_20 (0.1% formic acid) followed by semi-prep. HPLC (C-18 silica; 5-100% acetonitrile in water (0.1% formic acid)) to give the title compound as a white solid (23 mg, 19%); R_f = 0.35 (5% DCM in MeOH); M.p. 262-263 ^oC; λ_{max} (EtOH)/nm 237, 287; ν_{max}/cm^{-1}: 3248, 2929, 1650 (CO), 1591 (CONH), 1539; ^1H NMR (500 MHz, $CDCl_3$) δ 1.15 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 2.03 (4H, br s, 2 x CH_2), 2.58 (2H, q, *J* = 7.5 Hz, CH_2CH_3), 2.60-2.64 (5H, m, NCH_3 + CH_2N), 3.32 (2H, br s, CH_2), 6.80-6.84 (1H, m, H-Ar), 7.14-7.20 (1H, m, H-Ar), 7.26 (1H, s, H-3), 7.61 (1H, s, H-5), 8.44 (1H, br s, CONH), 10.63 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 14.3 (CH_2CH_3), 21.7 (CH_2CH_3), 29.4 (2 x CH_2), 43.8 (NCH_3), 44.3 (CH), 53.7 (2 x CH_2N), 111.2 (C-Ar), 111.4 (C-Ar), 117.9 (C-3), 127.1 (C-2 and C-5), 1.27.4 (d, *J* = 20.5 Hz, C-Ar), 127.9 (d, *J* = 4.5 Hz, C-Ar), 131.1 (C-4), 157.3 (d, J_{CF} = 202.1 Hz, CF), 160.1 (CONH), 167.2 (d, J_{CF} = 214.4 Hz, C-Ar), 183.3 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -116.7,-118.2; LRMS (ES^+) *m/z* 376.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{20}H_{24}F_2N_3O_2$ $[M+H]^+$ 376.1024, found 376.1023.

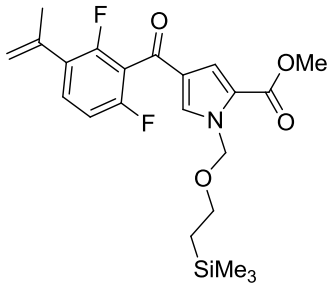
Methyl 4-(2,6-difluoro-3-vinylbenzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (276)



Methyl 4-(3-chloro-2,6-difluorobenzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (**269**) (1.0 g, 2.32 mmol) was dissolved in THF (18 mL) before potassium vinyltrifluoroborate (310 mg, 2.32 mmol) was added followed by Cs_2CO_3

(2.26 g, 6.96 mmol), XPhos (66 mg, 0.12 mmol) and $PdCl_2$ (8 mg, 0.04 mmol) and water (2 mL). The reaction was heated to 85 ^oC and left to stir for 1 h. Upon cooling, the mixture was filtered through Celite and the solvent removed *in vacuo*. The crude mixture was purified by MPLC (0-30% EtOAc in petrol). The pure product was obtained as a cream oil (778 mg, 80%); R_f = 0.56 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 236, 279; ν_{max}/cm^{-1}: 2954, 1720 (CO_2Me), 1661 (CO), 1544; ^1H NMR (500 MHz, $CDCl_3$) δ 0.00 (1H, s, $Si(CH_3)_3$), 0.92-0.96 (2H, m, CH_2), 3.57-3.60 (2H, m, CH_2), 3.87 (3H, s, CH_3), 5.45 (1H, d, *J* = 11.2 Hz, vinyl CH), 5.74 (2H, s, CH_2O), 5.83 (1H, d, *J* = 17.7 Hz, vinyl CH), 6.74 (1H, dd, *J* = 11.2 and 17.7 Hz, vinyl CH)**,** 6.97-7.02 (1H, m, H-5'), 7.35 (1H, s, H-3), 7.57 (1H, s, H-5), 7.58-7.60 (1H, m, H-4'); ^{13}C NMR (125 MHz, $CDCl_3$) δ (17.8 ($Si(CH_3)_3$), 51.7 (2 x CH_2), 66.9 (CH_3), 76.7 (CH_2O), 111.8 (CH_2 vinyl) 111.9 (C-Ar), 112.0 (C-Ar), 112.2 (CH vinyl), 119.1 (C-Ar), 124.0 (C-3), 125.0 (C-2 and C-5), 127.9 (C-4), 131.2 (C-Ar), 132.8 (C-Ar), 155.0 (d, J_{CF} = 247.5 Hz, CF), 163.0 (CO_2Me), 168.1 (d, J_{CF} = 218.4 Hz, C-Ar), 182.8 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.2, -117.4; LRMS (ES^+) *m/z* 422.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{12}H_{26}F_2NO_4Si$ $[M+H]^+$ 422.1108, found 424.1110.

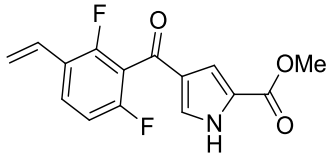
Methyl-4-(2,6-difluoro-3-(prop-1-en-2-yl)benzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (278)



Methyl 4-(3-chloro-2,6-difluorobenzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (**269**) (1.65 g, 3.84 mmol) was dissolved in THF (18 mL) before potassium isopropenyltrifluoroborate (568 mg, 3.84 mmol) was added followed by Cs_2CO_3 (3.75 g, 1.5 mmol), XPhos (110 mg, 0.23 mmol) and $PdCl_2$ (14 mg, 0.08 mmol) and water (2 mL). The reaction was heated to 85 ^oC and left to stir for 1 h. Upon cooling, the mixture was filtered through Celite and the solvent removed *in vacuo*. The crude mixture was purified by MPLC (0-30% EtOAc in petrol). The pure product was obtained as a colourless oil (699 mg, 42%); R_f = 0.57 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 235, 280; ν_{max}/cm^{-1}: 2965, 1695 (CO_2Me), 1653 (CO), 1521; ^1H NMR (500 MHz, $CDCl_3$) δ 0.00 (1H, s, $Si(CH_3)_3$), 0.92-0.96 (2H, m, CH_2), 2.15 (3H, s, CH_3), 3.56-

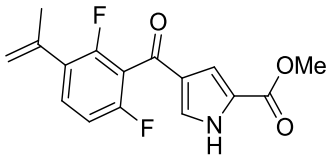
3.60 (2H, m, CH_2), 3.87 (3H, s, CH_3), 5.24 (1H, s, alkene-CH), 5.31 (1H, s, alkene-CH), 5.72 (2H, s, CH_2O), 6.95-6.99 (1H, m, H-5'), 7.36 (1H, s, H-3), 7.56 (1H, s, H-5), 7.59-7.61 (1H, m, H-4'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.5 ($Si(CH_3)_3$), 23.0 (CH_3), 51.5 (2 x CH_2), 68.0 (OCH_3), 77.2 (CH_2O), 112.0 (alkene-CH_2) 112.2 (C-Ar), 112.4 (C-Ar), 112.8 (C-Ar), 125.0 (C-3), 126.0 (C-2 and C-5), 127.9 (C-4), 131.3 (C-Ar), 134.0 (C-Ar), 156.0 (d, J_{CF} = 245.5 Hz, CF), 165.0 (CO_2Me), 169.0 (d, J_{CF} = 215.4 Hz, C-Ar), 182.8 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.2, -117.4; LRMS (ES^+) *m/z* 436.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{22}H_{28}F_2NO_4Si$ $[M+H]^+$ 436.1750, found 436.1747.

Methyl 4-(2,6-difluoro-3-vinylbenzoyl)-1*H*-pyrrole-2-carboxylate (278)



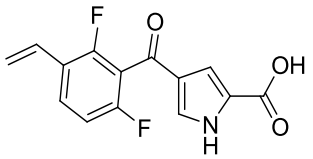
Methyl 4-(2,6-difluoro-3-vinylbenzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (**276**) (730 mg, 1.73 mmol) was dissolved in THF (15 mL) before TBAF (3.5 mL, 1.0 M in THF) was added and the reaction was heated to 65 ^{o}C for 18 h. Upon cooling to RT the solvent was removed *in vacuo.* The crude product was purified by MPLC (0-8 % MeOH in DCM) to give the pure product as a colourless oil (332 mg, 66%); R_f = 0.45 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 235, 280; ν_{max}/cm^{-1}: 2960, 1716 (CO_2Me), 1656 (CO), 1604; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.86 (3H, s, CH_3), 5.39 (1H, d, *J* = 11.4 Hz, vinyl CH), 5.76 (1H, d, *J* = 17.7 Hz, vinyl CH), 6.78 (1H, dd, *J* = 11.4 and 17.7 Hz, vinyl CH)**,** 6.94-6.99 (1H, m, H-5'), 7.21 (1H, s, H-3), 7.47 (1H, s, H-5), 7.54 (1H, dddd, *J* = 6.8, 6.9, 8.5 and 8.6 Hz, H-4'), 9.84 (1H, br s, NH);; ^{13}C NMR (125 MHz, $CDCl_3$) δ 65.2 (CH_3), 112.0 (CH_2 vinyl) 112.2 (C-Ar), 112.3 (C-Ar), 112.8 (CH vinyl), 119.5 (C-Ar), 124.0 (C-3), 125.6 (C-2 and C-5), 128.1 (C-4), 132.2 (C-Ar), 132.8 (C-Ar), 156.0 (d, J_{CF} = 245.6 Hz, CF), 165.2 (CO_2Me), 169.1 (d, J_{CF} = 225.4 Hz, C-Ar), 182.9 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.2, -117.4; LRMS (ES^+) *m/z* 292.3 $[M+H]^+$.

Methyl 4-(2,6-difluoro-3-(prop-1-en-2-yl)benzoyl)-1*H*-pyrrole-2-carboxylate (279)



methyl 4-(2,6-difluoro-3-(prop-1-en-2-yl)benzoyl)-1-((2-(trimethylsilyl)ethoxy)methyl)-1*H*-pyrrole-2-carboxylate (**277**) (350 mg, 0.80 mmol) was dissolved in THF (7 mL) before TBAF (1.7 mL, 1.0 M in THF) was added and the reaction was heated to 65 ^{o}C for 18 h. Upon cooling to RT the solvent was removed *in vacuo.* The crude product was purified by MPLC (0-8 % MeOH in DCM) to give the pure product as a colourless oil (170 mg, 70%); R_f = 0.46 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 236, 282; ν_{max}/cm^{-1}: 3300, 1710 (CO_2Me), 1654 (CO), 1596, 1516; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.13 (3H, s, CH_3), 3.85 (3H, s, OCH_3), 5.21 (1H, s, alkene-CH), 5.32 (1H, s, alkene-CH), 6.98-7.01 (1H, m, H-5'), 7.35 (1H, s, H-3), 7.58 (1H, s, H-5), 7.60-7.64 (1H, m, H-4'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 23.0 (CH_3), 67.0 (OCH_3), 111.9 (alkene-CH_2) 112.3 (C-Ar), 112.4 (C-Ar), 113.2 (C-Ar), 124.8 (C-3), 126.7 (C-2 and C-5), 129.5 (C-4), 130.9 (C-Ar), 133.0 (C-Ar), 153.2 (d, J_{CF} = 249.6 Hz, CF), 164.2 (CO_2Me), 169.6 (d, J_{CF} = 217.4 Hz, C-Ar), 184.6 (CO); ^{19}F NMR (470 Hz, $CDCl_3$) δ -114.1, -117.5; LRMS (ES^+) *m/z* 306.3 $[M+H]^+$.

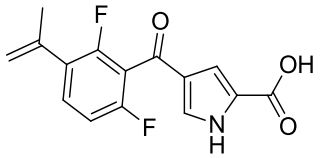
4-(2,6-Difluoro-3-vinylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (280)



Compound **280** was synthesised according to general procedure B using, Methyl 4-(2,6-difluoro-3-vinylbenzoyl)-1*H*-pyrrole-2-carboxylate (**278**) (165 mg, 0.57 mmol), THF (5 mL), LiOH (271 mg, 11.33 mmol) in H_2O (7 mL). The pure product was obtained as a white solid (156 mg, 99%); R_f = 0.35 (5% MeOH in EtOAc); M.p. 190-191 ^{o}C; λ_{max} (EtOH)/nm 236, 280; ν_{max}/cm^{-1}: 2965, 1720 (CO_2Me), 1658 (CO), 1610; ^{1}H NMR (500 MHz, DMSO-d_6) δ 5.41 (1H, d, *J* = 11.1 Hz, vinyl CH), 5.76 (1H, d, *J* = 17.5 Hz, vinyl CH), 6.87 (1H, dd, *J* = 11.1 and 17.5 Hz, vinyl CH)**,** 6.95-67.01 (1H, m, H-5'), 7.35 (1H, s, H-3), 7.48 (1H, s, H-5), 7.60 (1H, dddd, *J* = 6.6, 6.7, 8.6 and 8.7 Hz, H-4'), 12.75 (1H, br s, NH) 12.94 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.2

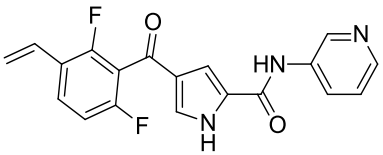
(CH_2 vinyl) 112.3 (C-Ar), 112.4 (C-Ar), 112.8 (CH vinyl), 119.0 (C-Ar), 120.9 (C-3), 125.4 (C-2 and C-5), 129.5 (C-4), 132.2 (C-Ar), 132.9 (C-Ar), 153.0 (d, J_{CF} = 235.6 Hz, CF), 165.2 (CO_2H), 169.1 (d, J_{CF} = 225.4 Hz, C-Ar), 183.9 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.6, -117.5; LRMS (ES^+) *m/z* 276.2 $[M-H]^-$; HRMS *m/z* calcd for $C_{14}H_8F_2NO_3$ $[M-H]^-$ 276.0478, found 276.0469.

4-(2,6-Difluoro-3-(prop-1-en-2-yl)benzoyl)-1*H*-pyrrole-2-carboxylic acid (281)



Compound **281** was synthesised according to general procedure B using, methyl 4-(2,6-difluoro-3-(prop-1-en-2-yl)benzoyl)-1*H*-pyrrole-2-carboxylate (**279**) (170 mg, 0.56 mmol), THF (5 mL), LiOH (267 mg, 11.1 mmol) in H_2O (7 mL). The pure product was obtained as a colourless oil (160 mg, 98%); R_f = 0.37 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 235, 279; ν_{max}/cm^{-1}: 3251, 1715 (CO_2H), 1656 (CO), 1524; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.14 (3H, s, CH_3), 5.21 (1H, s, alkene-CH), 5.30 (1H, s, alkene-CH), 6.96-6.99 (1H, m, H-5'), 7.36 (1H, s, H-3), 7.57 (1H, s, H-5), 7.64 (1H, dddd, *J* = 6.5, 6.6, 8.4 and 8.5 Hz, H-4'), 9.75 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.1 (CH_3), 112.5 (alkene-CH_2) 112.6 (C-Ar), 112.8 (C-Ar), 113.0 (C-Ar), 124.0 (C-3), 126.2 (C-2 and C-5), 130.0 (C-4), 130.8 (C-Ar), 132.1 (C-Ar), 157.2 (d, J_{CF} = 251.2 Hz, CF), 166.8 (CO_2H), 169.6 (d, J_{CF} = 218.4 Hz, C-Ar), 184.6 (CO); ^{19}F NMR (470 Hz, $CDCl_3$) δ -11401, -117.5; LRMS (ES^+) *m/z* 290.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{15}H_{10}F_2NO_3$ $[M-H]^-$ 290.0634, found 290.0624.

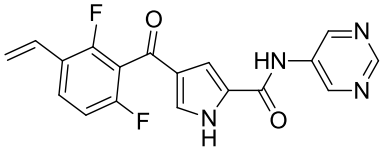
4-(2,6-Difluoro-3-vinylbenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (282)



Compound **282** was synthesised according to general procedure C using 4-(2,6-difluoro-3-vinylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**280**) (60 mg, 0.21 mmol), 3-aminopyridine (51 mg, 0.54 mmol), CDI (70 mg, 0.43 mmol) and THF (1 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) followed by (C-18 silica, 1:1 MeCN (0.1% formic acid):H20 (0.1% formic acid) followed by semi-prep. HPLC

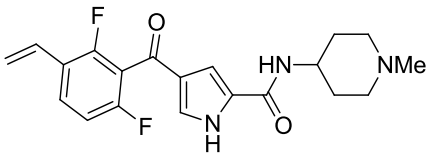
(C-18 silica; 5-100% acetonitrile in water (0.1% formic acid)) to give the title compound as a white solid (12 mg, 16%); R_f = 0.44 (5% DCM in MeOH); M.p. 255-256 ^oC; λ_{max} (EtOH)/nm 244, 293; ν_{max}/cm^{-1}: 3354, 3254, 3121, 1650 (CO), 1597 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 5.35 (1H, d, *J* = 11.3 Hz, vinyl CH), 5.73 (1H, d, *J* = 17.6 Hz, vinyl CH), 6.73 (1H, dd, *J* = 11.3 and 17.6 Hz, vinyl CH), 6.90-6.93 (1H, m, H-5'), 7.38 (d, *J* = 2.4 Hz, pyridine-CH), 7.39 (1H, s, H-3), 7.52 (1H, ddd, *J* = 6.4, 8.6 and 8.7 Hz, H-4'), 8.04-8.06 (1H, m, pyridine-CH), 8.17 (1H, s, H-5), 8.29 (1H, dd, *J* = 1.4 and 5.0 Hz, pyridine-CH), 8.69 (1H, d, J = 2.4 Hz, pyridine-CH), 9.90 (1H, br s, CONH); ^{19}F NMR (470 MHz, $CDCl_3$) δ -117.2, -113.1; LRMS (ES^+) *m/z* 354.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{14}F_2N_3O_2$ $[M+H]^+$ 354.1049, found 354.1051.

4-(2,6-Difluoro-3-vinylbenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (283)



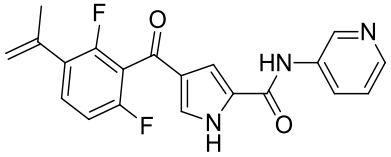
4-(2,6-Difluoro-3-vinylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (280) (60 mg, 0.21 mmol) was dissolved in acetonitrile (1 mL) before cyanuric fluoride (7 µL, 0.08 mmol) and pyridine (17 µL, 0.21 mmol) were added. The mixture was stirred at RT for 30 min before 5-aminopyrimidine (50 mg, 0.53 mmol) was added and the reaction was left to stir for 18 h. Brine was added and the product was extracted using ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude mixture was purified by semi-prep. HPLC (C-18 silica; 5-100% acetonitrile in water (0.1% formic acid)) to give the title compound as a white solid (15 mg, 20%); R_f = 0.39 (5% DCM in MeOH); M.p. 250-251 ^oC; λ_{max} (EtOH)/nm 243, 293; ν_{max}/cm^{-1}: 3333, 3252, 3118, 1626 (CO), 1586 (CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 5.49 (1H, d, J = 11.4 Hz, vinyl CH), 5.97 (1H, d, J = 17.7 Hz, vinyl CH), 6.84 (1H, dd, J = 11.4 and 17.7 Hz, vinyl CH), 7.26-7.31 (1H, m, H-5'), 7.50 (1H, s, H-3), 7.61 (1H, s, H-5), 7.85-7.90 (1H, m, H-4'), 8.46 (1H, br s, CONH), 8.91 (1H, s, CH-pyrimidine), 9.15 (2H, s, 2 x CH-pyrimidine), 10.60 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.5 (C-Ar), 112.6 (CH_2 vinyl), 118.2 (C-Ar), 121.7 (C-3), 125.9(C-2 and C-5), 127.5 (C-4), 129.2 (CH vinyl), 130.1 (C-Ar), 134.4, 141.8 (C-pyrimidine), 153.5 (d, J_{CF} = 258.7 Hz, CF), 158.9 (CON), 181.7 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -119.5, -114.7; LRMS (ES^+) *m/z* 355.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{13}F_2N_4O_2$ $[M+H]^+$ 355.1001, found 355.1003.4

(2,6-Difluoro-3-vinylbenzoyl)-*N*-(1-methylpiperidin-4-yl)-1*H*-pyrrole-2-carboxamide (284)



Compound **284** was synthesised according to general procedure C using 4-(2,6-difluoro-3-vinylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**280**) (60 mg, 0.21 mmol), 3-aminopyridine (51 mg, 0.54 mmol), CDI (70 mg, 0.43 mmol) and THF (1 mL). The crude mixture was purified by semi-prep. HPLC (C-18 silica; 5-100% acetonitrile in water (0.1% formic acid)) to give the title compound as a white solid (15 mg, 19%); R_f = 0.35 (5% DCM in MeOH); M.p. 233-234 ^oC; λ_{max} (EtOH)/nm 238, 290; ν_{max}/cm^{-1}: 3366 (NMe), 3261, 3117, 2954, 1588 (CO), 1587 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 2.03 (2H, br s, CH_2), 2.70 (4H, br s, CH_2 and CH_2N), 3.4 Hz (5H, s CH_2N and CH_3), 4.09 (2H, br s, CH), 5.32 (1H, d, *J* = 11.1 Hz, vinyl CH), 5.70 (1H, d, *J* = 17.6 Hz, vinyl CH), 6.72 (1H, dd, *J* = 11.1 and 17.6 Hz, vinyl CH), 6.86-6.89 (1H, m, H-5'), 7.24 (1H, s, H-3), 7.29 (1H, s, H-5), 7.45-7.49 (1H, m, H-4'), 8.38 (1H, br s, CONH), 10.79 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.0 (2 x CH_2), 42.6 (CH), 43.0 (CH_3), 110.5 (CH_2 vinyl), 111.0 (C-Ar), 116.1 (C-Ar), 117.1 (CH vinyl), 125.9 (C-3), 126.9 (C-2 and C-5), 127.1 (C-4), 127.2 (d, J_{CF} = 259.6 Hz, CF), 159.1 (CONH), 181.6 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -117.7, -113.5; LRMS (ES^+) *m/z* 374.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{20}H_{22}F_2N_3O_2$ $[M+H]^+$ 374.1675, found 374.1677.

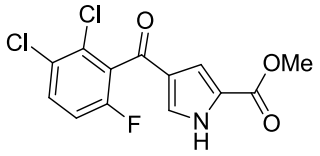
4-(2,6-Difluoro-3-(prop-1-en-2-yl)benzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (285)



4-(2,6-Difluoro-3-(prop-1-en-2-yl)benzoyl)-1*H*-pyrrole-2-carboxylic acid (**281**) (167 mg, 0.57 mmol) was dissolved in acetonitrile (4 mL) before cyanuric fluoride (20 µL, 0.23 mmol) and pyridine (46 µL, 0.57 mmol) were added. The mixture was stirred at RT for 30 min before 3-aminopyridine (135 mg, 1.43 mmol) was added and the reaction was left to stir for 18 h. Brine was added and the product was extracted using ethyl acetate. The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude mixture was purified by MPLC (0-8% MeOH in DCM) to

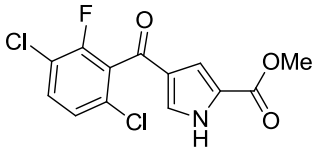
give the title compound as a white solid (84 mg, 0.23 mmol, 40%); R_f = 0.45 (5% DCM in MeOH); M.p. 269-270 ^{o}C; λ_{max} (EtOH)/nm 237, 293; ν_{max}/cm^{-1}: 3258, 2925, 2858, 1725, 1633 (CO), 1532 (CONH); ^{1}H NMR (500 MHz,$CDCl_3$) δ 2.13 (3H, s, CH_3), 5.23 (1H, s, alkene-CH), 5.29 (1H, s, alkene-CH), 6.96-6.70 (1H, m, H-5'), 7.42 (1H, ddd, J = 6.5, 8.5 and 8.6 Hz, H-4'), 7.46 (1H, s, H-3), 7.71 (1H, s, H-5), 8.17-8.20 (1H, m, pyridine-CH), 8.37 (1H, br s, pyridine-CH), 8.91 (2H, br s, pyridine-CH), 10.47 (1H, br s, CONH); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 23.0 (CH_3), 111.5 (C-Ar), 111.7 (alkene-CH_2), 117.7 (d, J_{CF} = 19.0 Hz, C-Ar), 123.9 (C-3), 127.5 (C-2 and C-5), 128.1 (C-pyridine), 129.6 (C-4), 131.3 (d, J_{CF} = 9.2 Hz, C-Ar), 135.1 (C-Ar), 138.7 (C-Ar), 141.0 (C-pyridine), 144.4, 158.3 (CH-N-pyridine), (d, J_{CF} = 242.5 Hz, CF), 158.9 (CH-N-pyridine), 163.6 (CONH), 183.3 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -116.2, -114.1; LRMS (ES$^+$) *m/z* 368.3 [M+H]$^+$; HRMS *m/z* calcd for $C_{20}H_{16}F_2N_3O_2$ [M+H]$^+$ 368.1205, found 368.1207.

Methyl 4-(2,3-dichloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (286)



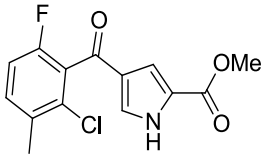
2,3-Dichloro-6-fluorobenzoic acid (2.0 g, 9.57 mmol) was dissolved in THF (20 mL) before $SOCl_2$ (2.1 mL, 28.7 mmol) and DMF (74 µL, 0.96 mmol) were added. The mixture was stirred at RT for 3 h before the solvent was removed *in vacuo.* The residue was re-dissolved in DCM (10 mL) and $AlCl_3$ (1.91 g, 14.4 mmol) was added followed by methyl pyrrole-2-carboxylate (**69**) (599 mg, 4.79 mmol) and the reaction was carried out as for general procedure A. The crude mixture was purified by MPLC (0-50% EtOAc in petrol) to give the pure product as a white solid (1.50 g, 99%); R_f = 0.41 (5% MeOH in EtOAc); M.p. 185-187 ^{o}C; λ_{max} (EtOH)/nm 234, 284; ν_{max}/cm^{-1}: 3260, 1715 (CO_2Me), 1597 (CO), 1546; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.87 (3H, s, CH_3), 6.86-6.87 (1H, m, H-5'), 7.21 (1H, br s, H-3), 7.50-7.56 (1H, m ,H-4'), 7.58 (1H, br s, H-5), 9.84 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 42.0 (CH_3), 111.9 (C-Ar), 112.4 (C-Ar), 116.0 (C-Ar), 124.6 (C-3), 128.9 (C-2 and C-5), 132.8 (C-4), 132.9 (C-Ar), 157.6 (d, J_{CF} = 246.2 Hz, CF), 167.4 (CO_2Me), 190.1 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -110.7; LRMS (ES$^+$) *m/z* 317.0 [M+H]$^+$; HRMS *m/z* calcd for $C_{13}H_9{}^{35}Cl_2FNO_3$ [M+H]$^+$ 316.1025, found 316.1029.

Methyl 4-(3,6-dichloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (287)



Compound **287** was synthesised according to general procedure A using methyl pyrrole-2-carboxylate (**69**) (250 mg, 1.99 mmol), 3,6-dichloro-2-fluorobenzoyl chloride (909 mg, 3.99 mmol), $AlCl_3$ (633 mg, 4.98 mmol) and DCM (10 mL). The crude mixture was purified by MPLC (0-50% EtOAc in petrol) to give the pure product as a white solid (539 mg, 86%); R_f = 0.42 (5% MeOH in EtOAc); M.p. 186-187 ^oC; λ_{max} (EtOH)/nm 235, 284; ν_{max}/cm^{-1}: 3259, 1704 (CO_2Me), 1591 (CO), 1552; ^1H NMR (500 MHz, $CDCl_3$) δ 3.85 (3H, s, CH_3), 6.85-6.86 (1H, m, H-5'), 7.20 (1H, br s, H-3), 7.46 (1H, ddd, *J* = 6.9, 8.5 and 8.6, H-4'), 7.50 (1H, br s, H-5), 9.75 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.1 (CH_3), 112.0 (C-Ar), 112.4 (C-Ar), 115.6 (C-Ar), 123.6 (C-3), 129.1 (C-2 and C-5), 131.8 (C-4), 132.0 (C-Ar), 155.1 (d, J_{CF} = 243.2 Hz, CF), 166.0 (CO_2Me), 189.6 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -110.3; LRMS (ES^+) *m/z* 316.9 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_9{}^{35}Cl_2FNO_3$ $[M+H]^+$ 316.1025, found 316.1025.

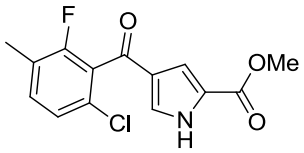
Methyl 4-(2-chloro-6-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylate (288)



Compound **288** was synthesised according to general procedure A using methyl pyrrole-2-carboxylate (**69**) (500 mg, 3.99 mmol), 2-chloro-6-fluoro-3-methylbenzoyl chloride (1.10 mL, 7.98 mmol), $AlCl_3$ (1.33 g, 9.98 mmol) and DCM (10 mL). The crude mixture was purified by MPLC (0-50% EtOAc in petrol) to give the pure product as a cream solid (706 mg, 60%); R_f = 0.41 (5% MeOH in EtOAc); M.p. 187-188 ^oC; λ_{max} (EtOH)/nm 233, 279; ν_{max}/cm^{-1}: 3285, 1750 (CO_2Me), 1634 (CO), 1521; ^1H NMR (500 MHz, $CDCl_3$) δ 2.20 (3H, s, CH_3), 3.71 (3H, s, OCH_3), 7.05 (1H, s, H-3), 7.10 (1H, s, H-5), 7.12-7.15 (1H, m, H-5'), 7.41 (1H, ddd, *J* = 6.6, 8.4 and 8.5 Hz, H-4'), 9.85 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3 (CH_3), 53.0 (OCH_3), 114.1 (C-Ar), 115.4 (C-Ar), 124.3 (C-3), 126.4 (C-2 and C-5), 127.6 (C-Ar) 129.0 (C-4), 132.0 (C-Ar), 157.0 (d, J_{CF} = 246.2 Hz, CF), 161.6 (CO_2Me), 186.0 (CO);^{19}F NMR (470 MHz,

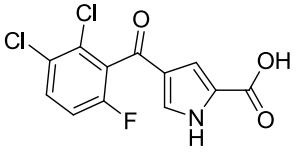
$CDCl_3$) δ -116.8; LRMS (ES^+) *m/z* 296.8 $[M+H]^+$; HRMS *m/z* calcd for $C_{14}H_{12}{}^{35}ClFNO_3$ $[M+H]^+$ 296.0489, found 296.0489.

Methyl 4-(6-chloro-2-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylate (289)



Compound **289** was synthesised according to general procedure A using methyl pyrrole-2-carboxylate (**69**) (500 mg, 3.99 mmol), 2-chloro-6-fluoro-5-methylbenzoyl chloride (1.10 mL, 7.98 mmol), $AlCl_3$ (1.33 g, 9.98 mmol) and DCM (10 mL). The crude mixture was purified by MPLC (0-50% EtOAc in petrol) to give the pure product as a cream solid (1.13 g, 96%); R_f = 0.41 (5% MeOH in EtOAc); M.p. 186-187 ^oC; λ_{max} (EtOH)/nm 233, 279; ν_{max}/cm^{-1}: 3263, 1711 (CO_2Me), 1636 (CO), 1549; ^1H NMR (500 MHz, $CDCl_3$) δ 2.20 (3H, s, CH_3), 3.69 (3H, s, OCH_3),7.05 (1H, s, H-3), 7.06 (1H, s, H-5), 7.10-7.12 (1H, m, H-5'), 7.37-7.39 (1H, m, H-4'), 9.96 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.3 (CH_3), 52.0 (OCH_3), 115.8 (C-Ar), 116.6 (C-Ar), 124.3 (C-3), 125.1 (C-2 and C-5), 126.9 (C-Ar) 128.5 (C-4), 132.4 (C-Ar), 156.3 (d, J_{CF} = 245.6 Hz, CF), 161.3 (CO_2Me), 185.3 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -116.8; LRMS (ES^+) *m/z* 296.8 $[M+H]^+$; HRMS *m/z* calcd for $C_{14}H_{12}{}^{35}ClFNO_3$ $[M+H]^+$ 296.0489, found 296.0486.

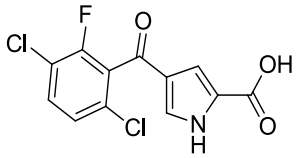
4-(2,3-Dichloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (290)



Compound **290** was synthesised according to general procedure B using, methyl 4-(2,3-dichloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**286**) (1.0 g, 3.17 mmol), THF (25 mL), LiOH (1.52 g, 63.5 mmol) in H_2O (40 mL). The pure product was obtained as a white solid (948 mg, 98%); R_f = 0.33 (5% MeOH in EtOAc); M.p. 190-191 ^oC; λ_{max} (EtOH)/nm 236, 285; ν_{max}/cm^{-1}: 3331, 1654 (CO_2H), 1593 (CO), 1558; ^1H NMR (500 MHz, DMSO-d_6) δ 7.02 (1H, s, H-3), 7.43-7.47 (1H, m, H-5'), 7.57 (1H, br s, H-5), 7.80 (1H, ddd, *J* = 6.9, 8.6 and 8.7,H-4'), 12.74 (1H, br s, NH), 13.01 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.5 (C-Ar), 112.4 (C-Ar), 117.0 (C-Ar), 124.6 (C-

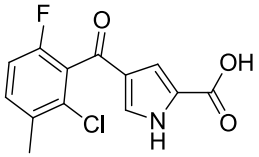
3), 128.9 (C-2 and C-5), 130.2 (C-4), 132.0 (C-Ar), 156.9 (d, J_{CF} = 250.1 Hz, CF), 168.2 (CO_2H), 189.7 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -110.4; LRMS (ES^+) *m/z* 301.2 $[M-H]^-$; HRMS *m/z* calcd for $C_{12}H_5{}^{35}Cl_2FNO_3$ $[M-H]^-$ 301.1011, found 301.1012.

4-(3,6-Dichloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (291)



Compound **291** was synthesised according to general procedure B using, methyl 4-(3,6-dichloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**287**) (330 mg, 1.05 mmol), THF (8 mL), LiOH (502 mg, 21.0 mmol) in H_2O (14 mL). The pure product was obtained as a white solid (314 mg, 99%); R_f = 0.32 (5% MeOH in EtOAc); M.p. 190-191 ^oC; λ_{max} (EtOH)/nm 234, 279; ν_{max}/cm^{-1}: 3267, 1649 (CO_2H), 1592 (CO) 1557; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.01 (1H, s, H-3), 7.45-7.48 (1H, m, H-5'), 7.56 (1H, br s, H-5), 7.77 (1H, ddd, *J* = 6.7, 8.4and 8.5 ,H-4'), 12.67 (1H, br s, NH), 13.04 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.0 (C-Ar), 112.9 (C-Ar), 116.9 (C-Ar), 123.6 (C-3), 128.9 (C-2 and C-5), 131.2 (C-4), 132.1 (C-Ar), 155.4 (d, J_{CF} = 254.2 Hz, CF), 169.0 (CO_2H), 188.4 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -110.3; LRMS (ES^+) *m/z* 301.3 $[M-H]^-$; HRMS *m/z* calcd for $C_{12}H_5{}^{35}Cl_2FNO_3$ $[M-H]^-$ 301.1056, found 301.1056.

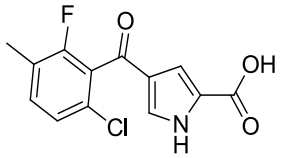
4-(2-Chloro-6-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (292)



Compound **292** was synthesised according to general procedure B using, methyl 4-(2-chloro-6-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylate (**288**) (400 mg1.33 mmol), THF (11 mL), LiOH (637 mg, 26.6 mmol) in H_2O (17 mL). The pure product was obtained as a cream solid (366 mg, 98%); R_f = 0.31 (5% MeOH in EtOAc); M.p. 195-196 ^oC; λ_{max} (EtOH)/nm 236, 279; ν_{max}/cm^{-1}: 3305, 1780, 1710 (CO_2H), 1562 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.27 (3H, s, CH_3), 6.96 (1H, s, H-3), 7.34 (1H, s, H-5), 7.35-7.37 (1H, m, H-5'), 7.41-7.45 (1H, m, H-4'), 12.65 (1H, br s, NH), 13.05 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.4 (CH_3), 115.2 (C-Ar), 115.8 (C-Ar), 124.6 (C-3), 125.2 (C-2 and C-5), 126.9 (C-Ar), 129.6 (C-4), 131.6 (C-Ar), 155.6 (d,

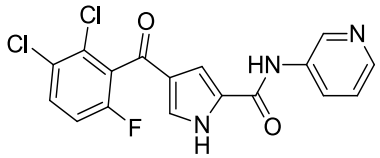
J_{CF} = 250.2 Hz, CF), 165.2 (CO_2H), 183.5 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -117.0; LRMS (ES^+) *m/z* 280.3 $[M-H]^-$; HRMS *m/z* calcd for $C_{13}H_8{}^{35}ClFNO_3$ $[M-H]^-$ 280.0172, found 280.0172.

4-(6-Chloro-2-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (293)



Compound **293** was synthesised according to general procedure B using, methyl 4-(6-chloro-2-fluoro-3-methylbenzoyl)-1H-pyrrole-2-carboxylate (**289**) (1.0 g, 3.32 mmol), THF (27 mL), LiOH (1.59 g, 66.5 mmol) in H_2O (43 mL). The pure product was obtained as a cream solid (914 mg, 98%); R_f = 0.30 (5% MeOH in EtOAc); M.p. 196-197 ^oC; λ_{max} (EtOH)/nm 235, 280; ν_{max}/cm^{-1}: 3300, 1753, 1701 (CO_2H), 1558 (CO); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.26 (3H, s, CH_3), 6.96 (1H, s, H-3), 7.31 (1H, s, H-5), 7.33-7.35 (1H, m, H-5'), 7.41-7.45 (1H, m, H-4'), 12.69 (1H, br s, NH), 13.25 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.3 (CH_3), 114.9 (C-Ar), 115.2 (C-Ar), 124.0 (C-3), 124.9 (C-2 and C-5), 126.9 (C-Ar), 128.5 (C-4), 132.0 (C-Ar), 155.4 (d, J_{CF} = 246.5 Hz, CF), 169.0 (CO_2H), 185.6 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -116.8; LRMS (ES^+) *m/z* 280.6 $[M-H]^-$; HRMS *m/z* calcd for $C_{13}H_8{}^{35}ClFNO_3$ $[M-H]^-$ 280.0171, found 280.0171.

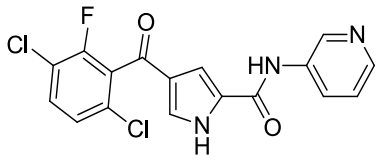
4-(2,3-Dichloro-6-fluorobenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (294)



Compound **294** was synthesised according to general procedure D using 4-(2,3-Dichloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**290**) (100 mg, 0.33 mmol), 3-aminopyridine (78 mg, 0.83 mmol), PCl_3 (29 µL, 0.33 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a white solid (60 mg, 48%); R_f = 0.49 (5% DCM in MeOH); M.p. 265-266 ^oC; λ_{max} (EtOH)/nm 295; ν_{max}/cm^{-1}: 3309, 2160, 1636 (CO), 1594 (CONH), 1551; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.39 (1H, dd, *J* = 4.7 and 8.2 Hz, CH-pyridine), 7.48-7.51 (2H, m, H-5' and H-3), 7.62 (1H, s, H-5), 7.86 (1H, dd, *J* = 4.7 and 8.2 Hz, CH-

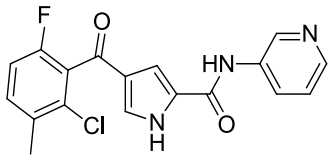
pyridine), 8.14 (1H, d, *J* = 7.3 Hz, H-4'), 8.30 (1H, d, *J* = 4.7 Hz, CH-pyridine), 8.89 (1H, s, CH-N-pyridine), 10.24 (1H, s, CONH), 12.70 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.5 (C-Ar), 116.6 (d, *J* = 23.1 Hz, C-Ar), 123.6 (C-3), 124.7(C-Ar), 127.0 (C-2 and C-5), 127.9 (C-pyridine), 128.3 (C-pyridine), 130.1 (C-4), 131.9 (d, *J* = 10.7 Hz, C-Ar), 135.5 (C-Ar and C-pyridine), 141.6 (C-N-pyridine), 144.4 (C-N-pyridine), 156.8 (d, *J* = 245.3 Hz, CF), 158.7 (CON), 182.9 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -115.8; LRMS (ES^+) *m/z* 378.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{11}{}^{35}Cl_2FN_3O_2$ $[M+H]^+$ 378.0210, found 378.0210.

4-(3,6-Dichloro-2-fluorobenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (295)



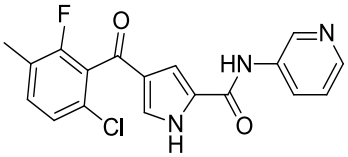
Compound **295** was synthesised according to general procedure D using 4-(3,6-Dichloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**291**) (90 mg, 0.30 mmol), 3-aminopyridine (71 mg, 0.75 mmol), PCl_3 (26 µL, 0.30 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a white solid (58 mg, 51%); R_f = 0.50 (5% DCM in MeOH); M.p. 262-263 ^oC; λ_{max} (EtOH)/nm 251, 293; ν_{max}/cm^{-1}: 3286, 3114, 3077, 2963, 1636 (CO), 1594 (CONH), 1529; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.39 (1H, dd, *J* = 4.5 and 7.6 Hz, CH-pyridine), 7.52 (1H, br s, H-5'), 7.54 (1H, br s, H-3), 7.64 (1H, s, H-5), 7.78-7.81 (1H, m, H-4'), 8.14 (1H, d, *J* = 7.6 Hz, CH-pyridine), 8.30 (1H, d, *J* = 4.5 Hz, CH-pyridine), 8.90 (1H, s, CH-N-pyridine), 10.25 (1H, br s, CONH), 12.77 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.5 (C-Ar), 119.3 (d, *J* = 18.7 Hz, C-Ar), 123.6 (C-3), 124.8 (C-Ar), 126.9 (C-2 and C-5), 128.5 (C-Ar), 129.2 (C-pyridine), 130.30 (C-Ar), 131.9 (C-4), 135.5 (C-pyridine), 141.6 (C-N-pyridne), 144.4 (C-N-pyridine), 153.8 (d, J_{CF} = 249.7, CF), 158.7 (CONH), 182.6 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -116.7; LRMS (ES^+) *m/z* 378.3 $[M+H]^+$ HRMS *m/z* calcd for $C_{17}H_{11}{}^{35}Cl_2FN_3O_2$ $[M+H]^+$ 378.0210, found 378.0210.

4-(2-Chloro-6-fluoro-3-methylbenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (296)



Compound **296** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**292**) (120 mg, 0.43 mmol), 3-aminopyridine (100 mg, 1.07 mmol), PCl_3 (38 µL, 0.43 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-5% MeOH in EtOAc) to give the title compound as a white solid (92 mg, 60%); R_f = 0.48 (5% MeOH in EtOAc); M.p. 267-268 ^oC; λ_{max} (EtOH)/nm 243, 292; ν_{max}/cm^{-1}: 3249, 3126, 2982, 1629 (CO), 1532(CONH); ^1H NMR (500 MHz, DMSO-d_6) δ 2.37 (3H, s, CH_3), 7.29-7.33 (1H, m, H-5'), 7.39 (1H, dd, *J* = 4.8 and 8.6 Hz, CH-pyridine), 7.46 (1H, br s, H-3), 7.48 (1H, br s, H-5), 7.54 (1H, dd, *J* = 6.5 and 8.5 Hz, H-4'), 8.13-8.15 (1H, m, CH-pyridine), 8.30 (1H, dd, J = 1.5 and 4.8 Hz, CH-pyridine), 8.90 (1H, d, *J* = 2.5 Hz, CH-pyridine), 10.20 (1H, s, CONH), 12.71 (1H, br s, NH; ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (CH_3), 111.6 (C-Ar), 114.5 (d, *J* = 20.2 Hz, C-Ar), 123.6 (C-3), 125.3 (C-Ar), 126.9 (C-2 and C-5), 128.1 (C-pyridine), 129.3 (C-pyridine), 130.0 (C-4), 132.3 (d, *J* = 10.7 Hz, C-Ar), 132.6 (C-Ar), 135.5 (C-pyridine), 141.6 (C-N-pyridine), 144.4 (C-N-pyridine), 156.8 (d, *J* = 246.3 Hz, CF), 158.7 (CONH), 184.3 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -118.4; LRMS (ES^+) *m/z* 358.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{14}{}^{35}ClFN_3O_2$ $[M+H]^+$ 358.0753, found 358.0755.

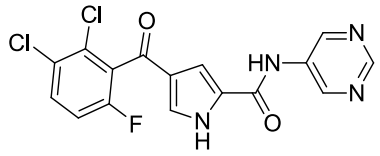
4-(6-Chloro-2-fluoro-3-methylbenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (297)



Compound **297** was synthesised according to general procedure D using 4-(6-chloro-2-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**293**) (120 mg, 0.43 mmol), 3-aminopyridine (100 mg, 1.07 mmol), PCl_3 (38 µL, 0.43 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-5% MeOH in EtOAc) to give the title compound as a pale yellow solid (122 mg, 80%); R_f = 0.50 (5% MeOH in EtOAc); M.p. 268-269 ^oC; λ_{max} (EtOH)/nm 250, 293; ν_{max}/cm^{-1}: 3124, 2978, 2858, 1985, 1633 (CO),

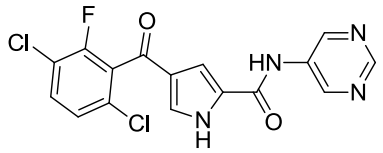
1533 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.29 (3H, s, CH_3), 7.36 (1H, d, *J* = 8.0 Hz, H-5'), 7.39 (1H, dd, *J* = 4.7 and 8.4 Hz, CH-pyridine), 7.45-7.49 (3H, m, H-3, H-5 and H-4'), 8.13-8.15 (1H, m, CH-pyridine), 8.30 (1H, dd, J = 1.4 and 4.7 Hz, CH-pyridine), 8.90 (1H, d, *J* = 2.5 Hz, CH-pyridine), 10.24 (1H, s, CONH), 12.70 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.9 (CH_3), 111.6 (C-Ar), 123.6 (C-3), 124.1 (d, *J* = 20.2 Hz, C-Ar), 125.2 (C-Ar), 127.0 (C-2 and C-5), 127.7 (C-pyridine), 128.1 (C-pyridine), 129.4 (C-4), 132.8 (d, *J* = 10.7 Hz, C-Ar), 135.5 (C-Ar), 141.6 (C-pyridine), 144.4 (C-N-pyridine), 156.8 (d, *J* = 245.3 Hz, CF), 158.7 (CONH), 184.2 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -118.4; LRMS (ES^+) *m/z* 358.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{14}{}^{35}ClFN_3O_2$ $[M+H]^+$ 358.0753, found 358.0754.

4-(2,3-Dichloro-6-fluorobenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (298)



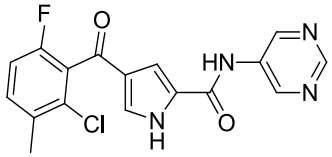
Compound **298** was synthesised according to general procedure D using 4-(2,3-Dichloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**290**) (100 mg, 0.33 mmol), 5-aminopyrimidine (79 mg, 0.83 mmol), PCl_3 (29 µL, 0.33 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a white solid (68 mg, 67%); R_f = 0.46 (5% DCM in MeOH); M.p. 259-260 ^{o}C; λ_{max} (EtOH)/nm 293; ν_{max}/cm^{-1}: 3115, 3058, 2971, 1651 (CO), 1590 (CONH), 1537; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.46-7.52 (2H, m, H-3 and H-5'), 7.68 (1H, br s, H-5), 7.87 (1H, dd, *J* = 5.5 and 9.1 Hz, H-4'), 8.93 (1H, s, CH-pyrimidine), 9.13 (2H, s, CH-pyrimidine), 10.45 (1H, br s, CONH), 12.89 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 112.0 (C-Ar), 116.6 (d, *J* = 23.5 Hz, C-Ar), 124.8 (C-3), 127.8 (C-2 and C-5), 128.7 (d, *J* = 6.3 Hz, C-Ar), 131.9 (C-Ar), 132.0 (d, *J* = 10.3 Hz, C-Ar), 134.3 (C-pyrimidine), 147.9 (C-N-pyrimidine), 153.2 (N-C-N-pyrimidine), 156.6 (d, J_{CF} = 252.4 Hz, CF), 158.8 (CON), 182.9 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -114.6; LRMS (ES^+) *m/z* 379.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{10}{}^{35}Cl_2FN_4O_2$ $[M+H]^+$ 379.1059, found 379.1061.

4-(3,6-Dichloro-2-fluorobenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (299)



Compound **299** was synthesised according to general procedure D using 4-(3,6-Dichloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**291**) (90 mg, 0.30 mmol), 5-aminopyrimidine (71mg, 0.75 mmol), PCl_3 (26 µL, 0.30 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a white solid (53 mg, 47%); R_f = 0.46 (5% DCM in MeOH); M.p. 258-259 ^oC; λ_{max} (EtOH)/nm 268, 292; ν_{max}/cm^{-1}: 3229, 3063, 2922, 1647 (CO), 1591 (CONH), 1536; ^1H NMR (500 MHz, DMSO-d_6) δ 7.53-7.55 (2H, m, H-3 and H-5'), 7.69 (1H, br s, H-5), 7.79-7.82 (1H, m, H-4'), 8.93 (1H, s, CH-pyrimidine), 9.13 (2H, s, CH-pyrimidine), 10.45 (1H, br s, CONH), 12.90 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 111.9 (C-Ar), 116.6 (d, *J* = 24.5 Hz, C-Ar), 123.8 (C-3), 126.9 (C-2 and C-5), 128.0 (d, *J* = 6.5 Hz, C-Ar), 132.9 (C-Ar), 133.0 (d, *J* = 10.5 Hz, C-Ar), 134.3 (C-pyrimidine), 148.2 (C-N-pyrimidine), 155.2 (N-C-N-pyrimidine), 157.9 (d, J_{CF} = 245.6 Hz, CF), 159.6 (CON), 183.6 (CO ^{19}F NMR (470 MHz, $CDCl_3$) δ -116.5; LRMS (ES^+) *m/z* 379.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{10}{}^{35}Cl_2FN_4O_2$ $[M+H]^+$ 379.1059, found 379.1060.

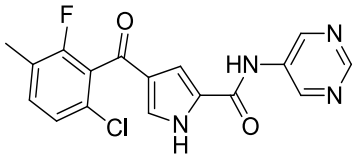
4-(2-Chloro-6-fluoro-3-methylbenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (300)



Compound **300** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**292**) (120 mg, 0.43 mmol), 5-aminopyrimidine (102 mg, 1.08 mmol), PCl_3 (38 µL, 0.43 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-5% MeOH in EtOAc) to give the title compound as a pale yellow solid (77 mg, 50%); R_f = 0.45 (5% MeOH in EtOAc); M.p. 256-257 ^oC; λ_{max} (EtOH)/nm 238, 300; ν_{max}/cm^{-1}: 3244, 2943, 2806, 1621 (CO), 1603 (CONH); ^1H NMR (500 MHz, MeOD) δ 2.30 (3H, s, CH_3), 7.03-7.06 (1H, m, H-5'), 7.33 (1H, s, H-3), 7.34-7.36 (1H, m, H-4'), 7.37 (1H, s, H-5). 8.77 (1H, s, CH-pyrimidine), 9.07 (2H, s, CH-pyrimidine); ^{13}C NMR (125 MHz, DMSO-d_6) δ 59.7

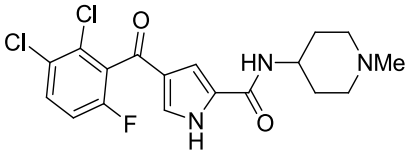
(CH_3), 112.1, 114.6 (C-Ar), 125.4 (C-3), 127.6 (C-2 and C-5), 129.5 (C-4), 132.3 (C-Ar), 134.3, 147.8 (C-pyrimidine), 153.2 (d, J_{CF} = 256.7 Hz, CF), 155.8 (CONH), 184.3 (CO); ^{19}F NMR (470 MHz, MeOD) δ -119.8; LRMS (ES^+) *m/z* 359.1 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{13}{}^{35}ClFN_4O_2$ $[M+H]^+$ 359.0712, found 359.0711.

4-(6-Chloro-2-fluoro-3-methylbenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (301)



Compound **301** was synthesised according to general procedure D using 4-(6-chloro-2-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**293**) (120 mg, 0.43 mmol), 5-aminopyrimidine (102 mg, 1.08 mmol), PCl_3 (38 µL, 0.43 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-5% MeOH in EtOAc) to give the title compound as a pale yellow solid (95 mg, 62%); R_f = 0.43 (5% MeOH in EtOAc); M.p. 255-256 ^oC; λ_{max} (EtOH)/nm 243, 298; ν_{max}/cm^{-1}: 3355, 2941, 2360, 1600 (CO), 1537 (CONH) , ,; ^{1}H NMR (500 MHz, MeOD) δ 2.20 (3H, s, CH_3), 7.10 (1H, d, *J* = 8.5 Hz, H-5'), 7.18-7.21 (1H, m, H-4'), 7.28 (1H, d, *J* = 3.4 Hz, , H-3), 8.04 (1H, s, H-5). 8.85 (1H, s, CH-pyrimidine), 8.98 (2H, s, CH-pyrimidine), 9.21 (1H, br s, CONH), 10.08 (1H, br s, NH); ^{13}C NMR (125 MHz, MeOD) δ 14.3 (CH_3), 111.4 (C-Ar), 124.6 (C-3), 125.4 (C-2 and C-5), 127.1 (C-4), 130.4, 133.1, 133.8 (C-Ar), 148.0, 153.8 (C-pyrimidine), 157.5 (d J_{CF} = 254.6 Hz, CF), 159.0 (CONH), 187.0 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -116.5; LRMS (ES^+) *m/z* 359.1 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{13}{}^{35}ClFN_4O_2$ $[M+H]^+$ 359.0712, found 359.0711.

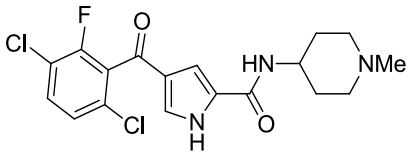
4-(2,3-Dichloro-6-fluorobenzoyl)-*N*-(1-methylpiperidin-4-yl)-1*H*-pyrrole-2-carboxamide (302)



Compound **302** was synthesised according to general procedure D using 4-(2,3-Dichloro-6-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**290**) (100 mg, 0.33 mmol), 4-amino-1-methylpiperidine (87 µL, 0.83 mmol), PCl_3 (29 µL, 0.33 mmol) and MeCN (2

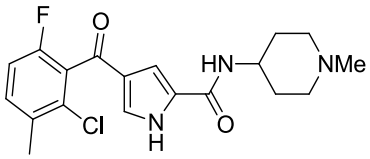
mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a white solid (66 mg, 50%); R_f = 0.40 (5% DCM in MeOH); M.p. 264-265 ^{o}C; λ_{max} (EtOH)/nm 237, 285; ν_{max}/cm^{-1}: 3204 (NMe), 2940, 2802, 2363, 2188, 1521 (CO), 1569 (CONH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.65-1.67 (2H, m, CH_2), 2.01-2.03 (2H, m, CH_2), 2.07-2.19 (2H, m, CH_2N), 2.36 (3H, s, NCH_3), 2.87-2.90 (2H, m, CH_2N), 3.51-3.53 (1H, m, CH), 6.04 (1H, br s, CONH), 7.00 (1H, s, H-5), 7.10 (1H, dd, *J* = 7.6 and 8.9 Hz, H-5'), 7.33 (1H, s, H-5), 7.54 (1H, dd, *J* = 5.2 and 8.9 Hz, H-4'); ^{13}C NMR (125 MHz, $CDCl_3$) δ 29.7 (2 x CH_2), 32.1 (NCH_3), 46.2 (CH), 54.4 (2 x CH_2N), 108.8 (C-Ar), 115.6 (d, *J* = 28.2 Hz, C-Ar), 126.1 (C-3), 127.9 (C-2 and C-5), 128.3 (C-4), 129.1 (C-Ar), 131.4 (d, *J* = 9.8 Hz, C-Ar), 156.7 (d, *J* = 244.9 Hz, CF), 159.6 (CON), 183.5 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -114.7; LRMS (ES$^+$) *m/z* 398.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{19}{}^{35}Cl_2FN_3O_3$ $[M+H]^+$ 398.0833, found 398.0835.

4-(3,6-Dichloro-2-fluorobenzoyl)-N-(1-methylpiperidin-4-yl)-1H-pyrrole-2-carboxamide (303)



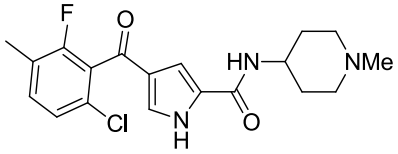
Compound **303** was synthesised according to general procedure D using 4-(3,6-Dichloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**291**) (150 mg, 0.50 mmol), 4-amino-1-methylpiperidine (130 µL, 1.24 mmol), PCl_3 (44 µL, 0.50 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a peach solid (99 mg, 50%); R_f = 0.39 (5% DCM in MeOH); M.p. 268-269 ^{o}C; λ_{max} (EtOH)/nm 230, 285; ν_{max}/cm^{-1}: 3230 (NMe), 3085, 2981, 1629 (CO), 1564 (CONH); ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.80 (2H, br s, CH_2), 1.91 (2H, br s, CH_2), 2.36 (5H, br s, CH_2N and CH_3), 2.97 (2H, br s, CH_2N), 2.98 (1H, br s, CH), 7.25 (1H, s, H-3), 7.48 (1H, s, H-5), 7.50 (1H, d, *J* = 8.4 Hz, H-5'), 7.76-7.79 (1H, m, H-4'), 8.21 (1H, d, J = 7.02 Hz, CONH), 12.51 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1 (2 x CH_2), 44.5 (CH), 45.0 (CH_3), 53.5 (2 x CH_2N), 119.2 (C-3), 124.5 (C-2 and C-5), 126.8 (C-4), 129.1 (C-Ar), 131.8 (C-Ar), 153.8 (d, J_{CF} = 254.6 Hz, CF), 159.2 (CONH), 182.6 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -116.8; LRMS (ES$^+$) *m/z* 398.4 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{19}{}^{35}Cl_2FN_3O_3$ $[M+H]^+$ 398.0833, found 398.0834.

4-(2-Chloro-6-fluoro-3-methylbenzoyl)-*N*-(1-methylpiperidin-4-yl)-1*H*-pyrrole-2-carboxamide (304)



Compound **304** was synthesised according to general procedure D using 4-(2-chloro-6-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**292**) (120 mg, 0.43 mmol), 4-amino-1-methylpiperidine (112 µL, 1.08 mmol), PCl_3 (38 µL, 0.43 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a white solid (85 mg, 52%); R_f = 0.40 (5% MeOH in DCM); M.p. 260-261 ^oC; λ_{max} (EtOH)/nm 235, 282; ν_{max}/cm^{-1}: 3361 (NMe), 3119, 2938, 1621 (CO), 1572 (CONH), 1538; ^1H NMR (500 MHz, $CDCl_3$) δ 1.69-1.78 (4H, m, 2 x CH_2), 2.07 (2H, br t, *J* = 11.1 Hz, CH_2N), 2.39 (3H, s, NCH_3), 2.41 (3H, s, CH_3), 2.97 (2H, br d, *J* = 11.1 Hz, CH_2N), 3.97-4.04 (1H, m, CH), 6.23 (1H, br d, *J* = 8.06 Hz, CONH), 7.00-7.04 (2H, m, H-4' and H-5'), 7.31 (1H, d, *J* = 2.4 Hz, H-3), 7.32 (1H, br s, H-5), 10.14 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 19.0 (2 x CH_2), 109.9, 114.4 (C-Ar), 125.0 (C-3), 127.9 (C-2 and C-5), 128.8 (C-4), 132.2, 132.5 (C-Ar), 154.8 (d, J_{CF} = 255.2 Hz, CF), 159.2 (CONH), 184.3 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -117.4; LRMS (ES^+) *m/z* 377.9 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{13}{}^{35}ClFN_4O_2$ $[M+H]^+$ 359.0712, found 359.0712.

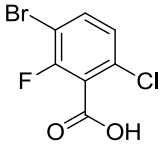
4-(6-Chloro-2-fluoro-3-methylbenzoyl)-*N*-(1-methylpiperidin-4-yl)-1*H*-pyrrole-2-carboxamide (305)



Compound **305** was synthesised according to general procedure D using 4-(6-chloro-2-fluoro-3-methylbenzoyl)-1*H*-pyrrole-2-carboxylic acid (**293**) (120 mg, 0.43 mmol), 4-amino-1-methylpiperidine (112 µL, 1.08 mmol), PCl_3 (38 µL, 0.43 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a white solid (78 mg, 48%); R_f = 0.40 (5% MeOH in DCM); M.p. 262-264 ^oC; λ_{max} (EtOH)/nm 236, 284; ν_{max}/cm^{-1}: 3242 (NMe), 3064, 2944, 2155, 1741 (CO), 1623 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 1.59-1.67 (2H, m, CH_2),1.90 (2H, br d, *J* = 11.1 Hz, CH_2), 2.14-2.19 (2H, m, CH_2N), 2.19 (3H, s, NCH_3), 2.27 (3H, s,

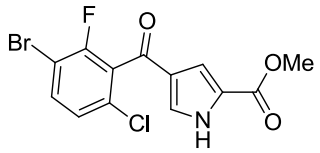
CH_3), 2.83(2H, br d, *J* = 11.1 Hz, CH_2N), 3.86-3.98 (1H, m, CH), 6.57 (1H, br d, *J* = 7.9 Hz, CONH), 7.05 (1H, d, *J* = 8.4 Hz, H-5'), 7.07 (1H, br s, H-3), 7.10-7.13 (1H, m, H-4'), 7.20 (1H, br s, H-5), 10.97 (1H, br s, NH); 14.3 (CH_3), 31.4 (2 x CH_2), 45.6 (NCH_3), 45.9 (CH), 54.1 (2 x CH_2N), 109.8 (C-3), 124.2 (d, *J* = 17.6 Hz, C-Ar), 125.0 (d, *J* = 3.3 Hz, C-Ar), 127.8 (d, *J* = 22.5 Hz, C-Ar), 128.3 (C-2 and C-5), 128.4 (C-4), 128.7 (d, *J* = 5.2 Hz, C-Ar), 132.3 (d, *J* = 5.2 Hz, C-Ar), 157.5 (d, *J* = 248.0 Hz, CF), 160.1 (CONH), 185.3 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -116.8; LRMS (ES^+) *m/z* 378.1 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{22}{}^{35}ClFN_3O_2$ $[M+H]^+$ 378.1379, found 378.1380.

3-Bromo-6-chloro-2-fluorobenzoic acid (307)



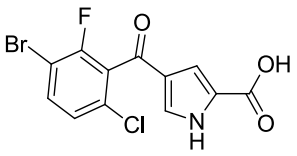
Diisopropylamine (0.68 mL, 4.8 mmol) was dissolved in THF (3.5 mL) at -60 °C before ^{n}BuLi (2.0 mL, 2.4 M in hexane) was added and the mixture was stirred for 30 min. The mixture was cooled to -70 °C and 1-bromo-4-chloro-2-fluorobenzene (298 µL, 2.4 mmol) was added and the mixture stirred for 2 h. The mixture was transferred *via* canula to a suspension of solid CO_2 in diethyl ether. The mixture was allowed to warm to RT before the pH was adjusted to 3 using 1.0 M HCl solution. The product was extracted using ethyl acetate and the organic fractions were dried over Na_2SO_4. The solvent was removed *in vacuo* to give the pure product as a white solid (548 mg, 90%); R_f = 0.25 (5% MeOH in DCM); M.p. 156-157 °C; λ_{max} (EtOH)/nm 231,280; ν_{max}/cm^{-1}: 3081, 1706 (CO_2H), 1639, 1590; ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.40-7.42 (1H, m, H-Ar), 7.83-7.87 (1H, m, H-Ar), 14.34 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 107.3 (C-Ar), 124.6 (C-Ar), 127.1 (C-Ar), 129.6 (C-Ar), 134.7 (C-Ar), 154.9 (d, J_{CF} = 256.2 Hz, CF), 162.9 (CO_2H); ^{19}F NMR (470 MHz, $CDCl_3$) δ -105.5; LRMS (ES^+) *m/z* 252.4 $[M-H]^-$.

Methyl 4-(3-bromo-6-chloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (308)



3-Bromo-6-chloro-2-fluorobenzoic acid (**307**) (0.50 g, 1.97 mmol) was dissolved in THF (5 mL) before $SOCl_2$ (429 µL, 5.92 mmol) and DMF (15 µL, 0.20 mmol) were added. The mixture was stirred at RT for 3 h before the solvent was removed *in vacuo.* The residue was re-dissolved in DCM (5 mL) and $AlCl_3$ (394 mg, 2.96 mmol) was added followed by methyl pyrrole-2-carboxylate 123 mg, 1.00 mmol) and the reaction was carried out as for general procedure A. The crude mixture was purified by MPLC (0-50% EtOAc in petrol) to give the pure product as a beige oil (314 mg, 87%); R_f = 0.34 (5% MeOH in DCM); M.p. 185-187 ^oC; λ_{max} (EtOH)/nm 235, 284; ν_{max}/cm^{-1}:3265, 1737 (CO_2Me), 1654, (CO) 1558; ^1H NMR (500 MHz, $CDCl_3$) δ 3.87 (3H, s, CH_3), 6.86-6.89 (1H, m, H-5'), 7.15 (1H, br s, H-3), 7.56 (1H, ddd, *J* = 6.9, 8.5 and 8.6,H-4'), 7.61 (1H, br s, H-5), 9.84 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.3 (CH_3), 112.0 (C-Ar), 112.4 (C-Ar), 115.5 (C-Ar), 124.2 (C-3), 128.6 (C-2 and C-5), 131.8 (C-4), 133.0 (C-Ar), 153.2 (d, J_{CF} = 250.1 Hz, CF), 165.2 (CO_2Me), 185.0 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -109.5; LRMS (ES^+) *m/z* 361.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_9{}^{79}Br^{35}ClFNO_3$ $[M+H]^+$ 360.1154, found 360.1157.

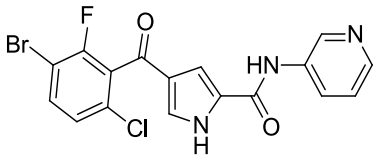
4-(3-Bromo-6-chloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (309)



Compound **309** was synthesised according to general procedure B using, methyl 4-(3-bromo-6-chloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylate (**308**) (310 mg, 0.86 mmol), THF (7 mL), LiOH (412 mg, 17.2 mmol) in H_2O (11 mL). The pure product was obtained as a cream solid (293 mg, 99%); R_f = 0.31 (5% MeOH in DCM); M.p. 196-197 ^oC; λ_{max} (EtOH)/nm 236, 284; ν_{max}/cm^{-1}:3350, 1740, 1645 (CO_2H), 1557 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 7.01-7.05 (1H, m, H-5'), 7.10 (1H, br s, H-3), 7.54 (1H, ddd, *J* = 6.9, 8.5 and 8.6,H-4'), 7.70 (1H, br s, H-5), 12.45 (1H, br s, NH), 12.98 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 53.0 (CH_3), 112.1 (C-Ar), 112.2 (C-Ar), 116.0 (C-Ar), 123.9 (C-3), 128.6 (C-2 and C-5), 131.5 (C-4), 133.4 (C-Ar), 153.5

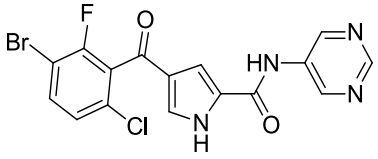
(d, J_{CF} = 250.5 Hz, CF), 166.7 (CO_2H), 187.0 (CO);^{19}F NMR (470 MHz, $CDCl_3$) δ -109.5; LRMS (ES^+) *m/z* 345.5 $[M-H]^-$; HRMS *m/z* calcd for $C_{12}H_7{}^{79}Br^{35}ClFNO_3$ $[M-H]^-$ 343.9124, found 343.9124.

4-(3-Bromo-6-chloro-2-fluorobenzoyl)-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (310)



Compound **310** was synthesised according to general procedure D using 4-(3-bromo-6-chloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**309**) (90 mg, 0.26 mmol), 3-aminopyridine (61 mg, 0.65 mmol), PCl_3 (23 µL, 0.26 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-5% MeOH in EtOAc) to give the title compound as a white solid (64 mg, 58%); R_f = 0.50 (5% MeOH in EtOAc); M.p. 273-274 ^oC; λ_{max} (EtOH)/nm 230, 285; ν_{max}/cm^{-1}: 3308, 2396, 2115, 1653 (CO), 1636 (CONH); ^{1}H NMR (500 MHz, MeOD) δ 7.24 (1H, dd, *J* = 1.3 and 8.7 Hz, H-5'), 7.31-7.34 (2H, m, H-3 and CH-pyridine), 7.42 (1H, d, *J* = 1.4 Hz, H-5), 7.66 (1H, dd, *J* = 7.5 and 8.9 Hz, H-4'), 8.11-8.14 (1H, m, CH-pyridine), 8.17 (d, *J* = 4.3 Hz, CH-pyridine), 8.78 (1H, d, J = 2.3 Hz, CH-N-pyridine); ^{13}C NMR (125 MHz, MeOD) δ 112.7 (C-Ar), 125.3 (C-3), 127.0 (C-Ar), 128.1 (d, J_{CF} = 5.7 Hz, C-Ar), 129.6 (C-2 and C-5), 130.7 (C-4), 135.9 (C-Ar), 142.3 (C-pyridine), 145.1 (C-pyridine), 156.8 (d, J_{CF} = 245.5 Hz, CF), 160.8 (CONH), 185.3 (CO); ^{19}F NMR (470 MHz, MeOD) δ -108.8; LRMS (ES^+) *m/z* 422.5 $[M+H]^+$; HRMS *m/z* calcd for $C_{17}H_{11}{}^{79}Br^{35}ClFN_3O_2$ $[M+H]^+$ 421.9702, found 421.9702.

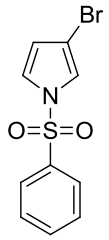
4-(3-Bromo-6-chloro-2-fluorobenzoyl)-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (311)



Compound **311** was synthesised according to general procedure D using 4-(3-bromo-6-chloro-2-fluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**309**) (90 mg, 0.26 mmol), 5-aminopyrimidine (62 mg, 0.65 mmol), PCl_3 (23 µL, 0.26 mmol) and MeCN (3 mL). The crude mixture was purified by MPLC (0-5% MeOH in EtOAc followed by NH-

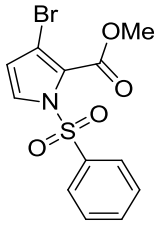
silica, 0-5% MeOH in EtOAc) to give the title compound as a pale yellow solid (33 mg, 30%); R_f = 0.48 (5% MeOH in EtOAc); M.p. 275-276 ^oC; λ_{max} (EtOH)/nm 231-291; ν_{max}/cm^{-1}:3301, 3031, 2942, 1646 (CO), 1528 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 7.21 (1H, dd, *J* = 1.4 and 8.7 Hz, H-5'), 7.41 (1H, d, *J* = 2.6 Hz, H-3), 7.64 (1H, dd, *J* = 7.3 and 8.7 Hz, H-4'), 7.82 (1H, s, H-5), 8.78 (1H, br s, CONH), 8.99 (1H, s, pyrimidine-CH), 9.09 (2H, s, pyrimidine-CH), 10.15 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 111.1 (C-Ar), 126.6 (C-3), 127.8 (C-Ar), 130.0 (C-2 and C-5), 133.4 (C-4), 134.8 (C-Ar), 148.0 (C-N-pyrimidine), 154.2 (d, J_{CF} = 250.1 Hz, CF), 158.6 (CONH), 184.2 (CO); ^{19}F NMR (470 MHz, MeOD) δ -105.7; LRMS (ES^+) *m/z* 422.5 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_9{}^{79}Br^{35}ClFN_4O_2$ $[M+H]^+$ 421.9810, found 421.9810.

3-Bromo-1-(phenylsulfonyl)-1*H*-pyrrole (313)



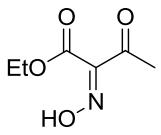
1-(Phenylsulfonyl)pyrrole (1.00 g, 4.83 mmol) was dissolved in AcOH (9 mL), and Br2 was added (0.251 mL, 4.83 mmol) as a solution in AcOH (3mL). The mixture was heated to reflux for 1 h. Upon cooling water was added (10 mL) and the mixture was neutralised using NaOH. The product was extracted using EtOAc (3 x 30 mL) and the combined organic extracts were dried over Na_2SO_4 before the solvent was removed *in vacuo.* The crude product was purified by MPLC (0-10% EtOAc in petrol) to give the pure product as a colourless oil that solidified on cooling (0.832 g, 2.90 mmol, 60%); R_f = 0.56 (5% MeOH in EtOAc); M.p. 65-66 ^oC (lit.[135] 66.5-67 ^oC) λ_{max} (EtOH)/nm 237; ν_{max}/cm^{-1}:3140, 1736, 1580, 1529; ^1H NMR (500 MHz, $CDCl_3$) δ 6.22 (1H, dd, *J* = 1.6 and 3.2 Hz, H-4), 7.01-7.02 (1H, m, H-5), 7.09-7.10 (1H, m, H-2), 7.44-7.49 (2H, m, H-Ar), 7.51-7.59 (1H, m, H-Ar), 7.78-7.82 (2H, m, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 116.4 (C-3), 119.7 (C-4), 120.8 (C-2), 121.3 (C-5), 126.9 (C-Ar), 127.2 (C-Ar), 129.3 (C-Ar), 129.7 (C-Ar), 134.6 (C-Ar); LRMS (ES^+) *m/z* 285.1 $[M+H]^+$.

Methyl 3-bromo-1-(phenylsulfonyl)-1*H*-pyrrole-2-carboxylate (314)



Diisopropylamine (0.785 µL, 5.60 mmol) was cooled to -78 ^{0}C before *n*-BuLi (2.13 M in hexane) (2.10 mL, 4.47 mmol) was added dropwise and the mixture was allowed to stir at the temperature for 1 h. 3-Bromo-1-(phenylsulfonyl)-1*H*-pyrrole (**313**) (0.800 g, 2.80 mmol) was added in THF (13 mL) and the mixture was allowed to stir at -78 ^{o}C for 1 h further. Methyl chloroformate (494 µL, 6.44 mmol) was added dropwise as a solution in THF (13 mL) and the reaction was stirred for 30 min before being allowed to warm to RT and being quenched with a saturated aqueous solution of NH_4Cl (10 mL). The product was extracted with EtOAc (3 x 30 mL) and washed with water (50 mL) followed by brine (50 mL). The combined organic extracts were dried over Na_2SO_4 before being concentrated *in vacuo*. The crude product was purified by MPLC (0-10% EtOAc in petrol) to give the title compound as a colourless oil that solidified on cooling (1.23 g, 3.59 mmol, 80%); R_f = 0.45 (5% MeOH in EtOAc); M.p. 77-78 ^{o}C (lit.[135] 77-77.5 ^{o}C) λ_{max} (EtOH)/nm 246; ν_{max}/cm^{-1}:3142, 2120, 1722 (CO_2Me), 1533; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.72 (3H, s, CH_3), 6.33 (1H, d, *J* = 3.4 Hz, H-4), 7.47-7.50 (3H, m, H-5 and H-Ar), 7.57-7.59 (1H, m, H-2), 7.87-7.91 (2H, m, H-Ar); ^{13}C NMR (125 MHz, $CDCl_3$) δ 52.4 (CH_3), 115.3 (C-3), 123.8 (C-4), 124.8 (C-2), 125.3 (C-5), 126.9 (C-Ar), 127.9 (C-Ar), 128.1 (C-Ar), 128.8 (C-Ar), 134.2 (C-Ar); LRMS (ES^{+}) *m/z* 344.2 [M+H]$^{+}$.

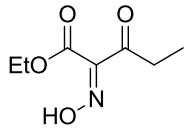
(*Z*)-Ethyl 2-(hydroxyimino)-3-oxobutanoate (317)



Ethyl acetoacetate (98 µL, 0.77 mmol) was cooled to 0 ^{o}C in AcOH (1 mL) before sodium nitrite (53 mg, 0.77 mmol) was added as a solution in water (1 mL) and the reaction left to stir for 4 h. Water was added (10 mL) and the product was extracted with diethyl ether (3 x 10 mL). The combined organic layers washed with brine (20 mL) before being dried over Na_2SO_4. The solvent was removed *in vacuo* to give the title

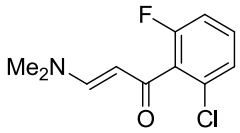
compound as a pale yellow oil (103 mg, 0.65 mmol 85%); R_f = 0.30 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 299; ν_{max}/cm^{-1}:3083, 2925, 2110, 1742 (CO_2Et), 1571; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.56 (3H, t, J = 7.2 Hz, CH_2CH_3), 2.01 (1H, s, NOH), 2.50 (3H, s, CH_3), 4.50 (2H, q, J = 7.2 Hz, CH_2CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.7 (CH_2CH_3), 20.8 (CH_3), 60.1 (CH_2CH_3), 144.0 (CNOH), 166.1 (CO_2Et), 196.1 (CO); LRMS (ES$^+$) *m/z* 160.3 [M+H]$^+$.

(*Z*)-Ethyl 2-(hydroxyimino)-3-oxopentanoate (318)



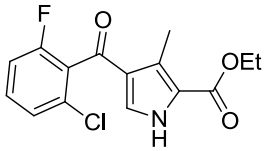
Ethyl propionylacetate (0.49 mL, 3.47 mmol) was cooled to 0 ^{o}C in AcOH (5 mL) before sodium nitrite (239 mg, 3.47 mmol) was added as a solution in water (5 mL) and the reaction left to stir for 4 h. Water was added (30 mL) and the product was extracted with diethyl ether (3 x 30 mL). The combined organic layers washed with brine (50 mL) before being dried over Na_2SO_4. The solvent was removed *in vacuo* to give the title compound as a yellow oil (0.49 g, 2.84 mmol 82%); R_f = 0.33 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 232; ν_{max}/cm^{-1}:3314, 2984, 2942, 1720 (CO_2Et), 1688; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.08 (3H, t, J = 7.2 Hz, CH_2CH_3), 1.31 (3H, t, J = 6.8 Hz, OCH_2CH_3), 2.10 (1H, s, NOH), 2.01 (1H, s, NOH), 2.79 (2H, q, J = 7.2 Hz, CH_2CH_3), 4.34 (2H, q, J = 6.8 Hz, OCH_2CH_3); ^{13}C NMR (125 MHz, DMSO-d_6) δ 7.7 (CH_2CH_3), 13.9 (OCH_2CH_3), 62.4 (CH_2CH_3), 77.1 (OCH_2CH_3), 150.3 (CNOH), 162.1 (CO_2Et), 197.0 (CO); LRMS (ES$^+$) *m/z* 174.1 [M+H]$^+$.

(*E*)-1-(2-Chloro-6-fluorophenyl)-3-(dimethylamino)prop-2-en-1-one (320)



N,*N*-Dimethylformamide dimethyl acetal (97 µL, 0.73 mmol) was added dropwise to a solution of 2'-chloro-6'-fluoroacetophenone (79 µL, 0.58mmol) in toluene (1 mL) and the mixture was heated to reflux for 18 h. Upon cooling, the solvent was removed *in vacuo* and the crude residue was purified by MPLC (0-50% EtOAc in petrol over 24 column volumes) to give the pure compound as a pale yellow solid (105 mg, 0.46, 80%); R_f = 0.35 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 305, 236; ν_{max}/cm^{-1}: 3077 (NMe_2), 2920, 2323, 1630 (CO), 1560; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.93 (6H, s, $N(CH_3)_2$), 6.86 (1H, br s, CH) Hz, 7.02-7.05 (2H, m, H-Ar), 7.26-7.30 (1H, m, H-Ar), 7.90 (1H, br s, CHN); ^{13}C NMR (125 MHz, $CDCl_3$) δ 45.4 ($N(CH_3)_2$), 114.1 (CH), 114.5 (C-Ar), 125.4 (C-Ar), 125.5 (C-Ar), 129.9 (C-Ar), 153.6 (CHN), 159.2 (d, *J* = 245.1 Hz, CF), 188.9 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.3; LRMS (ES^+) *m/z* 228.3 $[M+H]^+$.

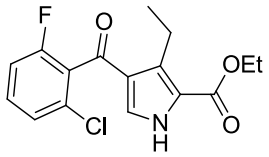
Ethyl 4-(2-chloro-6-fluorobenzoyl)-3-methyl-1*H*-pyrrole-2-carboxylate (321)



(*E*)-1-(2-Chloro-6-fluorophenyl)-3-(dimethylamino)prop-2-en-1-one (**320**) (100 mg, 0.44 mmol) was dissolved in AcOH (1 mL) before (*Z*)-Ethyl 2-(hydroxyimino)-3-oxobutanoate (**317**) (70 mg, 0.44 mmol) was added followed by NaOAc (108 mg, 1.32 mmol). Zn dust (247 mg, 3.78 mmol) was added portion-wise with heating to 140 ^oC over 6 h. Upon cooling water was added (5 mL) and the product was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo.* The crude product was purified by MPLC (0-30% EtOAc in petrol) to give the product as a yellow oil (40 mg, 0.13 mmol, 30%); R_f = 0.30 (5% MeOH in DCM); M.p. 256-257 ^oC; λ_{max} (EtOH)/nm 232; ν_{max}/cm^{-1}: 3057, 2925, 2110, 1681 (CO_2Me), 1571 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.33 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 2.37 (3H, s, CH_3), 4.28 (2H, q, *J* = 7.4 Hz, CH_2CH_3), 7.01 (1H, s, H-5), 7.21-7.26 (2H, m, H-3' and H-5'), 7.60 (1H, ddd, *J* = 6.2, 8.3 and 8.4Hz, H-4'), 9.65

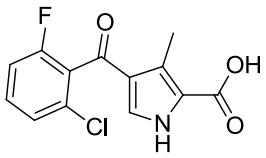
(1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 10.1 (CH_3), 15.2 (CH_2CH_3), 60.1 (CH_2CH_3), 113.7 (C-Ar), 118.2 (C-4), 122.1 (C-2), 123.5 (C-Ar), 125.8 (C-5), 128.4 (C-3), 136.2 (C-Ar), 136.9 (C-Ar), 157.3 (d, *J* = 256.3 Hz, CF), 186.2 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.2; LRMS (ES^+) *m/z* 310.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{15}H_{14}{}^{35}ClFNO_3$ $[M+H]^+$ 310.1023, found 310.1023.

Ethyl 4-(2-chloro-6-fluorobenzoyl)-3-ethyl-1*H*-pyrrole-2-carboxylate (322)



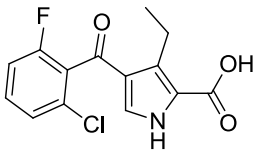
(*E*)-1-(2-Chloro-6-fluorophenyl)-3-(dimethylamino)prop-2-en-1-one (**317**) (264 mg, 1.16 mmol) was dissolved in AcOH (1 mL) before (*Z*)-ethyl 2-(hydroxyimino)-3-oxopentanoate (**318**) (100 mg, 0.58 mmol) was added followed by NaOAc (143 mg, 1.74 mmol). Zn dust (227 mg, 3.48 mmol) was added portion-wise with heating to 140 ^{o}C over 6 h. Upon cooling water was added (5 mL) and the product was extracted with ethyl acetate (3 x 10 mL). The combined organic layers were dried over Na_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by MPLC (0-30% EtOAc in petrol) to give the product as an orange oil (66 mg, 0.20 mmol, 35%); R_f = 0.32 (5% MeOH in DCM); M.p. 260-261 ^{o}C; λ_{max} (EtOH)/nm 239; ν_{max}/cm^{-1}: 2972, 2082, 1651 (CO_2Me), 1568 (CO); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.17 (3H, t, *J* = 7.1 Hz, CH_2CH_3), 1.31 (3H, t, *J* = 7.3 Hz, OCH_2CH_3), 3.15 (2H, q, *J* = 7.1 Hz, CH_2CH_3), 4.30 (2H, q, *J* = 7.3 Hz, OCH_2CH_3), 6.93 (1H, s, H-5), 6.97-7.00 (2H, m, H-3' and H-5'), 7.27 (1H, ddd, *J* = 6.0, 8.3 and 8.4 Hz, H-4'), 9.30 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.1 (CH_2CH_3), 15.04 (OCH_2CH_3), 18.7 (CH_2CH_3), 60.8 (OCH_2CH_3), 114.2 (C-Ar), 114.5 (C-Ar), 119.2 (C-4), 121.5 (C-2), 124.1 (C-Ar), 125.2 (C-Ar), 126.0 (C-5), 129.9 (C-3), 136.2 (C-Ar), 140.1 (C-Ar), 160.2 (d, *J* = 251.8 Hz, CF), 188.8 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.3; LRMS (ES^+) *m/z* 324.1 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{16}{}^{35}ClFNO_3$ $[M+H]^+$ 324.0804, found 324.0804.

4-(2-Chloro-6-fluorobenzoyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (323)



Ethyl 4-(2-chloro-6-fluorobenzoyl)-3-methyl-1*H*-pyrrole-2-carboxylate (**321**) (150 mg, 0.48 mmol) was dissolved in THF (4 mL) before LiOH (230 mg, 9.60 mmol) was added as a solution in H_2O (6 mL). The mixture was heated to 100 ^oC and left to stir for 18 h. The reaction was worked up according to general procedure B and the pure product was obtained as a yellow solid (105 mg, 0.37 mmol, 78%); R_f = 0.21 (5% MeOH in DCM); M.p. 271-272 ^oC; λ_{max} (EtOH)/nm 236; ν_{max}/cm^{-1}: 3343, 2921, 2087, 1638 (CO_2H), 1603 (CO); ^1H NMR (500 MHz, $CDCl_3$) δ 2.36 (3H, s, CH_3), 6.95 (1H, s, H-5), 7.01-7.25 (2H, m, H-3' and H-5'), 7.45 (1H, ddd, *J* = 6.3, 8.4 and 8.5 Hz, H-4'), 9.32 (1H, br s, NH), 9.56 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 9.7 (CH_3), 115.7 (C-Ar), 120.1 (C-4), 123.0 (C-2), 124.1 (C-Ar), 126.0 (C-5), 129.3 (C-3), 135.4 (C-Ar), 136.8 (C-Ar), 160.4 (d, *J* = 245.3 Hz, CF), 189.0 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -111.7; LRMS (ES^+) *m/z* 280.1 $[M-H]^-$; HRMS *m/z* calcd for $C_{13}H_8{}^{35}ClFNO_3$ $[M-H]^-$ 280.0182, found 280.0176.

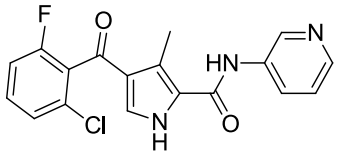
4-(2-Chloro-6-fluorobenzoyl)-3-ethyl-1*H*-pyrrole-2-carboxylic acid (324)



Ethyl 4-(2-chloro-6-fluorobenzoyl)-3-ethyl-1*H*-pyrrole-2-carboxylate (**322**) (337 mg, 1.04 mmol) was dissolved in THF (8 mL) before LiOH (499 mg, 20.9 mmol) was added as a solution in H_2O (14 mL). The mixture was heated to 100 ^oC and left to stir for 18 h. The reaction was worked up according to general procedure B and the pure product was obtained as a cream solid (230 mg, 0.78 mmol, 75%); R_f = 0.26 (5% MeOH in DCM); M.p. 275-276 ^oC; λ_{max} (EtOH)/nm 238; ν_{max}/cm^{-1}: 3323, 2090, 1704 (CO_2H), 1650 (CO), 1570; ^1H NMR (500 MHz, $CDCl_3$) δ 1.20 (3H, t, *J* = 7.4 Hz, CH_2CH_3), 3.20 (2H, q, *J* = 7.4 Hz, CH_2CH_3), 7.00 (1H, s, H-5), 7.03-7.05 (2H, m, H-3' and H-5'), 7.48 (1H, ddd, *J* = 6.1, 8.2 and 8.3 Hz, H-4'), 9.26 (1H, br s, NH), 9.75 (1H, br s, CO_2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.9 (CH_2CH_3), 18.2 (CH_2CH_3), 114.7 (C-Ar), 115.2 (C-

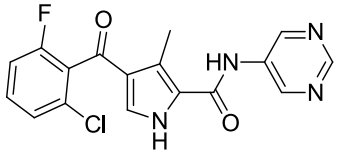
Ar), 119.9 (C-4), 122.1 (C-2), 124.9 (C-Ar), 126.2 (C-Ar), 127.0 (C-5), 130.2 (C-3), 137.2 (C-Ar), 140.3 (C-Ar), 157.8 (d, *J* = 256.7 Hz, CF), 189.1 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -112.0; LRMS (ES^+) *m/z* 294.3 $[M-H]^-$; HRMS *m/z* calcd for $C_{14}H_{10}{}^{35}ClFNO_3$ $[M-H]^-$ 294.0332, found 294.0331.

4-(2-Chloro-6-fluorobenzoyl)-3-methyl-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (325)



Compound **325** was synthesised according to general procedure D using 4-(2-Chloro-6-fluorobenzoyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (**323**) (80 mg, 0.28 mmol), 3-aminopyridine (66 mg, 0.70 mmol), PCl_3 (24 µL, 0.28 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a yellow solid (25 mg, 0.07 mmol, 25%); Rf = 0.43 (5% DCM in MeOH); M.p. 269-272 ^{o}C; λ_{max} (EtOH)/nm 256; ν_{max}/cm^{-1}: 3320, 3077, 2746, 1641, 1520, 1483; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.58 (3H, s, CH_3), 7.28 (1H, s, H-5), 7.35-7.46 (3H, m, H-3', H-5' and pyridine-CH), 7.52-7.60 (1H, m, H-4'), 8.10 (1H, d, *J* = 6.5 Hz, pyridine-CH), 8.31 (1H, d, *J* = 4.9 Hz, pyridine-CH-N), 8.82 (1H, s, pyridine-CH-N), 9.94 (1H, s, CONH), 12.27 (1H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 11.4 (CH_3), 114.8, 115.0 (C-Ar), 123.6 (C-3), 125.7 (C-2 and C-5), 126.8, (C-Ar), 130.5 (C-4), 135.5, 141.4, 144.4 (C-pyridine), 158.7 (d, JCF = 251.9 Hz, CF), 159.8 (CONH), 184.5 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -115.9; LRMS (ES^+) *m/z* 358.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{18}H_{13}ClFN_3O_2$ $[M+H]^+$ 358.0759, found 358.0758.

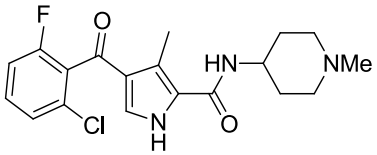
4-(2-Chloro-6-fluorobenzoyl)-3-methyl-*N*-(pyrimidin-5-yl)-1*H*-pyrrole-2-carboxamide (326)



Compound **326** was synthesised according to general procedure D using 4-(2-Chloro-6-fluorobenzoyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (**323**) (60 mg, 0.21 mmol), 5-aminopyrimidine (50 mg, 0.53 mmol), PCl_3 (18 µL, 0.21 mmol) and MeCN (1 mL).

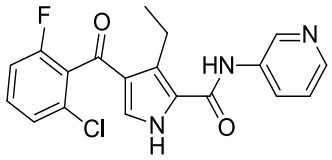
The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as an orange solid (15 mg, 0.04 mmol, 20%); R_f = 0.39 (5% MeOH in DCM); M.p. 281-282 ^{o}C; λ_{max} (EtOH)/nm 252; ν_{max}/cm^{-1}: 3292, 2921, 1643 (CO), 1508 (CONH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.83 (3H, s, CH_3), 6.98-7.05 (3H, m, H-5, H-3' and H-5'), 7.30 (1H, ddd, *J* = 6.0, 8.1 and 8.2 Hz, H-4'), 7.70 (1H, br s, CONH), 8.95 (1H, s, pyrimidine-CH), 9.03 (2H, s, 2 x pyrimidine-CH), 9.75 (1H, br s, NH); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.2; LRMS (ES$^+$) *m/z* 359.3 [M+H]$^+$; HRMS *m/z* calcd for $C_{17}H_{12}ClFN_4O_2$ [M+H]$^+$ 359.0712, found 359.0712.

4-(2-Chloro-6-fluorobenzoyl)-3-methyl-*N*-(1-methylpiperidin-4-yl)-1*H*-pyrrole-2-carboxamide (327)



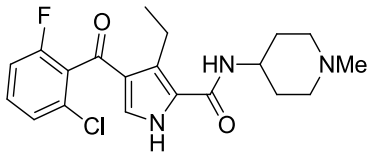
Compound **327** was synthesised according to general procedure D using 4-(2-Chloro-6-fluorobenzoyl)-3-methyl-1*H*-pyrrole-2-carboxylic acid (**323**) (60 mg, 0.21 mmol), 1-methyl-4-aminopiperidine (56 µL, 0.53 mmol), PCl_3 (18 µL, 0.21 mmol) and MeCN (1 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a pale yellow solid (24 mg, 0.06 mmol, 30%); R_f = 0.35 (5% DCM in MeOH); M.p. 254-255 ^{o}C; λ_{max} (EtOH)/nm 242; ν_{max}/cm^{-1}: 3218 (NMe), 2924, 2791, 1602 (CO), 1544 (CONH); ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.71-1.78 (2H, m, CH_2), 1.97-2.03 (2H, m, CH_2), 2.32-2.36 (2H, m, CH_2), 2.38 (3H, s, CH_3), 2.64 (3H, s, *N*-CH_3), 2.95 (2H, br s, 2H, m, CH_2), 3.94-4.00 (1H, m, CH), 6.43 (1H, br s, CONH), 6.86 (1H, s, H-5), 6.96-6.99 (1H, m, H-5'), 7.15 (1H, d, *J* = 8.1 Hz, H-3'), 7.25 (1H, ddd, *J* = 6.2, 8.1 and 8.2 Hz, H-4'), 10.49 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.7 (CH_3), 31.1 (2 x CH_2), 45.3 (*N*-CH_3 and CH), 114.3 (C-Ar), 114.5 (C-4), 123.7 (C-Ar), 124.7 (C-Ar), 124.8 (C-2), 129.0 (d, J_{CF} = 3.1 Hz, C-Ar), 129.2 (C-5), 130.7 (C-3), 131.9 (d, J_{CF} = 6.0 Hz, C-Ar), 159.2 (d, J_{CF} = 248.8 Hz, C-F), 161.0 (CONH), 185.5 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.3; LRMS (ES$^+$) *m/z* 378.4 [M+H]$^+$; HRMS *m/z* calcd for $C_{19}H_{21}ClFN_3O_2$ [M+H]$^+$ 378.1384, found 378.1384.

4-(2-Chloro-6-fluorobenzoyl)-3-ethyl-*N*-(pyridin-3-yl)-1*H*-pyrrole-2-carboxamide (328)



Compound **328** was synthesised according to general procedure D using 4-(2-Chloro-6-fluorobenzoyl)-3-ethyl-1*H*-pyrrole-2-carboxylic acid (**324**) (100 mg, 0.34 mmol), 3-aminopyridine (80 mg, 0.85 mmol), PCl_3 (30 µL, 0.34 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as a cream solid (38 mg, 0.10 mmol, 30%); R_f = 0.45 (5% DCM in MeOH); M.p. 269-272 ^oC; λ_{max} (EtOH)/nm 256, 289; ν_{max}/cm^{-1}: 3142, 3980, 1547 (CO), 1511 (CONH); ^1H NMR (500 MHz, $CDCl_3$) δ 1.48 (3H, t, *J* = 7.6 Hz, CH_3), 3.35 (2H, q, *J* = 7.6 Hz, CH_2), 7.08 (1H, d *J* = 3.4 Hz, CH-pyridine), 7.09-7.12 (1H, m, H-3'), 7.27 (1H, s, H-5), 7.35-7.40 (2H, m, H-4' and H-5'), 7.79 (1H, br s, CONH), 8.19 (1H, d, *J* = 7.2 Hz, CH-pyridine), 8.44 (1H, d, *J* = 4.9 Hz, CH-N-pyridine), 8.72 (1H, d, *J* = 2.2 Hz, CH-N-pyridine), 9.85 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 15.3 (CH_2), 19.6 (CH_3), 114.3 (C-Ar), 114.5 (C-4), 123.9 (C-2), 124.6 (C-Ar), 125.6 (C-Ar), 127.7 (d, J_{CF} = 3.2 Hz, C-Ar), 130.1 (C-5), 130.8 (d, J_{CF} = 9.2 Hz, C-Ar), 134.3 (C-pyridine), 141.5 (C-3), 145.7 (C-pyridine), 159.6 (CONH), 185.2 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.3; LRMS (ES^+) *m/z* 372.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{19}H_{15}ClFN_3O_2$ $[M+H]^+$ 372.0918, found 372.0917.

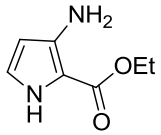
4-(2-Chloro-6-fluorobenzoyl)-3-ethyl-*N*-(1-methylpiperidin-4-yl)-1*H*-pyrrole-2-carboxamide (329)



Compound **329** was synthesised according to general procedure D using 4-(2-Chloro-6-fluorobenzoyl)-3-ethyl-1*H*-pyrrole-2-carboxylic acid (**324**) (100 mg, 0.34 mmol), 4-amino-1-methylpiperidine (89 µL, 0.85 mmol), PCl_3 (30 µL, 0.34 mmol) and MeCN (2 mL). The crude mixture was purified by MPLC (0-8% MeOH in DCM) to give the title compound as an orange solid (33 mg, 0.08 mmol, 25%); R_f = 0.36 (5% DCM in MeOH); M.p. 262-264 ^oC; λ_{max} (EtOH)/nm 242; ν_{max}/cm^{-1}: 3385 (NMe), 2925, 1571 (CO), 1535 (CONH), 1445, 1407; ^1H NMR (500 MHz, $CDCl_3$) δ 1.25-1.29 (2H, m,

CH_2), 1.32 (3H, t, *J* = 7.5 Hz, CH_2CH_3), 1.86-1.94 (2H, m, CH_2), 2.10-2.14 (2H, m, CH_2N), 2.52 (3H, s, NCH_3), 3.10-3.14 (2H, m, CH_2N), 3.24 (2H, q, *J* = 7.5 Hz, CH_2CH_3), 4.10-4.16 (1H, m, CH), 6.81 (1H, br s, CONH), 6.96 (1H, s, H-5), 7.05-7.06 (1H, m, H-5'), 7.08 (1H, d, *J* = 8.3 Hz, H-3'), 7.33 (1H, ddd, *J* = 5.9, 8.3 and 8.4 Hz, H-4'), 10.76 (1H, br s, NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ; 15.0 (CH_2CH_3), 18.9 (CH_2CH_3), 30.6 (2 x CH_2), 44.9 (NCH_3), 53.9 (2 x CH_2N), 114.2 (C-Ar), 114.4 (C-4), 124.1 (C-Ar), 124.4 (C-2), 125.5 (d, J_{CF} = 3.4 Hz, C-Ar), 129.3 (C-5), 130.5 (d, *J* = 9.0 Hz, C-Ar), 131.9 (d, J_{CF} = 6.2 Hz, C-Ar), 132.0 (C-3), 159.0 (d, J_{CF} = 250.5 Hz, C-F), 160.8 (CONH), 185.0 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -113.2; LRMS (ES^+) *m/z* 372.3 $[M+H]^+$; HRMS *m/z* calcd for $C_{20}H_{23}ClFN_3O_2$ $[M+H]^+$ 392.1538, found 392.1538.

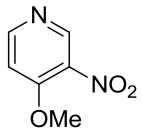
Ethyl 3-amino-1*H*-pyrrole-2-carboxylate (347)



Isoxazole (93 µL, 1.5 mmol) was dissolved in ethanol (4 mL) before a 21% by weight solution of NaOEt (109 mg, 1.6 mmol) in EtOH (0.5 mL) was added slowly at 0 ^oC. The mixture was stirred for 45 min before acetic acid (25 µL, 0.44 mmol) and diethyl 2-aminomalonate hydrochloride (192 mg, 0.91 mmol) were added and the mixture was stirred at RT overnight. The solvent was removed *in vacuo* and DCM (10 mL) was added to dissolve the residue, which was washed with water. The organic layer was dried over Na_2SO_4 and the solvent removed *in vacuo*. The residue was dissolved in EtOH (10 mL) and a 21% by weight solution of NaOEt (59 mg, 0.87 mmol) in EtOH (0.28 mL) was added. The reaction was left to stir at RT overnight before acetic acid (54 µL, 0.94 mmol) was added and the mixture was concentrated *in vacuo*. Water (10 mL) was added to the residue and this was neutralised using a saturated solution of $NaHCO_3$. The product was extracted with DCM (3 x 10 mL) and the organic extracts were dried over Na_2SO_4 before the solvent was removed *in vacuo*. The title compound was obtained as a yellow oil (190 mg, 85%); R_f = 0.75 (10% MeOH in EtOAc); λ_{max} (EtOH)/nm: 254; ν_{max}/cm^{-1}: 3370 (NH_2), 2987, 2202, 1732 (CO_2Et); ^{1}H NMR (500 MHz, MeOD) δ 1.24 (3H, t, *J* = 7.2 Hz, CH_3), 4.23 (3H, t, *J* = 7.2 Hz, CH_3), 5.49 (2H, br s, NH_2), 6.94-6.97 (2H, m, H-4 and H-5); ^{13}C NMR (125 MHz, MeOD) δ 14.0 (CH_3),

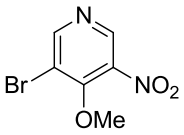
62.9 (CH_2), 119.9 (C-2, C-4, and C-5), 148.4 (C-3), 165.5 (CO); LRMS (ES^+) *m/z* 155.20 $[M+H]^+$.

4-Methoxy-3-nitropyridine (351)



Sodium (40 mg, 1.74 mmol) was dissolved in MeOH (1 mL) before 4-chloro-3-nitropyridine (250 mg, 1.58 mmol) was added as a solution in 3 mL MeOH:dioxane (1:1). The reaction mixture was heated to reflux for 2.5 h. After cooling, water was added and the product was extracted using EtOAc. The combined organic extracts were washed with brine and dried over Na_2SO_4 to give the pure compound as a pale yellow solid (235 mg, 6.50 mmol, 1.52 mmol, 96%); R_f = 0.43 (5% MeOH in DCM) M.p.: 71-72^{o}C (lit.[155]- 72-73 ^{o}C); λ_{max} (EtOH)/nm 241.5; ν_{max}/cm^{-1} 1594 (NO_2), 1511; ^{1}H NMR (500 MHz, DMSO-d_6) δ 4.03 (3H, s, OCH_3), 7.46 (1H, d, *J* = 6.1 Hz, CH-pyridine), 8.70 (1H, d, *J* = 6.1 Hz, CH-N-pyridine), 8.99 (1H, s, CH-N-pyridine); ^{13}C NMR (125 MHz, $CDCl_3$) δ 56.7 (OCH_3), 108.5 (C-5), 147.0 (C-2 and C-3), 154.8 (C-6), 158.9 (C-4); LRMS (ES^+) *m/z* 155.2 $[M+H]^+$.

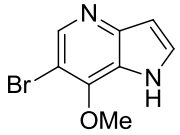
3-Bromo-4-methoxy-5-nitropyridine (352)



Sodium (292 mg, 0.23 mmol) was dissolved in MeOH (20 mL), before 3-bromo-4-chloro-5-nitropyridine (**356**) (2.74 g, 11.54 mmol) was added as a solution in 40 mL MeOH:Dioxane (1:1). The mixture was stirred at RT for 2.5 h before water was added and the product was extracted into EtOAc. The combined organic extracts were dried over Na_2SO_4 before the solvent was removed *in vacuo* to afford the pure product as a cream solid (2.30 g, 9.89 mmol, 86%); R_f = 0.35 (5% MeOH in DCM) M.p. 42-43 ^{o}C (lit.[151]- 39-40 ^{o}C); λ_{max} (EtOH)/nm 259; ν_{max}/cm^{-1} 3053, 1640, 1613, 1503 (NO_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.04 (3H, s, OCH_3), 8.78 (1H, s, CH-pyridine), 8.83 (1H, s,

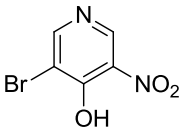
CH-pyridine); ^{13}C NMR (125 MHz, MeOD) δ 59.0 (OCH_3), 118.7 (C-3), 138.7 (C-5), 139.0 (C-6), 144.8 (C-2), 155.5 (C-4); LRMS (ES^+) *m/z* 234.1 $[M+H]^+$.

6-Bromo-7-methoxy-1*H*-pyrrolo[3,2-*b*]pyridine (353)



3-Bromo-4-methoxy-5-nitropyridine (**352**) (2.20 g, 9.44 mmol) was dissolved in THF (35 mL) and cooled to -78 °C. Vinylmagnesium bromide (1.0 M solution in THF, 35.0 mL, 35.0 mmol) was added dropwise and the mixture was left to stir for 5 h. The reaction was quenched using a saturated solution of NH_4Cl in MeOH (40 mL) before being allowed to warm to RT. The product was extracted using EtOAc and the combined organic extracts were dried over Na_2SO_4 before the solvent was removed *in vacuo*. The crude product was purified by MPLC (NH silica; 0-30% MeOH in DCM over 24 column volumes) to afford the pure product as an orange oil (857 mg, 3.77 mmol, 40%); R_f = 0.45 (5% MeOH in DCM); λ_{max} (EtOH)/nm 283; ν_{max}/cm^{-1} 2924, 1597, 1547, 1421; ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.10 (3H, s, CH_3), 6.67 (1H, dd, J = 2.1 and 3.3 Hz), 7.31-7.33 (1H, m, H-5), 8.41 (1H, s, CH-pyridine); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 60.7 (CH3), 91.5 (C-3), 104.7 (C-6), 112.4 (C-8), 127.0 (C-8), 127.0 (C-2), 127.7 (C-9), 147.1 (C-5), 165.6 (C-7); LRMS (ES^+) *m/z* 227.1 $[M+H]^+$; HRMS *m/z* calcd for $C_8H_8{}^{79}BrN_2O$ $[M+H]^+$ 227.1015, found 227.1018.

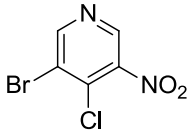
3-Bromo-5-nitropyridin-4-ol (355)



4-Hydroxy-3-nitropyridine (1.0 mg, 7.20 mmol) was suspended in water (20 mL) before Br_2 (482 µL, 9.4 mmol) was added. The reaction mixture was heated to 50 °C for 2.5 h. After cooling, the precipitate was filtered, washed with water followed by Et_2O and dried under vacuum to give the pure compound as a cream solid (1.42 g, 6.50 mmol, 90%); R_f = 0.47 (5% MeOH in DCM); M.p.: >300 °C (lit.[156] 370 °C); λ_{max} (EtOH)/nm: 332; ν_{max}/cm^{-1}: 2886 (OH), 1611, 1531 (NO_2); ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.39 (1H, s, CH-pyridine), 8.84 (1H, s, CH-pyridine), 12.80 (1H, br s, OH); ^{13}C NMR (125

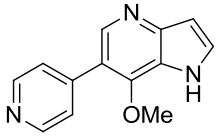
MHz, DMSO-d_6) 118.7 (C-3), 137.0 (C-5), 138.8 (C-6), 139.0 (C-2), 163.5 (C-4); LRMS (ES^+) *m/z* 221.1 $[M+H]^+$.

3-Bromo-4-chloro-5-nitropyridine (356)



3-Bromo-5-nitropyridin-4-ol (**355**) (200 mg, 0.92 mmol) and *N*,*N*-diethylaniline (140 µL, 0.92 mmol) were dissolved in $POCl_3$ (2 mL) and the mixture was heated under microwave irradiation at 140 ^oC for 15 min. The reaction mixture was added to vigourously stirring water (30 mL) at RT and the product was extracted into EtOAc. The combined organic layers were dried over Na_2SO_4 and the solvent removed *in vacuo* to afford the pure product as a clear oil, which crystallised upon cooling (195 mg, 0.82 mmol, 89%); R_f = 0.60 (5% MeOH in DCM); M.p.: 55-56 ^oC (lit.[151]- 49-50 ^oC); λ_{max} (EtOH)/nm 252; ν_{max}/cm^{-1} 2871, 1630, 1609, 1515 (NO_2); ^1H NMR (500 MHz, $CDCl_3$) δ 8.87 (1H, s, CH-pyridine), 8.88 (1H, s, CH-pyridine); LRMS (ES^+) *m/z* 238.1 $[M+H]^+$.

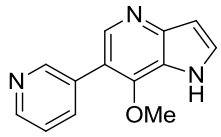
7-Methoxy-6-(pyridin-4-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (357)



6-Bromo-7-methoxy-1*H*-pyrrolo[3,2-*b*]pyridine (**352**) (150 mg, 0.66 mmol) was dissolved in dioxane (10 mL) before 4-pyridinyl-boronic acid (54 mg, 0.44 mmol) was added followed by Na_2CO_3 and water (1 mL). The mixture was degassed for 15 min before $Pd(PPh_3)_4$ (5mg, 0.04 mmol) was added and the mixture degassed for a further 15 min. The reaction was heated to 110 ^oC for 18 h before being cooled, filtered through Celite and the solvent removed *in vacuo*. The crude product was purified by MPLC (silica; 0-20% MeOH in EtOAc) to give the pure product as a yellow oil (111 mg, 0.49 mmol, 75%); R_f = 0.42 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 301, 254; ν_{max}/cm^{-1} 3404, 2925, 1599, 1552, 1517; ^1H NMR (500 MHz, $CDCl_3$) δ 3.77 (3H, s, CH_3), 6.73 (1H, d, *J* = 3.2 Hz, H-3), 7.42 (1H, d, *J* = 3.2 Hz, H-2), 7.48 (2H, d, *J* = 6.1 Hz, CH-pyridine), 8.35 (1H, s, H-5), 8.62 (2H, d, *J* = 6.1 Hz, CH-N-pyridine), 8.74 (1H, br s,

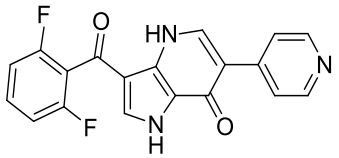
NH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 60.5 (CH_3), 103.7 (C-3), 111.7 (C-8), 118.3 (C-6), 123.4 (C-pyridine), 128.7 (C-2), 136.9 (C-N-pyridine), 145.8 (C-5 and C-9), 148.2 (pyridine-C), 150.2 (C-7); LRMS (ES^+) *m/z* 226.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_{12}N_3O$ $[M+H]^+$ 226.0975, found 226.0973.

7-Methoxy-6-(pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (358)



6-Bromo-7-methoxy-1*H*-pyrrolo[3,2-*b*]pyridine (**352**) (150 mg, 0.66 mmol) was dissolved in dioxane (10 mL) before 3-pyridinyl-boronic acid (54 mg, 0.44 mmol) was added followed by Na_2CO_3 and water (1 mL). The mixture was degassed for 15 min before $Pd(PPh_3)_4$ (5mg, 0.04 mmol) was added and the mixture degassed for a further 15 min. The reaction was heated to 110 ^oC for 18 h before being cooled, filtered through celite and the solvent removed *in vacuo*. The crude product was purified by MPLC (silica; 0-20% MeOH in EtOAc) to give the pure product as a yellow oil (110 mg, 0.49 mmol, 75%); R_f = 0.43 (5% MeOH in EtOAc); λ_{max} (EtOH)/nm 302, 254; ν_{max}/cm^{-1} 3404, 1599, 1552, 1515; ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.85 (3H, s, CH_3), 6.82 (1H, d, *J* = 3.2 Hz, H-3), 7.50 (1H, d, *J* = 3.2 Hz, H-2), 7.91-7.94 (2H, m, CH-pyridine), 8.41 (1H, s, H-5), 8.65 (1H, dd, *J* = 1.6 and 5.0 Hz, CH-N-pyridine), 8.84 (1H, br s, NH), 8.87 (1H, s, CH-N-pyridine); ^{13}C NMR (125 MHz, $CDCl_3$) δ 60.6 (CH_3), 103.7 (C-3), 111.7 (C-8), 118.1 (C-6), 124.4 (C-pyridine), 128.9 (C-2), 138.9 (C-N-pyridine), 145.6 (C-5 and C-9), 148.2 (pyridine-C), 155.2 (C-7); LRMS (ES^+) *m/z* 226.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{13}H_{12}N_3O$ $[M+H]^+$ 226.0975, found 226.0977.

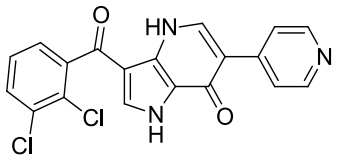
3-(2,6-Difluorobenzoyl)-6-(pyridin-4-yl)-1*H*-pyrrolo[3,2-b]pyridin-7(4*H*)-one (367)



Compound 367 was synthesised according to general procedure F using; (part i) 2,6-difluorobenzoyl chloride (446 µL, 3.55 mmol), 7-methoxy-6-(pyridin-4-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (357) (160 mg, 0.71 mmol), $AlCl_3$ (0.52 g, 3.91 mmol) and DCM (6 mL), (part ii) DCM (3 mL), TMSI (170 µL, 1.20 mmol). The crude product was

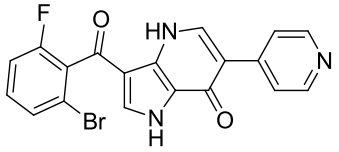
purified by semi-preparative HPLC (C-18 silica; 5-100% CH_3CN in water (0.1% formic acid)) to give the pure product as a white solid (19 mg, 0.06 mmol, 28%); R_f = 0.35 (5% MeOH in EtOAc); M.p. 235-236 ^oC; λ_{max} (EtOH)/nm 252; ν_{max}/cm^{-1} 3350, 3042, 2858, 1619 (CO), 1598 (CO-pyridone), 1565; ^1H NMR (500 MHz, MeOD) δ 7.17-7.20 (1H, m, H-Ar), 7.25 (1H, d, *J* = 8.9 Hz, H-Ar), 7.43-7.48 (1H, m, H-Ar), 7.43-7.48 (2H, m, CH-pyridine), 7.50 (1H, s, H-3), 7.61-7.63 (2H, m, CH-pyridine), 7.92 (1H, s, H-5), 8.06 (1H, d, J = 8.1 Hz, CH-pyridone), 8.41 (1H, br s, NH-pyridone), 8.74 (1H, br s, NH); ^{19}F NMR (470 MHz, MeOD) δ -115.4; LRMS (ES^+) *m/z* 368.1 $[M+H]^+$.

3-(2,3-Dichlorobenzoyl)-6-(pyridin-4-yl)-1*H*-pyrrolo[3,2-b]pyridin-7(4*H*)-one (368)



Compound **368** was synthesised according to general procedure F using; (part i) 2,3-dichlorobenzoyl chloride (0.74 g, 3.55 mmol), 7-methoxy-6-(pyridin-4-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (**357**) (160 mg, 0.71 mmol), $AlCl_3$ (0.52 g, 3.91 mmol) and DCM (6 mL), (part ii) DCM (3 mL), TMSI (181 µL, 1.28 mmol). The crude product was purified by semi-preparative HPLC (C-18 silica; 5-100% CH_3CN in water (0.1% formic acid)) to give the pure product as a white solid (20 mg, 0.05 mmol, 24%); R_f = 0.40 (5% MeOH in EtOAc); M.p. 140-141 ^oC; λ_{max} (EtOH)/nm 219; ν_{max}/cm^{-1} 3300, 2390, 2190, 1638 (CO), 1637 (CO-pyridone); ^1H NMR (500 MHz, DMSO-d_6) δ 7.06 (2H, dd, J = 1.3 and 8.0 Hz, H-5' and H-6'), 7.15-7.17 (2H, m, H-4' and H-5), 7.78 (2H, d, *J* = 5.2 Hz, CH-pyridine), 7.94 (2H, br s, CH-pyridine), 8.14 (1H, s, pyridone CH); LRMS (ES^+) *m/z* 384.2 $[M+H]^+$.

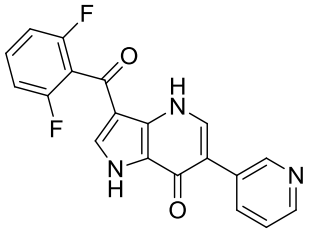
3-(2-Bromo-6-fluorobenzoyl)-6-(pyridin-4-yl)-1*H*-pyrrolo[3,2-b]pyridin-7(4*H*)-one (370)



2-Bromo-6-fluorobenzoic acid (1.36 g, 6.22 mmol) was dissolved in THF (12 mL) before $SOCl_2$ (1.36 mL, 18.7 mmol) and DMF (57 µL, 0.62 mmol) were added. The

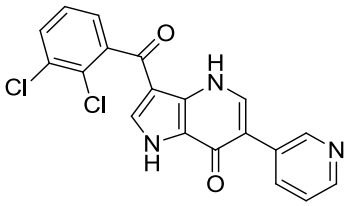
mixture was stirred at RT for 3 h before the solvent was removed *in vacuo.* The residue was re-dissolved in DCM (8 mL) and $AlCl_3$ (0.91 mg, 6.84 mmol) was added followed by 7-methoxy-6-(pyridin-4-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (**357**) (280 mg, 1.24 mmol) and the reaction was carried out as for general procedure F, (part ii) DCM (8 mL), TMSI (338 µL, 2.23 mmol). The crude product was purified by semi-preparative HPLC (C-18 silica; 5-100% CH_3CN in water (0.1% formic acid)) to give the pure product as a white solid (32 mg, 0.08 mmol, 21%); R_f = 0.41 (5% MeOH in EtOAc); M.p. 165-166 ^oC; λ_{max} (EtOH)/nm 253, 320; ν_{max}/cm^{-1} 3029, 2903, 2699, 1596 (CO), 1555 (CO-pyridone) ; ^1H NMR (500 MHz, DMSO-d_6) δ 7.43-7.46 (1H, m, H-3'), 7.54 (1H, ddd, *J* = 6.2, 8.4 and 8.5 Hz, H-4'), 7.63 (1H, d, *J* = 8.1 Hz, H-5'), 7.65 (1H, s, H-5), 7.79 (2H, d, *J* = 6.4 Hz, CH-pyridine), 7.96 (1H, s, CH-pyridone), 8.57 (2H, d, *J* = 6.4 Hz, CH-pyridine); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.4; LRMS (ES^+) *m/z* 414.2 $[M+H]^+$.

3-(2,6-Difluorobenzoyl)-6-(pyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-7(4H)-one (371)



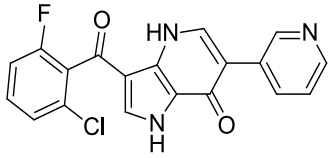
Compund 371 was synthesised according to general procedure F using; (part i) 2,6-difluorobenzoyl chloride (0.28 mL, 2.20 mmol), 7-Methoxy-6-(pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (358) (100 mg, 0.44 mmol), $AlCl_3$ (322 mg, 2.42 mmol) and DCM (4 mL), (part ii) DCM (1 mL), TMSI (50 µL, 0.36 mmol). The crude product was purified by semi-prepartive HPLC (C-18 silica; 5-100% CH_3CN in water (0.1% formic acid)) to give the pure product as a white solid (8 mg, 0.02 mmol, 39%); R_f = 0.31 (5% MeOH in EtOAc); M.p. 265-266 ^oC; λ_{max} (EtOH)/nm 252; ν_{max}/cm^{-1} 3532, 2874, 2160, 2015, 1618 (CO), 1589 (CO-pyridone); ^1H NMR (500 MHz, MeOD) δ 7.05-7.10 (3H, m, H-2 and H-Ar), 7.34 (1H, d, *J* = 8.8 Hz, CH-pyridine), 7.47-7.54 (2H, m, H-Ar and CH-pyridine), 7.61 (1H, s, H-5), 7.92 (1H, s, CH-N-pyridine), 8.08 (1H, d, *J* = 6.7 Hz, CH-N-pyridine); ^{19}F NMR (470 MHz, MeOD) δ -115.0 LRMS (ES^+) *m/z* 352.3 $[M+H]^+$.

3-(2,3-Dichlorobenzoyl)-6-(pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridin-7(4*H*)-one (372)



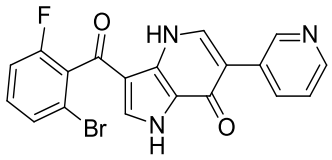
Compound **372** was synthesised according to general procedure F using; (part i) 2,3-dichlorobenzoyl chloride (461 mg, 2.20 mmol), 7-Methoxy-6-(pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (**358**) (100 mg, 0.44 mmol), $AlCl_3$ (322 mg, 2.42 mmol) and DCM (4 mL), (part ii) DCM (1 mL), TMSI (64 µL, 0.36 mmol). The crude product was purified by semi-prepartive HPLC (C-18 silica; 5-100% CH_3CN in water (0.1% formic acid)) to give the pure product as a white solid (10 mg, 0.03 mmol, 27%); R_f = 0.38 (5% MeOH in EtOAc); M.p. 251-252 ^oC; λ_{max} (EtOH)/nm 294; ν_{max}/cm^{-1} 3412, 2924, 2853, 2161, 1590 (CO); ^1H NMR (500 MHz, MeOD) δ 7.46-7.52 (3H, m, H-Ar), 7.56 (1H, s, H-2), 7.73 (1H, dd, *J* = 2.0 and 7.5 Hz, CH-pyridine), 8.01 (1H, s, H-5), 8.16 (1H, d, *J* = 7.5 Hz, CH-pyridine), 8.50 (1H, d, *J* = 4.9 Hz CH-N-pyridine), 8.83 (1H, s, CH-N-pyridine); LRMS (ES^+) *m/z* 384.2 $[M+H]^+$.

3-(2-Chloro-6-fluorobenzoyl)-6-(pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-7(4*H*)-one (373)



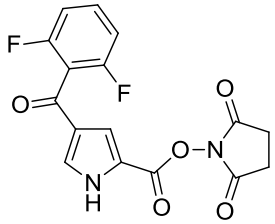
Compound 373 was synthesised according to general procedure F using; (part i) 2-chloro-6-fluorobenzoyl chloride (0.60 mL, 4.45 mmol), 7-methoxy-6-(pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (358) (200 mg, 0.89 mmol), $AlCl_3$ (0.65 g, 4.90 mmol) and DCM (8 mL), (part ii) DCM (5 mL), TMSI (189 µL, 1.33 mmol). The crude product was purified by semi-preparative HPLC (C-18 silica; 5-100% CH_3CN in water (0.1% formic acid)) to give the pure product as a white solid (24 mg, 0.07 mmol, 30%); R_f = 0.35 (5% MeOH in EtOAc); M.p. 235-236 ^oC; λ_{max} (EtOH)/nm 252; ν_{max}/cm^{-1} 3391, 3246, 3067, 2943, 2682, 1621 (CO); ^1H NMR (500 MHz, MeOD) δ 7.17-7.20 (1H, m, H-Ar), 7.25 (1H, d, *J* = 8.9 Hz, H-Ar), 7.43-7.48 (1H, m, H-Ar), 7.43-7.48 (2H, m, CH-pyridine), 7.50 (1H, s, H-3), 7.61-7.63 (2H, m, CH-pyridine), 7.92 (1H, s, H-5), 8.06 (1H, d, J = 8.1 Hz, CH-pyridone), 8.41 (1H, br s, NH-pyridone), 8.74 (1H, br s, NH); ^{19}F NMR (470 MHz, MeOD) δ -115.4; LRMS (ES^+) *m/z* 368.1 $[M+H]^+$.

3-(2-Bromo-6-fluorobenzoyl)-6-(pyridin-3-yl)-1*H*-pyrrolo[3,2-b]pyridin-7(4*H*)-one (374)



2-Bromo-6-fluorobenzoic acid (1.70 g, 7.76 mmol) was dissolved in THF (12 mL) before $SOCl_2$ (1.70 mL, 23.3 mmol) and DMF (70 µL, 0.77 mmol) were added. The mixture was stirred at RT for 3 h before the solvent was removed *in vacuo.* The residue was re-dissolved in DCM (8 mL) and $AlCl_3$ (1.14 g, 8.54 mmol) was added followed by 7-methoxy-6-(pyridin-3-yl)-1*H*-pyrrolo[3,2-*b*]pyridine (**358**) (350 mg, 1.54 mmol) and the reaction was carried out as for general procedure F, (part ii) DCM (8 mL), TMSI (379 µL, 2.68 mmol). The crude product was purified by semi-preparative HPLC (C-18 silica; 5-100% CH_3CN in water (0.1% formic acid)) to give the pure product as a white solid (35 mg, 0.09 mmol, 19%); R_f = 0.41 (5% MeOH in EtOAc); M.p. 165-166 ^oC; λ_{max} (EtOH)/nm 253, 320; ν_{max}/cm^{-1} 3029, 2903, 2699, 1596 (CO),1555 (CO-pyridone) ,; ^1H NMR (500 MHz, DMSO-d_6) δ 7.43-7.46 (1H, m, H-3'), 7.54 (1H, ddd, *J* = 6.2, 8.4 and 8.5 Hz, H-4'), 7.63 (1H, d, *J* = 8.1 Hz, H-5'), 7.65 (1H, s, H-5), 7.79 (2H, d, *J* = 6.4 Hz, CH-pyridine), 7.96 (1H, s, CH-pyridone), 8.57 (2H, d, *J* = 6.4 Hz, CH-pyridine); ^{19}F NMR (470 MHz, DMSO-d_6) δ -113.4; LRMS (ES^+) *m/z* 414.2 $[M+H]^+$.

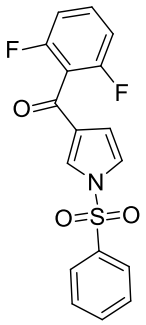
2,5-Dioxopyrrolidin-1-yl 4-(2,6-difluorobenzoyl)-1*H*-pyrrole-2-carboxylate (375)



4-(2,6-Difluorobenzoyl)-1*H*-pyrrole-2-carboxylic acid (**75**) (100 mg, 0.40 mmol) was dissolved in EtOAc (5 mL) at 0 ^oC in an ice bath. DCC (91 mg, 0.44 mmol) was then added followed by *N*-hydroxysuccinimide (51 mg, 0.44 mmol). The resulting yellow mixture was left to stir at 0 ^oC for 2 h before being warmed to RT and left to stir overnight. The cream suspension was filtered through a pad of Celite and the filtrate concentrated *in vacuo* to give the title compound as a pale orange gum (137 mg, 99%); R_f = 0.25 (50% MeOH in EtOAc); λ_{max} (EtOH)/nm 329, 283; ν_{max}/cm^{-1} 3606, 3102, 2933, 2646, 1724 (CO); ^1H NMR (500 MHz, DMSO-d_6) δ 2.80 (4H, br s, $(CH_2)_2$), 7.17-7.21 (2 H, m, H-3' and H-5'), 7.29 (1 H, br s, H-3), 7.54-7.57 (1 H, m, H-4'), 7.83 (

1H, br s, H-5), 13.41 (1 H, br s, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 25.5 (2 x CH_2), 114.6 (C-Ar), 118.1 (C-Ar), 118.8 (C-3), 124.5 (C-2 and C-5), 127.4 (C-4), 133.1 (C-Ar), 155.5 (d, J_{CF} = 257.3 Hz, CF), 170.4 (2 x CO - succinimide), 172.7 (CO_2N), 186.1 (CO); ^{19}F NMR (470 MHz, DMSO-d_6) δ -114.2; LRMS (ES^+) *m/z* 349.2 $[M+H]^+$; HRMS *m/z* calcd for $C_{16}H_{11}F_2N_2O_5$ $[M+H]^+$ 349.0632, found 349.0632.

(2,6-Difluorophenyl)(1-(phenylsulfonyl)-1*H*-pyrrol-3-yl)methanone (376)



1-(Phenylsulfonyl)pyrrole (100 mg, 0.48 mmol) was dissolved in DCM (3 mL) at 0 ^{o}C. $AlCl_3$ (70 mg, 0.53 mmol) was added followed by 2,6-difluorobenzoyl chloride (93 µL, 0.53 mmol) before the reaction was allowed to warm to RT overnight. The reaction mixture was quenched with water followed by a 1.0M aqueous solution of HCl until effervescence ceased before being extracted with DCM (3 x 5 mL). The combined organic extracts were washed with a saturated aqueous solution of $NaHCO_3$ followed by water and a saturated brine solution (2 x 5 mL respectively) before being dried over Na_2SO_4 and the solvent removed *in vacuo*. The crude product was purified using MPLC (silica, 2-100% EtOAc in petrol) to give the title compound as a beige solid (100 mg, , 60%); R_f = 0.81 (10% MeOH in EtOAc); M.p.: 101-102 ^{o}C; λ_{max} (EtOH)/nm: 246; ν_{max}/cm^{-1}: 3645, 3303, 3133, 1657 (CO), 1624; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.69 (1H, dd, *J* = 1.6 and 3.4 Hz, H-3), 6.89-6.95 (2H, m, H-3' and H-5'), 7.09 (1H, dd, *J* = 2.2 and 3.4 Hz, H-2), 7.36 (1H, dddd, *J* = 6.3, 6.4, 8.2 and 8.3 Hz, H-4'), 7.48-7.52 (3H, m, H-5 and H-Ar), 7.58-7.62 (1H, m, H-Ar), 7.81-7.83 (2H, m, H-Ar); ^{13}C NMR (125 MHz, MeOD) δ 111.9 (C-3), 112.9 (C-Ar), 117.5 (C-4), 122.0 (C-5 and C-2), 127.2 (C-Ar), 128.1 (C-Ar), 129.4 (C-Ar), 131.9 (C-Ar), 134.8 (C-Ar), 137.9 (C-Ar), 160.6 (C-Ar), 182.3 (CO); ^{19}F NMR (470 MHz, $CDCl_3$) δ -112.0; LRMS (ES^+) *m/z* 348.20 $[M+H]^+$.

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