Design, Synthesis and Biological Evaluation of Small-Molecules Targeting the Mdmx-p53 Interaction and the Atad2 Bromodomain for Cancer Therapy

This thesis is submitted to Newcastle University for the degree of Doctor of Philosophy

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Declaration

The work described in this thesis was carried out between September 2012 and November 2015 in the Northern Institute for Cancer Research Medicinal Chemistry Laboratories (Bedson Building, School of Chemistry, Newcastle University, Newcastle upon Tyne, UK, NE1 7RU) and in the Cancer Structural Biology Laboratories (Paul O’Gorman Building, Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, UK, NE2 4HH).

All the research described in this thesis is original and does not incorporate any material or ideas previously published or presented by other authors, except where acknowledged by references.

No part of this thesis is being, or has been previously, submitted for a degree, diploma or any qualification at any other university.
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I would like to dedicate this thesis to my parents Chhabilal Adhikari and Kamala Adhikari.
Abstract

In this thesis small molecule inhibitors of MDMX-p53 interaction, and ATAD2 bromodomain are investigated.

In many human cancers, the function of the tumour suppressor protein p53 is inhibited by the overexpression of MDM2 or MDMX. Modulation of MDM2-p53 and MDMX-p53 interactions is therefore an attractive strategy for anticancer drug discovery. A number of small molecule inhibitors of the MDM2-p53 interaction have been reported to date and several have entered clinical trials. Although MDM2 and MDMX have a high sequence homology, most of the small molecule MDM2 inhibitors show significantly lower binding affinity towards MDMX. A series of 2,4-aminothiazoles including compound 1 with modest inhibitory activity against the MDM2-p53 and MDMX-p53 interactions has been reported. The series was extended to a pyrrole series, which led to the discovery of compound 2 with low micromolar dual inhibition of MDM2/MDMX and structure-activity relationship studies were conducted.

An ELISA was used to examine potency against MDM2-p53 and MDMX-p53. Although, the assay gave results in agreement with literature values for some inhibitors of the MDM2-p53 interaction, the potencies of the published dual inhibitors RO-2443 3 and RO-5963 4 were around 1000-fold lower than reported. Therefore, an HTRF assay was developed, which provided IC\textsubscript{50} values comparable to the reported values for inhibition of MDM2-p53 and MDMX-p53 by RO-5963. Co-crystallisation experiments, using three different constructs of MDMX, were attempted using 15 compounds.
Bromodomains are protein modules that function as epigenetic readers of histone lysine acetylation. ATAD2, a bromodomain containing protein, is overexpressed in a wide range of human cancers including breast, lung, prostate, ovarian, liver, osteosarcoma and gastrointestinal carcinomas. Due to the polar and flexible nature of the binding surface, ATAD2 has been considered as a challenging target for ligand discovery. Therefore, very few potent and selective inhibitors of ATAD2 bromodomains have been reported to date.

Extensive SAR studies around hit 5 obtained from a fragment screening led to the identification of a sub-millimolar inhibitor 6 of ATAD2 bromodomain. Co-crystal structures of ATAD2 were used to guide compound design and synthesis.

5, ATAD2 IC$_{50}$ > 4000 µM  
6, ATAD2 IC$_{50}$ = 209 ± 26 µM
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<tbody>
<tr>
<td>ANCAA</td>
<td>AAA nuclear co-regulator cancer-associated protein</td>
</tr>
<tr>
<td>AID</td>
<td>Auto-inhibitory domain</td>
</tr>
<tr>
<td>BET</td>
<td>Bromodomain and extra-terminal</td>
</tr>
<tr>
<td>CML</td>
<td>Chronic myelogenous leukaemia</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
</tr>
<tr>
<td>DIBAL</td>
<td>Diisobutylaluminium hydride</td>
</tr>
<tr>
<td>DIC</td>
<td>$N,N'$-Diisopropylcarbodiimide</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine</td>
</tr>
<tr>
<td>DME</td>
<td>1,2-Dimethoxyethane</td>
</tr>
<tr>
<td>DMF</td>
<td>Dimethylformamide</td>
</tr>
<tr>
<td>DMP</td>
<td>Dess-Martin periodinane</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide</td>
</tr>
<tr>
<td>DTT</td>
<td>Dithiothreitol</td>
</tr>
<tr>
<td>EDC</td>
<td>1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide</td>
</tr>
<tr>
<td>ELISA</td>
<td>Enzyme linked immunosorbent assay</td>
</tr>
<tr>
<td>ELSD</td>
<td>Evaporative light scattering detector</td>
</tr>
<tr>
<td>ER</td>
<td>Estrogen receptor</td>
</tr>
<tr>
<td>(ES+)</td>
<td>Electrospray positive mode</td>
</tr>
<tr>
<td>(ES-)</td>
<td>Electrospray negative mode</td>
</tr>
<tr>
<td>EtOH</td>
<td>Ethanol</td>
</tr>
<tr>
<td>eq.</td>
<td>Number of molar equivalents</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and drug administration</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>FRET</td>
<td>Fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>FTIR</td>
<td>Fourier transform infrared</td>
</tr>
<tr>
<td>GIST</td>
<td>Gastrointestinal stromal tumour</td>
</tr>
<tr>
<td>GST</td>
<td>Glutathione S-transferase</td>
</tr>
<tr>
<td>HATU</td>
<td>1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate</td>
</tr>
<tr>
<td>HAT</td>
<td>Histone-acetyltransferase</td>
</tr>
<tr>
<td>HDAC</td>
<td>Histone deacetylase</td>
</tr>
<tr>
<td>HEPES</td>
<td>N-2-Hydroxyethylpiperazine-N’-2-ethanesulfonic acid</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HRMS</td>
<td>High-resolution mass spectrometry</td>
</tr>
<tr>
<td>HTRF</td>
<td>Homogenous time-resolved FRET</td>
</tr>
<tr>
<td>HTS</td>
<td>High-throughput screening</td>
</tr>
<tr>
<td>IPTG</td>
<td>Isopropyl β-D-1-thiogalactopyranoside</td>
</tr>
<tr>
<td>Kac</td>
<td>Acetyllysine</td>
</tr>
<tr>
<td>KHMDS</td>
<td>Potassium bis(trimethylsilyl)amide</td>
</tr>
<tr>
<td>LC-MS</td>
<td>Liquid chromatography-mass spectrometry</td>
</tr>
<tr>
<td>LE</td>
<td>Ligand efficiency</td>
</tr>
<tr>
<td>LHMDS</td>
<td>Lithium bis(trimethylsilyl)amide</td>
</tr>
<tr>
<td>µW</td>
<td>Microwave</td>
</tr>
<tr>
<td>MDM2</td>
<td>Murine double minute 2</td>
</tr>
<tr>
<td>MDMX</td>
<td>Murine doule minute X</td>
</tr>
<tr>
<td>MeCN</td>
<td>Acetonitrile</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
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<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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</tr>
<tr>
<td>mHBS</td>
<td>Modified HEPES-buffered saline</td>
</tr>
<tr>
<td>MOE®</td>
<td>Molecular operating environment</td>
</tr>
<tr>
<td>MPLC</td>
<td>Medium pressure liquid chromatography</td>
</tr>
<tr>
<td>m.p.</td>
<td>Melting point</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>NOE</td>
<td>Nuclear overhauser effect</td>
</tr>
<tr>
<td>NUT</td>
<td>Nuclear protein in Testis</td>
</tr>
<tr>
<td>LB</td>
<td>Luria-Bertani broth</td>
</tr>
<tr>
<td>PCC</td>
<td>Pyridinium chlorochromate</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PDGFR</td>
<td>Platelet derived growth factor receptor</td>
</tr>
<tr>
<td>PTM</td>
<td>Post-translational modification</td>
</tr>
<tr>
<td>quant.</td>
<td>Quantitative</td>
</tr>
<tr>
<td>N/A</td>
<td>Not available</td>
</tr>
<tr>
<td>NaHMDS</td>
<td>Sodium bis(trimethylsilyl)amide</td>
</tr>
<tr>
<td>NES</td>
<td>Nuclear export sequence</td>
</tr>
<tr>
<td>NLS</td>
<td>Nuclear localization signal</td>
</tr>
<tr>
<td>PCNA</td>
<td>Proliferating cell nuclear antigen</td>
</tr>
<tr>
<td>PPI</td>
<td>Protein-protein interaction</td>
</tr>
<tr>
<td>RING</td>
<td>Really interesting new gene</td>
</tr>
<tr>
<td>Rb</td>
<td>Retinoblastoma</td>
</tr>
<tr>
<td>RT</td>
<td>Room temperature</td>
</tr>
<tr>
<td>Rt</td>
<td>Retention time (HPLC)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
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</tr>
<tr>
<td>SAM</td>
<td>S-adenosylmethionine</td>
</tr>
<tr>
<td>SAR</td>
<td>Structure-activity relationship</td>
</tr>
<tr>
<td>SDS-PAGE</td>
<td>Dodecyl sulphate polyacrylamide gel electrophoresis</td>
</tr>
<tr>
<td>SEM</td>
<td>2-Trimethylsilylethoxyethylmethyl</td>
</tr>
<tr>
<td>TAD</td>
<td>Transactivation domain</td>
</tr>
<tr>
<td>TB</td>
<td>Terrific broth</td>
</tr>
<tr>
<td>TBS</td>
<td>Tert-butyldimethylsilyl</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>TMSCN</td>
<td>Trimethylsilyl cyanide</td>
</tr>
<tr>
<td>TR-FRET</td>
<td>Time-resolved fluorescence resonance energy transfer</td>
</tr>
<tr>
<td>vmax</td>
<td>Maximum vibrational frequency</td>
</tr>
<tr>
<td>v/v</td>
<td>Volume for volume</td>
</tr>
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</table>
Chapter 1. Introduction

1.1 Cancer

Cancer is a genetic disease caused by alterations in the genome such as point mutations, deletions, duplications, chromosome rearrangements and epigenetic changes. The number of genetic alterations in a cancer can vary from tens to thousands. A large number of mutations are known as ‘passengers’ that do not confer any growth advantage. The mutations that contribute to cancer development are known as ‘drivers’. A vast amount of research on the identification of ‘drivers’ has led to the discovery of various oncogenes and tumour suppressor genes. Oncogenes are dominant cancer genes that require only one of the two parental alleles present in the normal cell to be mutated, resulting in the activation of the encoded protein. Tumour suppressor genes are recessive genes that require mutations in both alleles resulting in the inactivation of the encoded protein.

Cancer was first described as an evolutionary process analogous to Darwinian natural selection by Peter Nowell almost forty years ago. Genetic variations that confer growth advantage lead to the progression of normal cells into cancer cells. An alternative to the Darwinian evolutionary model is the stem cell model, which proposes that a small population of cancer stem cells, irrespective of their origin, are responsible for cancer growth and progression. The stem cell model considers that a few sub-populations of cancer cells are tumorigenic, and responsible for cancer growth. The stem cell model and the evolutionary model are not mutually exclusive in cancers that follow stem cell model, in which stem cells can evolve by clonal evolution. Whereas, in cancers that do not follow stem cell model, the evolutionary model could explain the cancer growth. A better understanding of these models involved in various cancers will help to develop effective treatments.

Cancer research over the past few decades has led to the identification of a large number of phenotypic and genotypic variations involved in cancer. In 2000, Hanahan and Weinberg proposed six hallmarks of cancer that comprised of physiological changes or capabilities acquired during cancer development (Figure 1.1). In an update in 2011, two new hallmarks were added: the avoidance of immune destruction and the reprogramming of energy metabolism.
1.2 Cancer Chemotherapy

The era of chemotherapy began with the use of nitrogen mustards, including chlormethine 7, for the treatment of lymphomas.¹¹ These agents act by cross-linking DNA. Several other DNA-alkylating agents were subsequently introduced including chlorambucil 8 and cyclophosphamide 9.

Over the next few decades, several chemotherapeutic agents including antimetabolites: 6-mercaptopurine 10, 5-fluorouracil 11, methotrexate 12; antitumour antibiotics: doxorubicin; and antimitotic agents: Vinca alkaloids, taxanes were discovered.¹¹-¹² Antimetabolites mimic nitrogenous bases of DNA or other endogenous molecules and inhibit the synthesis of nucleic acids. Anti-mitotic agents act by inhibiting cell division of rapidly dividing cells. Conventional chemotherapy acts on the processes that contribute in the rapid division of cancer cells. However, many normal cells such as hair follicles, stomach epithelia, and bone-marrow cells are also rapidly replicating.¹³ Therefore, conventional chemotherapeutic agents also act on rapidly dividing non-cancerous cells causing serious side effects.
With the increase in the understanding of cancer biology at a molecular level, anti-cancer drug discovery transitioned from classical cytotoxic agents to targeted therapy. The new targets specific to cancer cells such as growth factors, signalling pathways, oncogenes, tumour suppressor genes, cell cycle proteins, apoptosis modulators and angiogenesis promoters were discovered. Imatinib (Glivec) 13, a landmark anticancer drug (based on targeted therapy) is used for the treatment of chronic myeloid leukaemia (CML).\textsuperscript{14-15} Imatinib acts by inhibiting Bcr-Abl kinase which is a product of \textit{bcr-abl} gene, formed by the fusion of a segment of the \textit{bcr} gene from chromosome 22 to the part of \textit{c-abl} gene on chromosome 9. Imatinib also inhibits c-KIT kinase and platelet derived growth factor receptor (PDGFR) kinase that are overexpressed in Gastrointestinal Stromal Tumour (GIST).\textsuperscript{16} Therefore, Imatinib is also used in the treatment of GIST.

The clinical success of Imatinib was a breakthrough in the field of targeted anticancer therapy. To date, more than 30 small-molecules have been approved, and several are in various stages of clinical trials, as targeted therapy for the treatment of variety of cancers.\textsuperscript{17}

1.3 Protein-Protein Interaction

Protein-protein interactions play an important role in most biological processes ranging from signal transduction to programmed cell death.\textsuperscript{18} Development of small molecules that modulate
protein-protein interactions involved in disease pathways has become an attractive strategy for drug discovery. However, there are several challenges in the discovery of small molecules that inhibit protein-protein interaction.

Protein-protein interfaces are generally flat with large surface areas around 1600 (± 400) Å² on each protein.¹⁹ There are numerous interactions including electrostatic, hydrophobic, hydrogen bonds, involved in protein-protein interaction. It is not possible to interfere with all of the interactions using small molecules that typically have a very small surface area (300-500 Å²) compared to the protein-protein interface.²⁰ Modulation of protein-protein interactions by small molecules can be successful by targeting ‘hot spots’ in the protein-protein interaction.¹⁸ Hot spots are the regions of key interactions at the protein-protein interface that contribute to the large component of the binding affinity. Therefore, even a molecule with a small surface area that can bind at hot spots can inhibit protein-protein interactions. For example, the p53-MDM2 interface area is approximately 1500 Å², whereas, the MDM2 inhibitor Nutlin 3a ¹⁴ has a surface area of approximately 800 Å².²¹-²² It is clear that Nutlin cannot cover the entire surface area; however, it is a strong inhibitor of the MDM2-p53 interaction, which suggests that interfering with all the interactions is not necessary.

![Nutlin 3a 14](image)

The other important examples of small molecule inhibitors of PPIs are inhibitors of Bcl-2 family proteins. These proteins play a major role in the regulation of apoptosis and are divided into three classes of proteins.²³

- The pro-apoptotic proteins Bax and Bak, which stimulates apoptosis.
- The anti-apoptotic proteins Bcl-2 and Bcl-xL, which inhibit the function of Bax and Bak.
- The BH3 only protein which inhibits Bcl-2 and Bcl-xL.

The Bcl-2 and Bcl-xL proteins contain a hydrophobic groove upon which Bax, Bak and BH3 only proteins bind.²⁴-²⁵ Bcl-2 and Bcl-xL are upregulated in cancer cells, and therefore, small
molecule inhibitors that can bind on the hydrophobic groove of the Bcl-2 and Bcl-xL proteins can induce apoptosis.\textsuperscript{23, 26}

To date, several small molecule inhibitors have been identified for inhibiting protein-protein interactions. Some of the small-molecule PPI inhibitors which are in various stages of clinical development are shown in Figure 1.2.\textsuperscript{20, 27}

\textbf{Figure 1.2}: Small molecule PPI inhibitors under clinical development.\textsuperscript{27}

Although, binding at hot spots is often sufficient for inhibiting protein-protein interactions, identifying a hot spot is another challenge. Binding sites that could fit small molecules are sometimes not visible in crystal structures due to the flexibility and adaptivity of proteins.\textsuperscript{18}

The drug leads developed by targeting the protein-protein interfaces often have high molecular weight and are hydrophobic. Inhibition of the protein-protein interaction is not enough for compound to become a drug. Small molecule PPI inhibitors should also possess drug-like
pharmacokinetic and pharmacodynamics properties. Therefore, besides being an attractive area for drug discovery, the development of small molecule inhibitors that can modulate protein-protein interactions has several challenges.

In this thesis, development of small molecules targeting two different protein-protein interactions are discussed: (a) MDMX-p53 interaction; (b) ATAD2-acetylated histone interaction.

### 1.4 Stages of Drug Discovery

The first stage in the drug discovery process involves the identification of a biological target, whose modulation might affect the disease progression (Figure 1.3). The target is then validated using data collected from bioinformatics, as well as several *in vivo* and *in vitro* experiments including genetically engineered knock-out animals, reducing the amount of target by short interfering siRNA experiments, or the target is modulated by a known small molecule or peptide inhibitor.28-30

Once a biological target is validated, a full drug discovery program can be commenced. The first step is the identification of a chemical starting point known as a ‘hit’, which is performed using various techniques like high-throughput screening (HTS), fragment screening, virtual screening, or structure based *de novo* design.31-34

In the hit to lead phase, structure-activity relationships around the hit are studied in-order to identify the effect of different groups in a molecule on the potency, selectivity and pharmacokinetic properties.34 A lead molecule, which has the potential to be developed as a drug is selected. In the lead optimisation stage, analogues of leads are synthesised by combining all of the desired properties in a molecule to achieve optimum potency, *in-vivo* pharmacokinetics, safety and pharmaceutical properties.34 Then, a candidate is selected for pre-clinical evaluation.
The two projects described in this thesis are in the target validation stage. The aim of the medicinal chemistry efforts was to develop chemical tools for use in \textit{in vitro} and \textit{in vivo} experiments to access the effect of inhibition of two protein-protein interactions: (a) MDMX-p53; (b) ATAD2 bromodomain-acetylated histone.
Chapter 2. Introduction to MDMX and Reported Inhibitors

2.1 p53, MDM2, and MDMX

The tumour suppressor gene p53 is mutated in almost half of all human cancers.\textsuperscript{35} In cancers with wild-type p53, the protein levels of p53 is controlled by its negative regulators, such as MDM2 and MDMX.\textsuperscript{36} p53 functions as a transcription factor responsible for the regulation of expression of a variety of genes involved in various stages of cell growth and progression.\textsuperscript{37} p53 is activated by DNA damage, abnormal growth signals, oncogene activation and other cellular stress, including hypoxia and nucleotide depletion (Figure 2.1).\textsuperscript{37-38} Following activation, tetrameric p53 binds to DNA,\textsuperscript{39} and mediates the transcription of genes that express proteins to accomplish a number of functions including cell cycle arrest, DNA repair, apoptosis and inhibition of angiogenesis.\textsuperscript{40} These responses either reverse potential mutagenic damage by cell cycle arrest and DNA repair, or signal the destruction of the damaged cells by apoptosis.\textsuperscript{37-38, 40-41}

![Figure 2.1: Upstream activators (blue) and downstream cellular effects (red) of p53.\textsuperscript{37}](image)

2.1.1 Cell Cycle Arrest Mediated by p53

During G\textsubscript{1} to S phase transition of the cell cycle, four proteins, p16, cyclin D\textsubscript{1}, CDK4 and Rb (Retinoblastoma protein) play a central role.\textsuperscript{42} p16 negatively regulates cyclin D\textsubscript{1} and CDK4 which activate Rb (Figure 2.2).\textsuperscript{42} After being activated by cyclin D\textsubscript{1} and CDK4, Rb protein releases several transcription factors responsible for the G\textsubscript{1} to S phase transition. In most
cancers, one of the four genes encoding for these four proteins is mutated. In response to upstream stress, p53 induces transcription of the p21 gene which encodes for p21 protein. The p21 protein then binds to and inhibits several cyclin and CDK complexes, which inhibit progression of the cell cycle to S phase causing cell cycle arrest. The p21 protein is also involved in inhibiting DNA replication by binding with PCNA (Proliferating Cell Nuclear Antigen).

![Figure 2.2: p53 mediated cell cycle arrest.](image)

### 2.1.2 Apoptosis Mediated by p53

Apoptosis is a type of programmed cell death and is mediated by two main pathways, the intrinsic pathway and extrinsic pathway. The extrinsic pathway is mediated by cell surface receptors, whereas, the intrinsic pathway is initiated by stress signals. The p53 protein is involved in induction of the proteins in both pathways, predominantly the intrinsic pathway. BAX and BH3-only proteins are apoptosis promoters involved in the intrinsic pathway, whereas, Bcl2 and Bcl-xL proteins are anti-apoptotic proteins. The p53 protein induces apoptosis by transcriptionally regulating the genes encoding for apoptotic and anti-apoptotic proteins.
2.1.3 The Structure of p53 Protein

Human p53 contains 393 amino acid residues divided into five domains (Figure 2.3).\textsuperscript{45-46}

![Relative frequency of missense mutation in human cancer](image)

**Figure 2.3:** Schematic representation of p53 protein domains. The black columns indicate the mutational hotspots.\textsuperscript{46}

(a) Transactivation domain: This domain is situated in the N-terminal region from residues 1 to 62 and can be divided into sub-domains TAD 1 and TAD 2.\textsuperscript{46} The transactivation domain interacts with a number of regulators of p53 including MDM2 and MDMX.\textsuperscript{47}

(b) Proline-rich domain: As the name suggests, this domain (residues 63-94) contains repeated proline residues. There are five repetitive sequence PXXP (P= Proline, X= any amino acid).\textsuperscript{46} Although this domain is not essential for transcriptional activity, deletion from p53 inhibits p53’s ability to reduce tumour cell growth in culture.\textsuperscript{48}

(c) DNA binding domain: The DNA binding domain (residues 94-292) is the central core domain and contains mutational hotspots. This domain binds to DNA response elements that contain the repetitive sequence 5’-Pu-Pu-Pu-C-(A/T)-(T/A)-G-Py-Py-Py-3’ (Pu represents A/G, Py represents T/C).\textsuperscript{46} p53 forms a tetramer and other domains also cooperate in binding at these DNA response elements.\textsuperscript{39}

(d) Tetramerization domain: This is present in the C-terminal region, residues 325-356, and regulates the formation of p53 tetramer.\textsuperscript{46}

(e) Auto-regulatory domain: This is present in the C-terminal region, residues 356-393. This domain contains lysine residues that get acetylated by histone acetyltransferase.
p300/CBP. Acetylation plays critical role in DNA-binding to the central core domain, and is also important in the transcription activity of p53.

2.2 MDM2

MDM2, a negative regulator of p53, induces proteasomal degradation of p53 by modifying the carboxyl terminus via ubiquitination (Figure 2.4). MDM2 contains a RING (Really Interesting New Gene) domain in the C-terminal with an E3 ubiquitin ligase activity important for proteasomal degradation of p53. MDM2 has ubiquitin ligase activity following homodimerization as well as hetero-dimerization with MDMX. In addition, MDM2 binds at the transactivation domain of p53 and inhibits its transcriptional activity. MDM2 contains nuclear export signal sequence that binds to p53, and exports it to the cytoplasm. The transcriptional activity of p53 can only occur inside the nucleus, therefore, p53 can no longer act as a transcription factor.

Figure 2.4: Regulation of p53 by MDM2 and MDMX.

MDM2 is a transcriptional target of p53; therefore, p53 increases the amount of MDM2, which in turn degrades p53. As a result, the level of p53 decreases which reduces the production of MDM2 that causes an increase in p53 activity forming an auto-regulatory feedback loop.
Inhibition of MDM2-p53 interaction is a well validated target, and several inhibitors (such as 17, 18, 19 and 20) are currently under clinical evaluation.\textsuperscript{36, 60-61} However, these compounds do not inhibit MDMX-p53 interactions and therefore, fail to restore p53 activities in cancers overexpressing MDMX.\textsuperscript{60}

\begin{center}
\begin{tabular}{ll}
AMG-232 17 & RG-7388 18 \\
RG7112 19 & MI-77301 20
\end{tabular}
\end{center}

\subsection*{2.3 MDMX and its Role in Cancer}

In order to identify new cellular proteins that regulate p53 activity, Shvarts \textit{et al.} screened a murine cDNA expression library with a radioactively labelled p53.\textsuperscript{62} A cDNA clone encoding MDMX was identified that had structural homology with MDM2. The human form, HDMX was identified by the same group a year later in 1997.\textsuperscript{63}

Like MDM2, MDMX also has a RING domain at its C-terminus but lacks intrinsic ubiquitin ligase activity. However, MDMX can mediate MDM2 ubiquitin ligase activity by hetero-oligomerisation with MDM2.\textsuperscript{53} MDMX acts as a negative regulator of p53 primarily due to the inhibition of the transcriptional activity of p53\textsuperscript{62}. 
MDMX is overexpressed in 15% of human cancers. Elevated levels of *MDMX* mRNA are found in 65% of retinoblastoma, 65% of cutaneous melanoma, 19% of breast carcinoma, 19% of colon carcinoma and 18% of lung carcinoma (Table 2.1).65-67

**Table 2.1:** Analysis of primary tumour samples to study MDMX overexpression.65-67

<table>
<thead>
<tr>
<th>Tumour Samples</th>
<th>Total Analysed</th>
<th>MDMX overexpressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinoblastoma</td>
<td>49</td>
<td>32</td>
</tr>
<tr>
<td>Breast carcinoma</td>
<td>218</td>
<td>41</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>27</td>
<td>5</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>88</td>
<td>16</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>25</td>
<td>0</td>
</tr>
<tr>
<td>Stomach carcinoma</td>
<td>14</td>
<td>6</td>
</tr>
<tr>
<td>Testis carcinoma</td>
<td>11</td>
<td>3</td>
</tr>
<tr>
<td>Larynx carcinoma</td>
<td>13</td>
<td>3</td>
</tr>
<tr>
<td>Uterus carcinoma</td>
<td>13</td>
<td>2</td>
</tr>
<tr>
<td>Melanoma</td>
<td>14</td>
<td>2</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

The deletion of *MDMX* in mice, despite having *MDM2*, causes embryonic lethality that can be rescued by loss of *Trp53*.68-69 This indicates that MDMX is a critical negative regulator of p53. The potent MDM2 inhibitor Nutlin 3 is inactive against MDMX-p53 interaction. Nutlin 3 failed to restore p53 activity in cancer cells overexpressing MDMX.70 A combination of Nutlin with MDMX-siRNA increases p53 activity which suggests that inhibition of MDMX is also an attractive approach for restoring p53 activity.70 It has been reported that dual inhibition of MDM2 and MDMX can increase the p53 activity more than the inhibitors of only MDM2-p53 interaction in cancer cells containing wild-type p53.71-72 Therefore, in order to achieve complete reactivation of p53 in cancers containing wild-type p53, inhibition of both MDM2 as well as MDMX is essential.
MDMX, independent of p53 and MDM2, is responsible for influencing genomic stability.\textsuperscript{64, 73} The overexpression of MDMX delayed DNA damage response signals and double strand DNA break repair.\textsuperscript{64}

### 2.4 Structure of MDMX: Comparison with MDM2

MDMX, containing 490 amino acids is structurally similar to MDM2 with 491 amino acids (Figure 2.5).\textsuperscript{74} MDMX and MDM2 have a very similar N-terminal p53 binding domain with 54% sequence homology. MDMX lacks nuclear export sequence (NES) and nuclear localisation signal (NLS) to travel in and out from the nucleus.\textsuperscript{75}

![Domain structure of MDM2 and MDMX highlighting different domains. NLS: Nuclear Localisation Signal; NES: Nuclear Export Sequence. (Adapted from Lee et al)\textsuperscript{75}](https://example.com)

N-terminal p53 binding domain of both MDM2 and MDMX have hydrophobic binding pockets into which the side-chains of three residues of p53; Phe19, Trp23 and Leu26 bind (Figure 2.6A/B).\textsuperscript{21, 76} The Trp23 nitrogen forms an H-bond with Leu54 in MDM2 (Figure 2.6 C), whereas, with Met53 in MDMX (Figure 2.6 D). The Leu26 pocket in MDMX is smaller and less-defined compared to MDM2 (Figure 2.6 E/F), and it is considered as the main hurdle to discovering a small-molecule inhibitor of MDMX-p53 interaction.\textsuperscript{77}
Figure 2.6: Crystal structure of p53-peptide (green) in complex with (A) MDM2, and (B) MDMX. The H-bond interaction between the nitrogen of Trp23 with (C) MDM2, and (D) MDMX. The p53 binding surface of (E) MDM2, and (F) MDMX. The surface and secondary structures of both MDM2 and MDMX are coloured cyan.
2.5 Reported Inhibitors of the MDMX-p53 Interaction

2.5.1 Peptides and Stapled Peptides

Using structure-based design and phage display methods several peptide and peptidomimetics with nanomolar inhibitory activity against MDMX-p53 interactions have been identified.\textsuperscript{78-79} Despite having high potency against MDMX, peptides are less likely to become cancer therapeutics because of their instability \textit{in vivo} and poor pharmacokinetic properties.\textsuperscript{80} SAH-p53-8 is a stapled peptide with improved stability and pharmacokinetic properties (Figure 2.7).\textsuperscript{81} SAH-p53-8 acts as a dual inhibitor of MDM2-p53 and MDMX-p53 interactions \textit{in vitro}. S20 and P27 residues in WT-p53 were replaced with synthetic olefinic residues, which were stapled using olefin metathesis. SAH-p53-8 showed nanomolar inhibitory activity against both MDM2- and MDMX-p53 interactions. The WT-p53 and mutant SAH-p53-8\textsubscript{F19A} did not show any inhibition of MDMX at that dose range. However, SAH-p53-8 requires Nutlin-3a 14 for optimal inhibition of MDM2-p53 interactions in cells.\textsuperscript{81}

![Figure 2.7: Composition of wild-type p53, SAH-p53-8 and SAH-p53-8\textsubscript{F19A} peptides. The olefinic residues used for stapling are shown by stars.\textsuperscript{81}]

A stapled peptide ATSP-7041, which demonstrated p53 dependent tumour growth suppression in MDM2 or MDMX-overexpressing xenograft models was reported in 2013.\textsuperscript{72} ATSP-7041 binds with MDM2 and MDMX with nanomolar affinities \textit{in vitro} and demonstrates sub-micromolar activities in cancer cells overexpressing MDM2 and MDMX.\textsuperscript{72} The stapled peptide also demonstrated favourable pharmacokinetic properties when tested \textit{in vivo} in rat, mouse, and monkey.
Figure 2.8: Sequences of a series of stapled peptides and their potency against MDM2 and MDMX. R8 and S5 are the olefinic residues stapled using olefin metathesis.\textsuperscript{72}

The crystal structure of ATSP-7041 in complex with MDMX (15-106) showed that Phe19, Trp23 and Cba26 were bound on the three hydrophobic pockets of MDMX (Figure 2.9).\textsuperscript{72} The Tyr\textsuperscript{22} of ATSP-7041 forms a water-mediated H-bond with His68 of MDMX. The staple group occupies a binding pocket on the surface of MDMX formed by Lys47, Met50, Gly54, Gln55 Ile57, and Met58.
Figure 2.9: Crystal structure of ATSP-7041 (green) bound to MDMX (PDB: 4N5T). The MDMX surface is coloured based on the charge of amino acid residues: blue: positive; red: negative.

2.5.2 SJ-172550

SJ-172550 21 was the first small-molecule inhibitor of MDMX discovered from the high-throughput screening of 295,848 unique compounds.82 It increased p53 activity in MDMX-amplified retinoblastoma cells. However, further studies on the compound showed that it forms a covalent adduct with cysteine residues of MDM2 and MDMX at the p53 binding domain.83 Due to the reactivity of SJ-172550 with thiols, further development was discontinued.
**2.5.3 Imidazole-Indoles**

Imidazole-indoles are the class of MDM2-p53 interaction inhibitors developed by Novartis.\(^84\)-\(^85\) WK298 22, an imidazole-indole is the first small-molecule whose crystal structure in complex with MDMX was solved (Figure 2.10).\(^86\) The chloro-indole substituent binds at the Trp23 pocket. The nitrogen atom of the indole forms a hydrogen bond with Met53 in MDMX. The chlorine atom in the indole ring induces a conformational change in key residues including Leu54, Met53, Leu 98 and Leu102, which helps in enlargement of the central Trp23 binding pocket. The phenyl ring occupies the Phe19 pocket and the chlorobenzyl group, the Leu26 pocket in Mdmx. Although the compound was not a potent inhibitor of MDMX-p53 interaction, the crystal structure of WK298 bound to MDMX provided a basis for the structure based-design of small molecule MDMX inhibitors.
Figure 2.10: Crystal structure of WK298 (cyan) bound to MDMX (PDB: 3LBJ). The solvent accessible surface of MDMX is coloured based on the charge of the amino acid residues: positive: blue; negative: red.

2.5.4 Pyrrolidones

Pyrrolidones are a class of compounds identified by structure-based virtual screening. Structure based design led to the identification of compounds with low nanomolar activity against MDM2-p53 interaction. Several compounds (such as 23 and 24) also showed low-micromolar binding affinity for MDMX.

![Chemical structures](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>MDMX $K_i$</th>
</tr>
</thead>
<tbody>
<tr>
<td>23</td>
<td>2.6 μM</td>
</tr>
<tr>
<td>24</td>
<td>2.1 μM</td>
</tr>
</tbody>
</table>
2.5.5 *Indolylhydantoin*

Graves *et al* identified two compounds, RO-2443 (3) and RO-5963 (4), with low nanomolar potency against both MDM2 and MDMX.\(^\text{71}\) RO-5963 is an analogue of RO-2443 with improved solubility. RO-5963 restored p53 activity in MDMX overexpressing cell-lines SJSA-X and MCF-7. However, due to poor pharmacological characteristics, the compound did not progress for clinical development.\(^\text{72}\) The crystal structure of RO-2443 bound to MDMX showed that the compound forms a dimer, and MDMX also forms a dimer (Figure 2.11). The details of the crystal structure are discussed in detail in Chapter 8.

![Crystal structure of RO-2443 bound to MDMX](image)

**Figure 2.11**: Crystal structure of RO-2443 (grey) bound to MDMX (PDB: 3U15). The two monomers of MDMX are coloured cyan and blue.

\(\text{RO-2443 (3)}\)

\[\text{MDM2 } \text{IC}_{50} = 33 \text{ nM} \]

\[\text{MDMX } \text{IC}_{50} = 41 \text{ nM} \]

\(\text{RO-5963 (4)}\)

\[\text{MDM2 } \text{IC}_{50} = 17 \text{ nM} \]

\[\text{MDMX } \text{IC}_{50} = 24 \text{ nM} \]
2.5.6 Nutlin-3a Derivative

Qin et al reported a Nutlin 3a derivative 25 as a dual inhibitor of MDM2- and MDMX-p53 interactions.\textsuperscript{88} However, the paper was retracted recently (Oct 30, 2015) due to the intellectual property issues.\textsuperscript{89} The compound was rationally designed using the crystal structure of Nutlin-3a bound to MDM2. The compound demonstrated p53-dependent activity on various cell lines.\textsuperscript{88} After the withdrawal of RO-5963 from clinical evaluation, compound 25 is the only low-nanomolar inhibitor of MDMX reported to date with therapeutic potential.

\begin{align*}
\text{Nutlin 3a 14} & \\
\text{MDMX } K_d & = 6500 \text{ nM} \\
\text{MDM2 } K_d & = 19 \text{ nM}
\end{align*}

\begin{align*}
\text{25} & \\
\text{MDMX } K_d & = 27 \text{ nM} \\
\text{MDM2 } K_d & = 6 \text{ nM}
\end{align*}

The docking studies of compound 25 on p53 binding surface of MDMX revealed that the compound mimics the binding mode of p53 occupying the three key hydrophobic pockets.\textsuperscript{88}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2.12}
\caption{Docking of compound 25 (green) on the p53 binding surface of MDMX.\textsuperscript{88}}
\end{figure}
2.5.7 Pyrroles

The diaryl- and triaryl-pyrroles were identified within the group from a screening of 800 compounds using MDM2-p53 ELISA assay.\textsuperscript{90} Compound 26 with an IC\textsubscript{50} of 12 \(\mu\)M against MDM2-p53 was identified as a hit. Extensive SAR studies around the hit were conducted to identify triaryl-pyrroles 27 and 28 as the most potent compounds in the series. SAR investigation revealed that the barbituric acid was important for the activity. However, the barbituric acid substituent is considered as a promiscuous binder in screening campaigns.\textsuperscript{91} Although, the compounds showed sub-micromolar potency against MDM2 and MDMX, the compounds were not selective on cancer cells with WT-p53 over cells containing mutant-p53. In this thesis, the structural features of the reported triaryl pyrroles were taken into consideration while designing compounds against MDMX-p53 interaction (discussed in detail in the next chapter).

![Chemical structures]

- **26**: MDM2 IC\textsubscript{50} = 12 \(\mu\)M
- **27**: MDM2 IC\textsubscript{50} = 0.11 \(\mu\)M, MDMX IC\textsubscript{50} = 4.2 \(\mu\)M
- **28**: MDM2 IC\textsubscript{50} = 0.15 \(\mu\)M, MDMX IC\textsubscript{50} = 0.68 \(\mu\)M
2.6 MDMX Project Aim

As mentioned earlier, the MDM2-p53 interaction inhibitors cannot restore p53 activities in cancer cells overexpressing MDMX. The aim of the project was to design and develop small-molecule inhibitors of the MDMX-p53 interaction. The knowledge obtained from the SAR studies around the thiazole series and the pyrrole series (both series developed within the group) was utilised to design and synthesise a new set of compounds (details in next chapter).

The other aim of the project was to synthesise the reported dual inhibitors RO-2443 3 and RO-5963 4 to benchmark the in-house ELISA assay and develop a Homogenous Time Resolved FRET (HTRF) assay as an alternative assay.

\[
\text{3} \\
\text{MDM2 IC}_{50} = 33 \text{ nM} \\
\text{MDMX IC}_{50} = 41 \text{ nM}
\]

\[
\text{4} \\
\text{MDM2 IC}_{50} = 17 \text{ nM} \\
\text{MDMX IC}_{50} = 24 \text{ nM}
\]

After almost 20 years since the discovery of MDMX, co-crystallisation of MDMX with small molecules has only been reported two times: one is with a micromolar inhibitor WK298 22,86 and the other is with RO-2443 3,71 and the features of the co-crystal structure with RO-2443 could not be utilised in our series of compounds because it binds via a unique dimerization mode as discussed earlier. The final aim of the project was to co-crystallise MDMX with small-molecule inhibitors developed within the research group. A co-crystal structure would provide an insight into the binding mode of the molecules guiding the design of potent inhibitors of the MDMX-p53 interaction.
Chapter 3. Development of Inhibitors of MDMX-p53 Interaction

3.1 Rationale

The aminothiazole series was identified from virtual screening of a large library of compounds. SAR studies around hit 1 led to the identification of compound 29 with low-micromolar inhibitory activity against the MDMX-p53 interaction. However, due to the challenging chemistry at the 5-position of thiazoles, tri-substituted thiazoles that would be necessary to target the three hydrophobic pockets on the binding site of MDMX could not be synthesised. It was decided to change the scaffold from thiazole to pyrrole because the synthesis of tri-substituted pyrroles had been previously achieved within the group as described below.

The pyrrole scaffold had previously been used within the group for developing MDMX-p53 interaction inhibitors. Tetra-substituted pyrrole 26 was identified as a hit from the screen of 800 compounds using Enzyme-Linked Immunosorbent Assay (ELISA). Extensive SAR studies led to the identification of compound 28 that showed sub-micromolar inhibitory activity against MDMX-p53 interaction, but suffered from low solubility in both aqueous medium, as well as organic solvents.
It was decided to remove the barbituric acid group, which has been identified as a component of ‘frequent hitters’ in high-throughput screening campaigns.\textsuperscript{91} Compound 30 was designed and docked in the binding site of MDMX (PDB: 3DAC) using Molecular Operating Environment (MOE) software (Figure 3.1). The binding site was defined automatically by MOE around the region where p53 peptide was bound to MDMX. Ten different poses were generated by MOE using the default London dG and GBVI/WSA dG scoring systems,\textsuperscript{93} and the top scoring pose was selected. The docking study suggested that a CH\textsubscript{2} linker provides a perfect angle for the phenyl ring to occupy the Phe19 pocket in the binding site of MDMX. Besides, it was identified later on that a CH\textsubscript{2} linker improved the organic solubility of the compounds. The 4-chlorophenyl group occupied the Trp23 pocket and the phenyl ring occupied the Leu26 pocket.
Figure 3.1: Compound 30 (carbons: magenta) docked on the binding site of MDMX (PDB: 3DAC) using Molecular Operating Environment (MOE) software. The binding surface is coloured based on the charge of the amino acid residue; positive: blue, negative: red.

The docked structure of compound 30 on MDMX was superimposed on the crystal structure of a p53 peptide in complex with MDMX (Figure 3.2). The superimposed image suggested that the substituents at 1,2,5-positions of the pyrrole ring occupied the same region as the three key amino acids in p53 (Leu26, Trp23 and Phe19).
Figure 3.2: Superimposition of crystal structure of MDMX in complex with p53 peptide (PDB: 3DAC) and docked structure of compound 30 (carbons: magenta). MDMX secondary structure is coloured blue; p53 peptide is coloured green; three key amino acids in p53 are coloured grey.

Therefore, the first strategy was to access the three key hydrophobic pockets in the MDMX binding surface with aromatic substituents at 1,2, and 5-positions of pyrroles.

Figure 3.3: 1,2,5-trisubstituted pyrroles to be synthesised.

3.2 Synthesis of 1,2,5-trisubstituted pyrroles

Synthesis of compound 30 started with the Grignard reaction on commercially available phenylacetaldehyde 31 followed by oxidation to give α,β-unsaturated ketone 32 (Scheme 3.1). A Stetter reaction using a thiazolium salt provided the desired diketone 34. Finally, the Paal-Knorr pyrrole synthesis afforded pyrrole 30.
Scheme 3.1: Reagents and conditions: (a) vinyl magnesium chloride, THF, -78 °C, 30 min, then r.t., 2h, 61%; (b) DMP, DCM, 0 °C-r.t., 3.5 h, quant.; (c) 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, benzaldehyde, NEt₃, EtOH, 80 °C, 19 h, 65%; (d) 4-chloroaniline, AcOH, reflux, 1.5 h, 30%.

Compounds 40, 41, and 42 were synthesized using the same route. The synthesis started with the oxidation of 4-chlorophenethyl alcohol 35 (Scheme 3.2). Subsequently, following the previously described reaction sequence, diketone 39 was obtained. Finally, the Paal-Knorr pyrrole synthesis afforded desired pyrroles 40, 41 and 42. The Paal-Knorr condensation condition used for synthesising compound 30, 40 was low yielding, therefore, microwave conditions described by Minetto et al were employed. Diketone 39 was heated with the corresponding amine under microwave irradiation for 10 min to obtain desired pyrroles 41 and 42 in good isolated yields.
Scheme 3.2: Reagents and conditions: (a) DMP, DCM, 0 °C, 15 min, then r.t., 1 h, 66%; (b) vinyl magnesium chloride, THF, -78 °C, 30 min, then r.t., 2h, 39%; (c) DMP, DCM, 0 °C, 15 min, then r.t., 3.5 h; (d) 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride, benzaaldehyde, NEt₃, EtOH, 80 °C, 19 h, 30% over 2 steps; (e) 4-chloroaniline, AcOH, reflux, 8 h, 31% (40); or, 4-bromoaniline or 3,4-difluoroaniline, AcOH, 170 °C µW, 10 min, 83% (41), 77% (42).

Compound 46 with a 6-chloroindole at the pyrrole-\(N^1\) position was designed based on the published inhibitor WK-298 22, which has a 6-chloroindole group to access the Trp23 pocket in MDMX. The proposed synthetic route for compound 46 is shown in Scheme 3.3. The synthesis started with the nitration of the commercially available indole 43. There is literature precedent for nitration at multiple positions of indole under nitration conditions using nitric acid. Therefore, milder conditions were attempted which provided desired 3-nitroindole 44 in 10% yield. The low yield was due to the difficulty in purification of the tarry crude material as mentioned in the literature. A thiol SPE cartridge was used to separate silver impurities generated in the reaction and the remaining material was purified by reverse phase chromatography which helped to increase the yield to 42%. The reduction of 3-nitroindole 44 was attempted with various reducing agents such as Zn/acetic acid, Zn/NH₄Cl in MeOH, and Pd/C and H₂, but resulted in a complex mixture of products.
Scheme 3.3: Reagents and conditions: (a) AgNO₃, benzoyl chloride, CH₃CN, 0 °C, 30 min., 42%.

There is a literature precedent for the Paal-Knorr condensation using nitrobenzenes involving in-situ reduction of the nitrobenzene to anilines. Using the literature conditions, the Paal-Knorr condensation using Zn/Acetic acid was attempted (Scheme 3.4) but the reaction provided complex mixture of products.

Scheme 3.4: Reagents and conditions: (a) Zn, AcOH, 90 °C, 20 h.

It was proposed that the 3-aminoindole 45, due to its electron rich nature, would be involved in various side reactions. Therefore, the protection of indole 43 with an electron withdrawing protecting group was proposed (Scheme 3.5). The Boc protection of indole 43, followed by nitration afforded compound 48. Subsequent reduction of compound 48 using Zn/AcOH was unsuccessful. Following a literature procedure by Lee et al., nitroindole 48 was used directly in the Paal-Knorr condensation using indium/acetic acid, however, a mixture of unidentified products was obtained. While this work was underway, it was determined that 1,2,5-
trisubstituted pyrroles (30, 40, 41 and 42) were not potent inhibitors of MDMX-p53 interaction as shown in Table 3.1. Therefore, the synthesis of compound 46 was abandoned. The focus was shifted towards other regions of the pyrroles with modifications designed to improve the solubility which will be discussed in detail in the next chapter.

![Chemical structures and reactions]

**Scheme 3.5: Reagents and conditions:** (a) Boc$_2$O, DMAP, MeCN, r.t., 2 h, 97%; (b) HNO$_3$, Ac$_2$O, -78 °C-0 °C, 17 h, 51%; (c) Zn, AcOH, r.t., 45 min.

### 3.3 Biological Evaluation

1,2,5-Trisubstituted pyrroles were tested against MDMX-p53 interaction using an Enzyme-Linked Immunosorbent Assay (ELISA). Compounds 30, 40, 41 and 42 demonstrated weak inhibition of the MDMX-p53 interaction (Table 3.1). The compounds were very lipophilic with average clogP around 9. Due to high lipophilicity, the compounds had low-solubility in assay buffer conditions resulting in weak inhibition of the MDMX-p53 interaction.
Table 3.1: Results of the ELISA for 1,2,5-trisubstituted pyrroles.

\[
\text{R}^1 \quad \text{R}^2 \quad \text{R}^3 \quad \text{MDMX IC}_{50} (\mu\text{M})
\]

<table>
<thead>
<tr>
<th>Compound</th>
<th>( \text{R}^1 )</th>
<th>( \text{R}^2 )</th>
<th>( \text{R}^3 )</th>
<th>MDMX IC(_{50}) ((\mu\text{M}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>(\text{C}_6\text{H}_5\text{CH}_2)</td>
<td>(\text{Ph})</td>
<td>(4\text{-ClC}_6\text{H}_4)</td>
<td>(&gt;200)</td>
</tr>
<tr>
<td>40</td>
<td>(4\text{-ClC}_6\text{H}_4\text{CH}_2)</td>
<td>(4\text{-FC}_6\text{H}_4)</td>
<td>(4\text{-ClC}_6\text{H}_4)</td>
<td>197</td>
</tr>
<tr>
<td>41</td>
<td>(4\text{-ClC}_6\text{H}_4\text{CH}_2)</td>
<td>(4\text{-FC}_6\text{H}_4)</td>
<td>(4\text{-BrC}_6\text{H}_4)</td>
<td>168</td>
</tr>
<tr>
<td>42</td>
<td>(4\text{-ClC}_6\text{H}_4\text{CH}_2)</td>
<td>(4\text{-FC}_6\text{H}_4)</td>
<td>(3.4\text{-F}_2\text{C}_6\text{H}_3)</td>
<td>281</td>
</tr>
</tbody>
</table>
Chapter 4. Introduction of Water Solubilising Group

4.1 Rationale

It was postulated that 1,2,5-trisubstituted pyrroles were inactive against MDMX because of the poor solubility of the compounds in assay buffer conditions. Therefore, it was decided to introduce a water solubilising group in the molecule. The 3-position of the pyrrole was chosen for introducing a water solubilising group for reasons of synthetic accessibility. The water solubilising group in the published MDMX inhibitor RO-5963 4 was chosen and compound 51 was designed.

4.2 Synthesis

The synthesis of compound 51 started with the preparation of 1,4-diketone 54 following a route described by Minetto et al (Scheme 4.1).\textsuperscript{94} 1,4-Diketone 54 was synthesised from the commercially available β-keto ester 52 using Et₂Zn and CH₂I₂, followed by oxidation. Then, Paal-Knorr condensation of 1,4-diketone 54 with 4-chloroaniline provided pyrrole 55 which was hydrolysed to compound 2. Subsequent amide coupling of the carboxylic acid 2 with the amine 115 produced compound 57. The synthesis of amine 115 is discussed in detail in Chapter 7 (Scheme 7.2). Finally, the deprotection of acetal 57 using HCl provided desired target 51.
Scheme 4.1: Reagents and conditions: (a) 36, Et₂Zn, CH₂I₂, DCM, 0 °C, 1.5 h; (b) PCC, DCM, r.t., 41 h, 33% over 2 steps; (c) 4-chloroaniline, AcOH, 170 °C µW, 10 min, 48%; (d) LiOH, THF, MeOH, H₂O, r.t., 72 h, 91%; (e) 115, DIC, DMAP, DCM, 0 °C, 15 min, then r.t., 16 h, 70%; (f) HCl, H₂O, THF, r.t., 3h, 94%.
Minetto et al proposed a mechanism for the formation of diketone using Et₂Zn and CH₂I₂ (Scheme 4.2). The reaction proceeds via the formation of a cyclopropyl intermediate 59 which ring opens to intermediate 60. The nucleophilic attack of intermediate 60 on the aldehyde affords alcohol 61, which can be oxidised to give desired 1,4-diketone 62.

Scheme 4.2: Proposed mechanism for the conversion of β-Keto ester to 1,4-Diketone. 

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4.3 Biological Evaluation

MDMX inhibitory activity of compound 51 along with the intermediates obtained during the synthesis were determined (Table 4.1). A significant increase in potency for MDM2 and MDMX, at least 12-fold, was observed upon the hydrolysis of ester 55 to carboxylic acid 2. Amides 51 and 57 were at least 4-fold less potent than the carboxylic acid 2. Interestingly, both the amides were more potent against MDMX than MDM2, compound 51 was the most MDMX selective compound. Compound 2 was considered as the lead in the pyrrole series and SAR studies around the compound were carried out, discussed in detail in following chapters.

Table 4.1: Results of the ELISA for tetra-substituted pyrroles.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>MDM2 IC₅₀ (μM)ᵃ</th>
<th>MDMX IC₅₀ (μM)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>H</td>
<td>&gt;200</td>
<td>197</td>
</tr>
<tr>
<td>55</td>
<td>-COOMe</td>
<td>&gt;200</td>
<td>188</td>
</tr>
<tr>
<td>2</td>
<td>-COOH</td>
<td>11ᵇ</td>
<td>15ᶜ</td>
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<tr>
<td>57</td>
<td></td>
<td>104</td>
<td>61</td>
</tr>
<tr>
<td>51</td>
<td></td>
<td>&gt;200</td>
<td>40</td>
</tr>
</tbody>
</table>

ᵃ Determinations (n = 1 unless otherwise stated);ᵇ n = 2;ᶜ n = 3
Chapter 5. SAR Studies around Compound 2

5.1 Rationale

The increase in potency due to a carboxylic acid at the 3-position, encouraged SAR studies around lead 2. Compound 63 with a barbituric acid group at the 3-position could be used for direct comparison with the previously described pyrrole series. Compound 64, with two carboxylic acids was designed to study the effect of an additional acid at the 4-position. To understand the effect of homologation of carboxylic acid compound 65 and 66 were designed.

Introduction of a succinimide ring on the 3/4-positions could be accessible via dicarboxylic acid compound 67. 3,4-Pyrroledicarboximides 68 has an unsubstituted nitrogen that could be used as a vector to introduce various water solubilising groups. Removal of fluorine is discussed in detail in Chapter 6.
It was postulated that compound 69 with a CH$_2$ linker at the N$^1$-position would mimic the indole ring of Trp23 in p53 (Figure 5.2).

**Figure 5.2:** Overlay of compound 69 on the side chain of Trp23 of p53.

### 5.2 Synthesis

#### 5.2.1 Synthesis of Tetra-Substituted and Penta-Substituted Pyrroles

1,4-Diketone 74 and pyrrole 75 were synthesised in bulk quantities, and used for the synthesis of various compounds. The synthesis started with the preparation of β-keto ester 72 using acid chloride 70 and Meldrum’s acid 71 (Scheme 5.1). The nucleophilic attack of the enolate of β-keto ester 71 on compound 73 afforded desired 1,4-diketone 74 in good isolated yield. Finally, the Paal-Knorr pyrrole synthesis using 4-chloroaniline provided pyrrole 75. The scheme was high yielding, and the purification of the products was easier compared to the previously
described route (Scheme 4.1). In addition, this route was versatile in terms of synthesising different analogues of 1,4-diketone.

Scheme 5.1: Reagents and Conditions: (a) (i) pyridine, DCM, 0 °C, 1 h, then r.t., 2.5 h; (ii) EtOH, reflux, 2 h, then r.t., 16 h, 78% over 2 steps; (b) NaH, THF, r.t., 2.5 h. 91%; (c) 4-chloroaniline, AcOH, 170 °C µW, 10 min, 67%.

Compound 63 was synthesised following a four-step reaction sequence starting from intermediate 75 (Scheme 5.2). DIBAL reduction of ester 75 gave alcohol 76, and subsequent oxidation with Dess-Martin periodinane provided aldehyde 77. Knoevenagel condensation of aldehyde 77 with barbituric acid in the presence of acetic acid afforded desired pyrrole 63.
Scheme 5.2: Reagents and Conditions: (a) DIBAL, DCM, 0 °C, 2 h, 95%; (b) DMP, DCM, 0 °C-r.t., 2 h, 61%; (c) barbituric acid, AcOH, reflux, 2.5 h, 48%.

Compound 69 was synthesised following a two-step reaction sequence starting from intermediate diketone 74 (Scheme 5.3). Paal-Knorr condensation of diketone 74 with amine 78 afforded pyrrole 79. Subsequent hydrolysis of the ester group provided desired pyrrole 69.
Scheme 5.3: *Reagents and Conditions:* (a) AcOH, 170 °C µW, 10 min, 50%; (b) NaOH, THF, MeOH, H₂O, 75 °C, 18 h, 70%.

The synthesis of di-carboxylic acid compound 64 started with the formylation of intermediate 75 using Vilsmeier-Haack conditions (Scheme 5.4). Pinnick oxidation of aldehyde 80 provided mono-carboxylic acid 81. Subsequent hydrolysis provided desired di-carboxylic acid 64.
Scheme 5.4: \textit{Reagents and conditions:} (a) POCl\textsubscript{3}, DMF, 70 °C, 4 h, 71%; (b) NaClO\textsubscript{2}, sulfamic acid, MeCN, H\textsubscript{2}O, r.t., 2 h, quant.; (c) NaOH, MeOH, H\textsubscript{2}O, reflux, 4 h, 97%.

Compound 65 was synthesised by the condensation of aldehyde 77 and malonic acid using pyridine and piperidine (Scheme 5.5).

Scheme 5.5: \textit{Reagents and Conditions:} (a) malonic acid, pyridine, piperidine, reflux, 2.5 h, 75%.
5.2.2 Synthesis of pyrroles with succinimide ring at the 3/4-position

Compound 68 was synthesised following a five step reaction sequence starting from intermediate 82 (Scheme 5.6). Di-carboxylic acid 67 was synthesised using the previously described chemistry. Acetic anhydride mediated ring closure afforded compound 85. Nucleophilic attack of ammonia on anhydride 85 opens the ring producing an intermediate amide, which was converted to succinimide 68 upon thionyl chloride-mediated ring closure.

Scheme 5.6: Reagents and conditions: (a) POCl₃, DMF, 70 °C, 4 h, quant.; (b) NaClO₂, sulfamic acid, MeCN, H₂O, r.t., 2 h, 62%; (c) NaOH, MeOH, H₂O, reflux, 4 h, quant.; (d) Ac₂O, reflux, 2 h, 82%; (e) (i) NH₃, AcOH, 120 °C µW, 30 min; (ii) SOCl₂, THF, r.t., 1.5 h, 62%.
In order to examine the versatility of the reaction scheme, compound 86 was synthesised. Compound 86 has a 4-chlorophenyl ring at the $N^1$-position of the succinimide ring that could flip the binding mode of the molecule such that the 4-chlorophenyl ring at the $N^1$-position the succinimide ring would occupy the Trp23 pocket in MDMX.

![Chemical structure of compound 86](image)

Compound 86 was synthesised in moderate isolated yield from anhydride 85 using 4-chloroaniline as a nucleophile (Scheme 5.7).

![Reactions scheme](image)

**Scheme 5.7:** *Reagents and Conditions:* (a) (i) 4-chloroaniline, AcOH, 120 °C µW, 20 min; (ii) SOCl₂, THF, r.t., 18 h, 64%.
5.2.3 One-Carbon Homologation of 3-Carboxylic Acid

A classical route for one-carbon homologation of a carboxylic acid includes the conversion of the carbonyl compound to nitrile, which can be hydrolysed to get the desired homologated carboxylic acid (Scheme 5.8).

\[
\begin{align*}
\text{R-O} & \rightarrow \text{R-OH} \rightarrow \text{R-CN} \rightarrow \text{R-COOH} \\
\end{align*}
\]

**Scheme 5.8**: One carbon homologation via nitrile.

Synthesis of homologated carboxylic acid analogue 66 started with the reduction of ester 75 to alcohol 76. Conversion of alcohol 76 to nitrile 87 proved to be extremely challenging. Initial attempts to convert the alcohol group of 76 to a leaving group (LG) such as chloro, mesyl or tosyl resulted in a mixture of unidentified products.

**Scheme 5.9**: Reagents and conditions: (a) DIBAL, DCM, 0 °C, 2 h. (95%)
It was proposed that in the presence of a good leaving group, the lone pair of nitrogen pushes the electron density, eliminates the leaving group producing a reactive electrophile, which can be involved in unwanted side-reactions (Scheme 5.10).

\[ \text{Scheme 5.10: Proposed mechanism for the formation of a reactive electrophile.} \]

Simply heating alcohol 76 with various cyanide sources like Et₄N⁺CN⁻, NaCN, and TMSCN did not produce any reaction. Cheng et al have described the direct conversion of alcohols to nitriles using TMSCN and catalytic InBr₃. The reaction was attempted, but a complex mixture of products was obtained.

Cafiero et al has reported one-carbon homologation via an aldehyde. One of the examples in the paper was aldehyde 88, which has very similar electronic character to the pyrrole scaffold. The oxygen lone pair in methoxy group of compound 89 can push electron density, similar to the nitrogen of pyrroles (Scheme 5.10).

The procedure described by Cafiero et al (Scheme 5.11) was used for the synthesis of the one-carbon homologated carboxylic acid 92. The aldehyde 90 was trichloromethylated to afford compound 91, which under sodium borohydride and diphenyldiselenide and sodium hydroxide provided desired acid 92 in good isolated yield. The diphenyldiselenide was important in the reaction. The reaction in the absence of diphenyldiselenide gave alcohol. Final compound 92 does not contain fluorine because it was identified simultaneously that the fluorine was not essential for potency (discussed in detail in next chapter).
Scheme 5.11: Reagents and conditions: (a) (i) CHCl$_3$, DMF, -10 °C, 15 min; (ii) KOH, MeOH, -10 °C, 3 h, 64%; (b) (PhSe)$_2$, NaBH$_4$, NaOH, EtOH, r.t., 24 h, 90%.

5.3 Biological Evaluation

Compounds along with the intermediates obtained during the synthesis were analysed by ELISA (Table 5.1). Compounds 76 and 80 without any acidic groups showed a significant loss in potency, suggesting that an acidic group at 3- or 4-position of pyrroles is essential for activity. Compound 81 with an acidic group as well as an ester group showed a reduction in potency against MDMX; however acidic groups at both 3- and 4-positions in compound 64 were tolerated. Interestingly, compound 69 with a CH$_2$ linker at the N$^1$-position was at least 10-fold more potent against MDM2 than MDMX. A crystal structure of compound 69 in complex with MDMX would be beneficial to understand the selectivity observed.
Table 5.1: Results of the ELISA for tetra- and penta-substituted pyrrole derivatives.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R¹</th>
<th>R²</th>
<th>R³</th>
<th>MDM2 IC₅₀ (µM)ᵃ</th>
<th>MDMX IC₅₀ (µM)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>COOH</td>
<td>H</td>
<td>4-ClC₆H₄</td>
<td>11ᵇ</td>
<td>15ᶜ</td>
</tr>
<tr>
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<td>COOH</td>
<td>H</td>
<td>4-ClC₆H₄</td>
<td>3.5</td>
<td>4.5</td>
</tr>
<tr>
<td>69</td>
<td>COOH</td>
<td>H</td>
<td>4-ClC₆H₄</td>
<td>4.9</td>
<td>53</td>
</tr>
<tr>
<td>80</td>
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<td>CHO</td>
<td>4-ClC₆H₄</td>
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<td>187</td>
</tr>
<tr>
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<td>COOH</td>
<td>4-ClC₆H₄</td>
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<td>55.9</td>
</tr>
<tr>
<td>64</td>
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<td>COOH</td>
<td>4-ClC₆H₄</td>
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<tr>
<td>76</td>
<td>CH₂OH</td>
<td>H</td>
<td>4-ClC₆H₄</td>
<td>&gt; 200</td>
<td>&gt; 200</td>
</tr>
</tbody>
</table>

ᵃ Determinations (n = 1 unless otherwise stated; ᵇ n = 2; ᶜ n = 3)

Before the biological evaluation of homologated carboxylic acid compounds 65 and 92, the antibody used in the MDMX-ELISA was withdrawn from the market. Therefore, it was decided to develop an alternate Homogenous Time Resolved FRET (HTRF) assay (Chapter 8).
Chapter 6. SAR Studies around the Phenyl Rings at 2 and 5-Positions

6.1 Rationale

The docking studies with compound 30 suggested that there is not enough space (highlighted by red arrow in Figure 6.1) to accommodate any atoms in the para-position of the phenyl ring into the Leu26 pocket of MDMX.

Figure 6.1: Compound 30 (carbons: magenta) docked on the binding site of MDMX (PDB: 3DAC) using Molecular Operating Environment (MOE) software. The binding surface is coloured based on the charge of the amino acid residue; positive: blue, negative: red. The space between the phenyl ring and Leu26 pocket is highlighted by a red arrow.

The role of the fluoro group from the para position of 2 was investigated, and compound 93 was designed.
The docking studies with compound \textbf{30} also suggested that the phenyl ring in the 5-position was close to the oxygen of Gln68 (Figure 6.2). It was postulated that an electron donating group might interact with Gln68 improving the potency.

\textbf{Figure 6.2}: Compound \textbf{30} (carbons: magenta) docked on the binding site of MDMX using Molecular Operating Environment (MOE) software. The secondary structure of MDMX is coloured green. The carbons of key amino acids in the binding site are coloured grey. The red arrow highlights the distance between the phenyl ring and Gln68.

Compound \textbf{94} was designed replacing the chloro group with a hydroxyl group that might provide an H-bond interaction with Gln68. In addition, the hydroxyl group also reduced the lipophilicity of the molecule.
6.2 Synthesis

Compound 93 was synthesised using the previously described chemistry (Scheme 6.1). β-Keto ester was synthesised using acid chloride 70 and Meldrum’s acid 71 (Scheme 5.1). The nucleophilic attack of the enolate of β-keto ester 72 on compound 95 afforded desired 1,4-diketone 96 in good isolated yield. The Paal-Knorr pyrrole synthesis using 4-chloroaniline provided pyrrole 82, which was hydrolysed to desired pyrrole 93.

![Chemical structures](image)

**Scheme 6.1: Reagents and Conditions:** (a) NaH, THF, r.t., 2.5 h, 79%; (b) 4-chloroaniline, AcOH, 170 °C µW, 10 min, 73%; (c) NaOH, MeOH, H2O, 65 °C, 24 h, 98%.

Similarly, compound 94 was synthesised following a five step reaction sequence (Scheme 6.2). The synthesis started with the formation of β-keto ester 98 from the commercially available acid chloride 97 and Meldrum’s acid 71. Following the chemistry described before, compound 101 was obtained. Finally, BBr3-mediated removal of methyl group from compound 101 afforded desired target 94 in good isolated yield.
Scheme 6.2: Reagents and conditions: (a) (i) pyridine, DCM, 0 °C, 1 h, then r.t., 2.5 h; (ii) EtOH, reflux, 2 h, then r.t., 16 h, 72% over 2 steps; (b) NaH, THF, r.t., 2.5 h, 76%; (c) 4-chloroaniline, AcOH, 170 °C µW, 10 min, 75%; (d) NaOH, MeOH, H₂O, 65 °C, 24 h, quant.; (e) BBr₃, DCM, 0 °C, 1 h, 88%.
6.3 Biological Evaluation

Compound 93, retained the potency suggesting that the fluoro group at the *para*-position was not important for the inhibition of MDMX-p53 interaction.

As mentioned in the last chapter, before the biological evaluation of compounds 94, the antibody used in the MDMX-ELISA was withdrawn from the market. Therefore, it was decided to develop an alternate Homogenous Time Resolved FRET (HTRF) assay (Chapter 8).
Chapter 7. Synthesis and Biological Evaluation of RO-2443 3 and RO-5963 4

7.1 Rationale

During the project work, RO-2443 3 and RO-5963 4 were the only reported dual small-molecule nanomolar inhibitors of MDM2 and MDMX. Therefore, in order to benchmark the biochemical assay ELISA, which was used for measuring the potency of in-house compounds, it was decided to synthesise RO-2443 3 and RO-5963 4.\textsuperscript{71}

7.2 Synthesis

7.2.1 Synthesis of RO-2443 3

The syntheses of RO-2443 3 and RO-5963 4 were not described in the paper by Graves et al.\textsuperscript{71} Initial retrosynthetic analysis of RO-2443 3 suggested that the condensation of aldehyde 104 and substituted-hydantoin 107 could provide the desired target (Figure 7.1). The mono-alkylation of hydantoin 105 could be performed by K\textsubscript{2}CO\textsubscript{3}-mediated procedure reported by Fraile et al.\textsuperscript{101}
The synthesis started with a Bartoli reaction on 1-chloro-2-methyl-3-nitrobenzene 102 to give indole 103. Formylation under Vilsmeier-Haack conditions afforded aldehyde 104 in good yield. The monoalkylation of hydantoin 105 was achieved by using K$_2$CO$_3$ as a base to afford compound 107. Di-alkylation was observed when NaH was used. The final condensation reaction with NH$_4$OAc in acetic acid was not successful. The condensation reaction was attempted with various reagents and conditions (Table 7.1). The desired target 3 was obtained in 24% yield using NH$_4$OAc in toluene. The best results were obtained with 3 equivalents of piperidine for condensation to give RO-2443 3 in 92% isolated yield.
Scheme 7.1: Reagent and condition: (a) vinyl magnesium bromide, THF, -40 °C, 1 h, 43%; (b) POCl₃, DMF, 0 °C, then 70 °C µW, 10 min, 93%; (c) K₂CO₃, CH₃CN, reflux, 6 h; r.t., 16 h, 57%; (d) piperidine, 130 °C, 30 min, 92%.
Table 7.1: Various reagents and conditions used in step d of Scheme 7.1 for the condensation of aldehyde 104 and hydantoin 107.

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Solvent</th>
<th>Temperature (°C)</th>
<th>Time</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>NH₄OAc</td>
<td>AcOH</td>
<td>180 µW</td>
<td>30 min</td>
<td>Complex mixture of products</td>
</tr>
<tr>
<td>NH₄OAc</td>
<td>Toluene</td>
<td>200 µW</td>
<td>30 min</td>
<td>24% isolated yield</td>
</tr>
<tr>
<td>NH₄OAc</td>
<td>DME</td>
<td>100 µW</td>
<td>30 min</td>
<td>Complex mixture of products</td>
</tr>
<tr>
<td>NH₄OAc</td>
<td>MeCN</td>
<td>100 µW</td>
<td>30 min</td>
<td>Complex mixture of products</td>
</tr>
<tr>
<td>Piperidine (excess)</td>
<td>-</td>
<td>Reflux</td>
<td>24 h</td>
<td>Complex mixture of products</td>
</tr>
<tr>
<td>Piperidine (3 eq)</td>
<td>-</td>
<td>Reflux</td>
<td>30 min</td>
<td>92% yield</td>
</tr>
</tbody>
</table>

7.2.2 X-ray structure of RO-2443 3

A small molecule X-ray crystal structure of RO-2443 3 was obtained (Figure 7.2). The torsion angle between the bond formed by C8 and C9 and the bond formed by C10 and C11 is 0.4° which suggests that the indolylhyantoin moiety is planar. The crystal structure, along with NMR and HRMS data confirmed that the compound synthesised was RO-2443 3.
Figure 7.2: Crystal structure of RO-2443 3. Atoms are coloured as: carbon: grey, oxygen: red, nitrogen: blue, hydrogen: white; chlorine: green.

### 7.2.3 Synthesis of RO-5963 4

Initial retrosynthetic analysis of RO-5963 4 suggested that the nucleophilic substitution reaction using indolylhydantoin 118 and bromo-derivative 110 would provide the desired target (Figure 7.3). Indolylhydantoin 118 could be prepared using the chemistry described for the synthesis of RO-2443 3. The bromo-compound 110 could be synthesised via the amide coupling of carboxylic acid 109 and 2-aminopropane-1,3-diol 112.

![Figure 7.3: Retrosynthetic analysis of RO-5963 4.](image-url)
The synthesis of RO-5963 4 started with the preparation of bromo-compound 110 (Scheme 7.2). 3,4-Difluorophenylglycine 108, was brominated via the formation of the diazonium salt, followed by a nucleophilic attack with bromide. The amide coupling of carboxylic acid 109 and 2-amino propane-1,3-diol 112 in presence of DIC and EDC was unsuccessful. Therefore, it was decided to protect the diol. The amine group of 2-amino propane-1,3-diol 112 was protected as a trifluoroacetate 113. Subsequently, acetal protection afforded compound 114. Finally, the trifluoroacetyl group was removed using LiOH to liberate the free amine 115, which was used for amide coupling with carboxylic acid 109 to afford compound 116.

Scheme 7.2: Reagents and condition: (a) HBr, KBr, NaNO₂, H₂O, 0 °C-r.t., 3 h, 36%; (b) THF, r.t., 48 h, 98%; (c) 2,2-dimethoxypropane, camphor-10-sulfonic acid, DCM, r.t., 96 h, 54%; (d) LiOH, THF, H₂O, r.t., 1.5 h, 54%; (e) DIC, DMAP, DCM, 0 °C-r.t., 16 h, 79%.
Condensation of aldehyde 104 with hydantoin afforded indolylhydantoin 118 (Figure 7.3). The nucleophilic substitution reaction with indolylhydantoin 118 and bromo-compound 116 was very slow, and desired product 119 was obtained in poor yield. Acid-catalysed acetal deprotection afforded RO-5963 4. Although, the scheme was low yielding in a few steps, the reactions were reproducible and was successfully repeated by a colleague (Dr Stephanie Myers).

Scheme 7.3: **Reagents and condition:** (a) hydantoin, piperidine, 130 °C, 1 h, 86%; (b) 116, K₂CO₃, DMF, r.t., 96 h, 17%; (c) HCl, THF, H₂O, 0 °C-r.t., 3 h, 36%.
7.3 Biological Evaluation

Upon the biochemical evaluation by ELISA, RO-2443 3 and RO-5963 4 were at least 1000 fold less potent compared to the HTRF values reported by Graves et al (Table 7.2).71

Table 7.2: Comparison of the reported HTRF assay and in-house ELISA assay.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Published HTRF IC₅₀ (µM)</th>
<th>In-house ELISA IC₅₀ (µM)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDM2</td>
<td>MDMX</td>
</tr>
<tr>
<td>RO-2443 3</td>
<td>0.033</td>
<td>0.041</td>
</tr>
<tr>
<td>RO-5963 4</td>
<td>0.019</td>
<td>0.024</td>
</tr>
</tbody>
</table>

¹ Determinations (n = 1 unless otherwise stated);  
⁷² n = 2;  ⁷³ n = 3;  ⁷⁴ n = 4

Although, the potencies of RO-2443 and RO-5963 were significantly lower in NCL-ELISA, the cellular data were in line with the published results. RO-2443 3 and RO-5963 4 were potent in MDMX overexpressing cell lines JEG3 and MCF-7. Although less potent, the compounds also exhibited growth inhibition in MDM2 overexpressing cell line SJSA-1. Therefore, in order to develop an alternative assay, a structural biology placement was undertaken (Chapter 8).
Chapter 8. MDM2 and MDMX Structural Biology

8.1 Aims

Although MDM2 and MDMX have high sequence homology, most of the small molecule MDM2 inhibitors show significantly lower binding affinity towards MDMX.\(^7\), \(^102\)-\(^103\) An extensive SAR on four different series (benzenoids, thiazoles, pyrroles and isoindolinones) did not produce any low nanomolar inhibitors of the MDMX-p53 interaction.\(^92\), \(^104\)-\(^105\) To guide rational drug design, a co-crystal structure of a small molecule bound to MDMX was essential.

To date, co-crystal structures of only two small molecules bound to MDMX have been reported. Compound WK298 22 with low micromolar potency against MDMX is the first small molecule for which a structure bound to MDMX has been reported.\(^86\) The co-crystal structure of WK-298 22 bound to MDMX revealed many key features which are important for designing small molecule inhibitors of MDMX (Figure 8.1). The chloro-indole substituent binds in the Trp23 pocket. The nitrogen atom of the indole forms a hydrogen bond with Met53 in MDMX. The chlorine atom in the indole ring induces a conformational change in key residues including Leu54, Met53, Leu98 and Leu102 which helps to enlarge the Trp23 binding pocket. The phenyl ring occupies the Phe19 pocket and the chlorobenzyl group occupies a Leu23 pocket.

**Figure 8.1:** The X-ray co-crystal structure of WK-298 22 bound to MDMX. WK-298 22 is drawn in skeleton mode with carbon, nitrogen, oxygen and chlorine atoms coloured cyan, blue and red and green, respectively. The solvent accessible surface of MDMX (probe radius 1.5 Å) around WK-298 22 is coloured based on the atoms; carbon: grey, oxygen: red, nitrogen blue, sulphur: yellow. The structure is taken from PDB code: 3LBJ.
Compound RO-2443 3 developed by Roche is the other small molecule for which a co-crystal structure with MDMX has been reported and it is a potent inhibitor of MDMX with an IC₅₀ of 41 nM. The compound interacts with MDM2 and MDMX by dimerization avoiding the challenging Leu26 pocket in both MDM2 and MDMX (Figure 8.2). The compound contains an indolylhydantoin and difluorophenyl groups. The indolylhydantoin moiety occupies the Phe19 pocket in one monomer of MDMX, and the difluorophenyl moiety occupies the Trp23 pocket of the other monomer of MDMX. The molecules also form aromatic stacking interactions between two indolylhydantoin moieties and two tyrosine residues from two MDMX monomers to generate a four level π-sandwich. The other key interaction is between the σ-hole of the chlorine substituent of one monomer of the small molecule with difluorophenyl ring of the other monomer of the small molecule. This interaction along with the aromatic stacking interaction helps in the dimerization of small molecule as well as MDMX protein.

**Figure 8.2:** Co-crystal structure of RO-2443 3 bound to MDMX. MDMX dimers are represented as ribbons and coloured based on secondary structure: helices are red, beta sheets are cyan, turns are green, and coils are white. Two molecules of RO-2443 3 and two tyrosine residues from two MDMX monomers form a four level π-sandwich. The four-level sandwich is visible in this view holding the two MDMX molecules together. The chlorine atom is labelled green which forms an interaction via its σ-hole with the difluorophenyl ring of the other monomer of RO-2443 3. The structure is taken from PDB code: 3U15.
Due to the unique nature of the RO-2443 3 binding mode, the key features of the crystal structure could not help rational drug design of the compound series developed at Newcastle. Therefore, a co-crystal structure of a small molecule inhibitor developed at Newcastle bound to MDMX would be valuable. To attempt to achieve this aim, a 5-month placement in structural biology at the Northern Institute for Cancer Research was completed.

8.2 Expression and Purification of MDM2 and MDMX

Three different constructs of MDM2 and MDMX encoding the N-terminal p53-binding domain were selected for co-crystallisation trials. This selection was based on the literature data as well as successes in protein expression experienced in-house.\textsuperscript{71, 86, 104-106} The constructs expressed and purified for co-crystallisation trials are listed in Table 8.1.

Table 8.1: Constructs of MDMX and MDM2 used for co-crystallisation trials.

<table>
<thead>
<tr>
<th>Protein</th>
<th>Construct</th>
<th>Amino acid range</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMX</td>
<td>1</td>
<td>18-111</td>
<td>-</td>
</tr>
<tr>
<td>MDMX</td>
<td>2</td>
<td>22-111</td>
<td>-</td>
</tr>
<tr>
<td>MDMX</td>
<td>3</td>
<td>26-111</td>
<td>-</td>
</tr>
<tr>
<td>MDM2</td>
<td>1</td>
<td>17-125</td>
<td>K51A</td>
</tr>
<tr>
<td>MDM2</td>
<td>2</td>
<td>17-109</td>
<td>E69A/K70A</td>
</tr>
<tr>
<td>MDM2</td>
<td>3</td>
<td>17-125</td>
<td>E69A/K70A</td>
</tr>
</tbody>
</table>

The pre-cloned DNA coding for each construct of MDMX and MDM2 were inserted into the pGEX-6P-1 vector and expressed in \textit{E. coli} Rosetta(DE3)pLys S and BL21(DE3) pLys S strains, respectively.

The pGEX-6P-1 plasmid contains several genes essential for the expression of the desired protein. The plasmid possesses genes encoding for ampicillin resistance, a glutathione-S-
transferase (GST) tag, a 3C viral protease cleavage site and the \textit{lac} operon (Figure 8.3). The expression of N-GST tagged MDM2 and MDMX was induced by the addition of IPTG (isopropyl \(\beta\)-D-1-thiogalactopyranoside).

\begin{figure}
\centering
\includegraphics[width=\textwidth]{pGEX-6P-1.png}
\caption{Map of the pGEX-6P-1 expression vector. The vector map shows the positions for the genes encoding ampicillin resistance (Amp\(^\text{r}\)), the GST protein tag, the cleavage codon (PreScission\textsuperscript{TM} Protease) and the \textit{lac} operon (lac 1\(^\text{q}\)).\textsuperscript{107}}
\end{figure}

The expressed proteins were first purified by the addition of lysozyme, DNase and RNase followed by sonication and centrifugation. The addition of these three enzymes and sonication lyses the \textit{E. coli} cells and subsequent centrifugation separates insoluble cell debris from soluble proteins, which include MDM2 or MDMX. To separate N-GST tagged MDMX or MDM2 from other undesired proteins, the supernatant obtained after centrifugation was incubated with glutathione-agarose and washed with mHBS buffer, pH 7.4 containing glutathione. SDS-PAGE analysis of the soluble cell lysate and the eluate from the glutathione column (Figure 8.4, lanes 2-7) confirmed the expression and purification of N-GST tagged MDMX or MDM2. Subsequent incubation with 3C protease successfully cleaved the GST tag from MDMX and MDM2 (Figure 8.4, lanes 8-10).

66
**Figure 8.4:** Purification of MDMX. SDS-PAGE (12% acrylamide) analysis of MDMX constructs 1, 2 and 3 at various stages during purification. Lanes 2-4, soluble cell lysate; lanes 5-7, glutathione elution; lanes 8-10, proteins post 3C digestion. Molecular weight markers, lane 1, uncleaved GST-MDMX (xkDa) is identified by a star.

The protein fractions containing MDMX obtained after elution from the glutathione column with mHBS buffer, pH 7.4 containing glutathione were pooled and incubated with 3C protease to cleave the GST tag from the desired protein. The protein was purified by size exclusion chromatography to separate the GST tag (Figure 8.5A). SDS-PAGE analysis of selected fractions confirmed the purity of the protein (Figure 8.5B). The pure protein fractions were concentrated by ultrafiltration and stored at -80 °C.
Figure 8.5: Purification of MDMX by size-exclusion chromatography. Construct C3 (MDMX\textsubscript{26-111}) was subjected to size-exclusion chromatography as a final purification step. The sample was run on a Superdex HR 16/60 column (GE Healthcare) equilibrated in mHBS buffer. (A) Chromatogram, (B) SDS-PAGE of selected fractions. Fractions analysed in (B) are identified by the black line under the chromatogram in (A).

8.3 Co-Crystallisation of MDM2 and MDMX with Pyrrole Inhibitors

Compounds for co-crystallisation trials were selected based on the LogD values (calculated using Stardrop and the IC\textsubscript{50} values against MDMX measured by ELISA assay (Table 8.2). The compounds with LogD values greater than 4 showed visible precipitation in the presence of MDM2 or MDMX. Compound 68 has a logD value greater than 4 but it was selected for the co-crystallisation trials because of a unique molecular feature: It has a succinimide ring on the 3\textsuperscript{rd} and 4\textsuperscript{th} position of the pyrrole. The nitrogen of the succinimide ring would be a good vector to reach other favourable interactions or to introduce any water solubilising groups. A co-crystal structure of compound 68 bound to MDMX would be valuable to design a series of compounds with a fused ring system.
<table>
<thead>
<tr>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
</tr>
<tr>
<td>67</td>
</tr>
<tr>
<td>81</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>93</td>
</tr>
<tr>
<td>68</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MDMX ELISA IC(_{50}) (µM)</th>
<th>LogD</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.4</td>
<td>1.6</td>
</tr>
<tr>
<td>56</td>
<td>3.2</td>
</tr>
<tr>
<td>15</td>
<td>3.3</td>
</tr>
<tr>
<td>7.9</td>
<td>3.4</td>
</tr>
<tr>
<td>Not tested</td>
<td>5.5</td>
</tr>
</tbody>
</table>
The inhibitors were incubated with the concentrated proteins in a 3:2 molar ratio at 4 °C overnight. The inhibitor-protein complexes were concentrated to between 1.5 to 18 mg/ml (Table 8.3). The concentrations of the protein-inhibitor complexes with MDM2 were between 10-20 mg/ml, however, precipitation was observed with MDMX complexes at concentrations higher than 4-5 mg/ml. Precipitation was mostly observed with compounds 68 and 93 and as a result crystal trays were not set up for these inhibitors.

Table 8.3: Protein-inhibitor concentration for co-crystallisation trials.

<table>
<thead>
<tr>
<th>Compound</th>
<th>MDMX constructs</th>
<th>MDM2 Constructs</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
<td>3.7</td>
<td>3.1</td>
</tr>
<tr>
<td>68</td>
<td>3.5</td>
<td>-</td>
</tr>
<tr>
<td>67</td>
<td>2.9</td>
<td>2.7</td>
</tr>
<tr>
<td>81</td>
<td>2.9</td>
<td>2.9</td>
</tr>
<tr>
<td>93</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

In an attempt to identify novel crystallisation conditions, MDMX-inhibitor complexes were screened against three commercial crystallisation screens, JCSG+ (Molecular Dimensions), Index (Hampton Research) and ammonium sulfate (Qiagen) and MDM2-inhibitor complexes were screened against JCSG+ and ammonium sulfate. 2-subwell 96-well trays were used for co-crystallisation trials (Figure 8.6). Screening solutions were first added to the 96 wells by hand. The solutions were also mixed with protein-inhibitor complexes in 1:1 and 2:1 ratios (protein-inhibitor complex:screen) and the mixtures dispensed into the subwells using a Mosquito robot. Trays were stored at 4 °C in a Rikagun Minstrel tray hotel which also imaged the subwells at set time intervals. The images were observed for 8 weeks to monitor crystal formation.
Figure 8.6: Crystallisation of MDM2 and MDMX with small molecule inhibitors. Complexes of MDM2 and MDMX bound to small molecule inhibitors were screened against selected commercial crystallisation screens in 96 well, 2 subwell plates. (A) The MRC crystallisation plate. (B) Close-up to show the juxtaposition of the two subwells and the screen well.

In total, 24 MDMX or MDM2-inhibitor complexes were subjected to crystallisation trials using three commercial screens for MDMX and two screens for MDM2. Overall, around 14,000 different conditions were used for co-crystallisation trials of MDMX and MDM2 with pyrrole-based inhibitors.

8.4 Co-Crystallisation of MDM2 and MDMX with Autoinhibitory domains

It was reported recently that MDMX contains an autoinhibitory sequence which interferes with its binding to p53. The autoinhibitory sequence is a tryptophan-rich segment within residues 190-210 of MDMX (FEEWDVAGLPWWFLGNLR). The tandem tryptophan residues at positions 200 and 201 have been called the WW element. Bista et al (2013) performed isothermal titration calorimetry (ITC) experiments to measure the binding affinity (K_d) of MDMX with p53 in the presence or absence of the autoinhibitory sequence. Full length MDMX (1-340) with an autoinhibitory sequence bound to p53 with a measured K_d of 2700 nM. MDMX (1-111) without the autoinhibitory sequence was 100-fold more tightly bound to p53 (K_d = 30.3 nM). MDMX without residues 193-210 had binding affinity of 92.6 nM with p53 (Table 8.4).
Table 8.4: Binding of different MDMX sequences to the transactivation domain of p53. The p53 transactivation domain is defined as residues 17-32.\textsuperscript{108}

<table>
<thead>
<tr>
<th>Protein</th>
<th>$K_d$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDMX 1-340</td>
<td>2700 ± 531</td>
</tr>
<tr>
<td>MDMX 1-111</td>
<td>30.3 ± 3.9</td>
</tr>
<tr>
<td>MDMX Δ193-210\textsuperscript{*}</td>
<td>92.6 ± 11.7</td>
</tr>
<tr>
<td>MDMX W200D/201D</td>
<td>385 ± 43.5</td>
</tr>
</tbody>
</table>

Residues 193-210 deleted.

Based on these findings reported by Bista et al (2013), it was hypothesised that the autoinhibitory sequence could stabilise MDMX and help in its crystallisation. Three autoinhibitory sequences (AID1, AID2 and AID3, Table 8.5) of different lengths were kindly supplied by Astex pharmaceuticals. All three autoinhibitory sequences contain the WW element which was reported as the most essential sequence for binding to the N-terminal domain of MDMX.

Co-crystallisation trials of AID with MDMX were set up using the JCSG+ and AmSO$_4$ screens (Table 8.5). Precipitation was observed when AID1 was incubated with MDMX construct 3. Similarly, AID3 showed precipitation with constructs 1 and 2.

Table 8.5: MDMX-AID co-crystallisation. Protein-autoinhibitory domain concentration for co-crystallisation trials.

<table>
<thead>
<tr>
<th>Autoinhibitory Domain</th>
<th>Sequence</th>
<th>MDMX-Peptide concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>MDMX constructs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>AID1</td>
<td>FEEWDVAGLPWWFLGN</td>
<td>17.7</td>
</tr>
<tr>
<td>AID2</td>
<td>GLPWWFL</td>
<td>15</td>
</tr>
<tr>
<td>AID3</td>
<td>FEEWDVAGLPWWFLGNLRSNY</td>
<td>-</td>
</tr>
</tbody>
</table>
The images generated by the Rikagu Minstrel tray hotel were reviewed at irregular intervals for 8 weeks. Unfortunately, from more than 15,000 different conditions, crystal formation was not observed. In most of the cases, precipitation of the protein-inhibitor complex was observed (Figure 8.7A). In one case, a more promising precipitation was found (Figure 8.7B).

![Image A](image1.png) ![Image B](image2.png)

**Figure 8.7**: MDMX crystallisation trials. Images taken by the Rikagu Minstrel tray hotel. (A) MDMX C1 + 67, (B) MDMX C2 + 81.

Based on the most promising leads from the initial screen, an optimisation screen was prepared by altering the concentration of the Li$_2$SO$_4$ (0-0.3 M), PEG400 (30-100%), sodium acetate (0.1 M) and pH (4-5.5) of the final solution. The five pyrrole-based inhibitors, AID1 and AID2 were used for co-crystallisation trials with MDMX constructs 1, 2 and 3 using this optimised screen. However, crystal formation was not observed after 6 weeks of monitoring.

### 8.5 Development of Homogenous Time-Resolved FRET assay

RO-2443 3 and RO-5963 4 are reported as dual inhibitors of MDM2 and MDMX with low nanomolar IC$_{50}$ values against both MDM2 and MDMX. However, the IC$_{50}$ values generated using the in-house ELISA were around 500-1000 fold less than the reported IC$_{50}$ values (Table 8.6). To investigate the discrepancies in IC$_{50}$ values between Roche’s homogenous time-resolved FRET (HTRF) assay and the NCL-ELISA assay, an HTRF assay was developed.
Table 8.6: IC₅₀ values of RO-2443 3 and RO-5963 4 obtained from ELISA assay and published HTRF values.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Roche’s HTRF assay</th>
<th>Newcastle ELISA assay</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MDM2 IC₅₀ (µM)</td>
<td>MDMX IC₅₀ (µM)</td>
</tr>
<tr>
<td>RO-2443</td>
<td>0.033</td>
<td>0.041</td>
</tr>
<tr>
<td>RO-5963</td>
<td>0.019</td>
<td>0.024</td>
</tr>
</tbody>
</table>

The HTRF (Homogenous Time Resolved Fluorescence) assay relies on the Fluorescence Resonance Energy Transfer (FRET) from an excited donor molecule to an acceptor molecule.¹¹⁰⁻¹¹¹ The donor molecule absorbs energy and transfers it to an acceptor. FRET efficiency (E) is inversely proportional to the sixth power of the distance between the donor and acceptor as shown in the equation below.¹¹² E is the FRET efficiency, R₀ is the Förster distance at which half of the energy is transferred and r is the actual distance between the donor and acceptor. When the donor and acceptor are not in close proximity, the FRET efficiency is reduced (Figure 8.8).

\[
E = \frac{R₀^6}{R₀^6 + r^6}
\]

HTRF is a time resolved FRET in that there is a time delay of 50-150 µs between the excitation of the donor and measurement of the FRET signal.¹¹¹ This time delay prevents measurement of any short-lived fluorescence which would otherwise interfere with measurement of the authentic signal.
In the HTRF assay established at Newcastle for measuring the potency of MDMX-p53 interaction inhibitors, GST-tagged MDMX (residues 22-111) was mixed with fluorescein-labelled IP3 peptide (Ac-16-MPRFMDYWQGLN-27-NH₂). IP3 peptide is a mutant form of p53 with high affinity for MDM2/MDMX.¹¹³ The fluorescein label on IP3 acts as an acceptor. A terbium-labelled anti-GST antibody which binds to GST-tagged MDMX was added to the mixture. The terbium label acts as a donor. Upon excitation, there is a transfer of FRET from donor to acceptor which can be measured (Figure 8.9A).

In the absence of an inhibitor, the donor (terbium) and the acceptor (fluorescein) are in close proximity. The donor terbium absorbs energy and transfers it to fluorescein. The transfer of energy is measured as a FRET signal. In the presence of an inhibitor, the donor and acceptor are separated which results in a decrease in the amount of FRET (Figure 8.9B).
Figure 8.9: Schematic representation of MDMX-FRET assay. (A) Without inhibitor, (B) With inhibitor.

The ELISA was performed by Dr Yan Zhao. In the assay, biotinylated IP3 peptide was bound to streptavidin coated plate (Figure 8.10). MDM2 and MDMX were incubated with the compound being tested and added to the plate containing IP3 peptide. Then a primary mouse antibody that binds to MDM2 or MDMX was added. Subsequently, a secondary goat-antimouse antibody, conjugated to horseradish peroxidase (HRP) was bound to the primary antibody. The plate was analysed using a luminometer to detect signals from HRP (see chapter 16.2.1 for details). The data obtained from the luminometer were analysed and the IC50 values derived.

There were key differences between the protocols of the ELISA and HTRF assays. The major difference is the length of MDMX used, and it is believed to be the major cause of discrepancies between the IC50s obtained from ELISA and HTRF assays. In the ELISA, full length MDMX was used, whereas, in the HTRF assay, the MDMX construct (residues 22-111) was used. The full length MDMX contains an autoinhibitory domain (described in section 8.3), which binds on the p53 binding domain of MDMX, thus, preventing the binding of p53 to MDMX. The MDMX construct used in the HTRF assay did not contain the autoinhibitory domain. The use of full length MDMX in HTRF assay development would require the optimisation of expression and purification of the desired protein, therefore, it was outside the scope of the structural biology placement undertaken. However, other differences in the assay protocols like the incubation order and times, different buffers were addressed during the HTRF assay development (described later in this chapter).
The HTRF assay development was done by Dr Judith Reeks. In a typical HTRF experiment, the transfer of FRET signals were measured with the decreasing concentration of inhibitors. The maximum value (positive control) was obtained when p53 and MDMX formed a complex in the absence of inhibitors. The minimum value (negative control) was obtained in the absence of both MDMX and inhibitor. The values were converted to % inhibition using the formula given below and plotted against the concentration of the inhibitor to get a dose-response curve and IC\textsubscript{50} value.

\[
\text{% Inhibition} = \frac{\text{FRET(Average of positive control)} - \text{FRET (inhibitor)}}{\text{FRET(Average of positive control)} - \text{FRET(Average of negative control)}} \times 100
\]

The assay had provided reasonable dose-response curves with stapled peptides and published small-molecule inhibitors, although the IC\textsubscript{50} values obtained from the assay were not consistent (Figure 8.11A).\textsuperscript{114} RO-5963 showed greater than 50% inhibition at the lowest concentration used in the assay. However, the other Roche compound, RO-2443 was significantly less potent in our HTRF assay compared to the published data. The IC\textsubscript{50} values from the assay were not reproducible, therefore, the data should not be over-interpreted. However, the discrepancies in the data may be due to the different constructs of MDMX used, but the construct of MDMX used in the assay was not disclosed by Roche. The investigation on the discrepancies observed,
and the development of other assay formats is currently ongoing. In favour of the assay, the signal to noise ratio of the assay was 25 and Z’ of the assay was 0.61. The published inhibitors showed dose-response relationships, and the error bars were low. However, when the Newcastle small molecule inhibitors were tested in this assay, the measurements had associated high error bars (Figure 8.11B). Following assay optimisation, the final assay concentrations were MDMX-3nM, IP3 peptide-125 nM, 1 nM Tb-antibody and DMSO-2%.
Figure 8.11: MDM2/X inhibitor evaluation by HTRF. HTRF assay results for (A) RO-2443 3, WK-298 22, and stapled peptides YSO1 and YSO2115, (B) Newcastle inhibitors. The assay was done in duplicates shown by the error bars.

The assay protocol required the inhibitor samples (provided in DMSO) to be diluted into assay buffer so that the final concentration of DMSO was 2%. Precipitation was observed with most of the Newcastle inhibitors when the DMSO solution of the inhibitors were added to the assay buffer. This resulted in high error bars and inhibition could not be observed with any Newcastle inhibitors.

In order to decrease precipitation of the inhibitors, the DMSO concentration was increased to 5%. However, this increase in the concentration of DMSO, decreased the $Z'$ and signal to noise (S/N) ratio. Therefore, the assay had to be redeveloped to improve the $Z'$ and S/N ratio. Initially,
MDMX at a range of concentrations was titrated against various concentrations of IP3 peptide to identify conditions that yielded a robust FRET readout at the lowest concentration of MDMX. The lowest concentration of MDMX and p53 after which the curve starts to flatten was chosen. Thus MDMX concentration was selected to be 10 nM as shown in Figure 8.12. The concentration of IP3 peptide was selected to be 300 nM as it was the rough average of the Kd values obtained.

**Figure 8.12:** Titration of IP3 against different concentration of MDMX.\(^{114}\) (A), Graph showing the FRET values at different concentration of IP3 and MDMX, (B) Table associated with the figure A.

To determine the optimal concentration of Tb antibody, the S/N ratio was determined at various antibody concentrations with decreasing concentration of MDMX (Figure 8.13). At around 10 nM MDMX, 10 nM antibody provided maximum S/N ratio. Therefore the final concentration of the Tb antibody was selected to be 10 nM.
Based on these results, the final assay condition was changed to 10 nM MDMX, 300 nM IP3 peptide, 5% DMSO in buffer A. After 1 h incubation of MDMX, IP3 and inhibitor, the terbium-labelled antibody was added to a final concentration of 10 nM and the final assay volume was brought to 20 µl. This new protocol provided good reproducible Z’ values (0.5-0.7) and two inhibitors showed measurable inhibition (Figure 8.14).

Figure 8.13: Signal to noise ratio at different concentrations of Tb antibody and MDMX.\textsuperscript{114}
Figure 8.14: HTRF results for Newcastle small molecule inhibitors. (A) Compounds without any significant inhibition (B) Compounds that show inhibition.

The assay was repeated again with selected Newcastle inhibitors with low logD values (Table 8.7). The compounds with low logD values showed dose-response curves. The lipophilic compound 305 which was the most potent isoindolinone inhibitor of MDMX-p53 interaction in the ELISA showed no measurable inhibition in the HTRF assay (Figure 8.15).
Table 8.7: List of Newcastle inhibitors with their logD values and MDMX ELISA IC$_{50}$ values.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>Structures</th>
<th>LogD</th>
<th>MDMX IC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>302</td>
<td><img src="image1.png" alt="Structure 302" /></td>
<td>2.7</td>
<td>29</td>
</tr>
<tr>
<td>64</td>
<td><img src="image2.png" alt="Structure 64" /></td>
<td>1.7</td>
<td>13</td>
</tr>
<tr>
<td>303</td>
<td><img src="image3.png" alt="Structure 303" /></td>
<td>1.9</td>
<td>110</td>
</tr>
<tr>
<td>304</td>
<td><img src="image4.png" alt="Structure 304" /></td>
<td>2.5</td>
<td>50% inhibition @ 200</td>
</tr>
</tbody>
</table>
Although, hydrophilic compounds provided reasonable dose-response curves, the results were not reproducible and the derived IC\textsubscript{50} values did not agree with the ELISA values. There were some major differences between the protocols of the ELISA and HTRF assay which are tabulated in Table 8.8.

**Table 8.8:** Protocol differences between the MDMX ELISA and HTRF assays.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>ELISA</th>
<th>HTRF</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>PBS buffer was used.</td>
<td>Tris buffer was used.</td>
</tr>
<tr>
<td></td>
<td>Inhibitors were incubated with MDMX for 45 min, then p53 peptide was added.</td>
<td>Inhibitors, MDMX and p53 peptide were added to the final reaction plate and incubated for 1 h.</td>
</tr>
<tr>
<td>2.</td>
<td>Full length MDMX was used.</td>
<td>MDMX construct was used.</td>
</tr>
<tr>
<td>3.</td>
<td>Proteins are immobilised.</td>
<td>Proteins are in solution.</td>
</tr>
</tbody>
</table>

Following this comparative analysis, the buffer was changed to PBS and the incubation sequence was changed in the HTRF assay to make the two assay protocols similar. However, these changes did not improve the HTRF assay results (Figure 8.16).
Figure 8.16: HTRF assay of Newcastle small molecule inhibitors. (A) With Tris buffer, (B) with PBS buffer. For both conditions, the order of addition of reagents was similar to that used in the ELISA as described in the 2nd point of Table 8.8.

The HTRF assay provided reasonable dose-response curves, with low error bars for literature inhibitors and peptides, but the IC\textsubscript{50} values were inconsistent. Most of the Newcastle inhibitors did not return a measurable IC\textsubscript{50} from the HTRF assay. Newcastle inhibitors provided high error bars and the results were not reproducible. The discrepancies in IC\textsubscript{50} values obtained from the ELISA and HTRF assay may be because of the different constructs of MDMX used. The discrepancies in the IC\textsubscript{50} values obtained for RO-2443 in the published HTRF assay and our HTRF assay might also be due to the difference in the length of MDMX used, but the MDMX construct was not disclosed by Roche. Further investigations on the different assay formats are ongoing. In order to get a good comparison between ELISA and HTRF assays, full length MDMX should be used in the HTRF assay. In addition, a label free assay such as Isothermal titration calorimetry (ITC) should be considered as an orthogonal assay. ITC does not rely on the fluorescence and depends on the change in enthalpy produced after the binding of the two
protein partners. Therefore, an assay with a different principle such as ITC might be beneficial to compare with the results obtained from ELISA and HTRF.
9.1 Introduction to Epigenetics

Every cell in an organism inherits the same genetic material, yet form structurally different tissues and organs with unique biological functions. This is due to the differences in the heritable epigenetic modifications, which include DNA methylation, histone modification and nucleosome positioning but do not include changes in the DNA sequence.\textsuperscript{116-118}

To understand the role of epigenetic modifications in producing different phenotypes, and its disease linkage, it is important to understand the architecture of chromatin formed by DNA and several proteins, most importantly, histones.

9.1.1 Chromatin Architecture

The diploid human genome contains approximately 6 billion base pairs of DNA packaged into 23 pairs of chromosomes per cell.\textsuperscript{119-120} Each base pair is around 0.34 × 10\textsuperscript{-9} metre long, therefore, each diploid cell contains approximately 2 meters of DNA ((0.34 × 10\textsuperscript{-9}) × (6× 10\textsuperscript{9})).\textsuperscript{119} The histone proteins play an important role in the packaging of 2 meters of DNA into a small volume of cell nucleus. The dimers of four histone proteins H2A, H2B, H3 and H4 form an octamer which wraps approximately 147 base pairs of DNA to form nucleosomes which are the basic subunit of chromatin.\textsuperscript{121-123}

Nucleosomes are connected by short DNA segments known as ‘linker DNA’. The linker DNA is either naked or bound to histone protein H1.\textsuperscript{123} The nucleosomes assemble to form chromatin fibres, which are further packaged to form chromosomes (Figure 9.1).\textsuperscript{120-121}
9.1.2 **Post-Translational Modifications of Histones**

The histone proteins undergo several post translational modifications which affect DNA centred processes, including DNA transcription, replication and repair as well as genomic architecture. The histone post-translational modifications also known as ‘histone marks’ are regulated by three groups of proteins known as writers, erasers and readers (Figure 9.3). Although, there are several post-translational modifications of histones including phosphorylation and ubiquitylation, the most important and the most widely studied are acetylation and methylation.

9.1.2.1 **Histone Acetylation**

Histone acetyltransferases (HATs) are the writer proteins responsible for the acetylation of histones. Acetylation takes place at the amino group of the lysine side chain of the histone tail (Figure 9.2). Acetylation neutralises the positive charge on lysine reducing the affinity of the histone tail for DNA, resulting in a relaxed chromatin architecture. Acetylation of histones has several effects depending on the site of acetylation. For example, acetylation of
H4K5 (means Histone H4, Lysine-5) and H4K12 are important for chaperone recognition during the formation of nucleosome core. Acetylation of H3K56 occurs on the histone within the nucleosome core and affects the interaction between histones and DNA.\(^{118,130}\)

![Chemical structure of L-Lysine and ε-N-acetylated lysine](image)

**Figure 9.2:** Neutralisation of the positive charge on lysine due to acetylation.

Histone deacetylases (HDACs) are the eraser proteins that help to remove the acetylation mark from the lysine residues of histones. HDACs are well-studied targets in the field of drug discovery and several clinically successful inhibitors of HDACs are reported to date (Table 9.1).\(^{131-133}\)

**Table 9.1:** Selected inhibitors of HDAC and their clinical status.\(^{133}\)

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vorinostat</td>
<td>Approved by FDA in 2006</td>
</tr>
<tr>
<td>Romidepsin</td>
<td>Approved by FDA in 2009</td>
</tr>
<tr>
<td>Panobinostat</td>
<td>Approved by FDA in 2015</td>
</tr>
<tr>
<td>Entinostat</td>
<td>Phase III</td>
</tr>
<tr>
<td>Mocetinostat</td>
<td>Phase II</td>
</tr>
<tr>
<td>Pracinostat</td>
<td>Phase III</td>
</tr>
<tr>
<td>Ricolinostat</td>
<td>Phase II</td>
</tr>
<tr>
<td>Resminostat</td>
<td>Phase II</td>
</tr>
</tbody>
</table>

### 9.1.2.2 Histone Methylation

There are different types of S-adenosylmethionine (SAM)-dependent methyl transferases that write one, two or three methyl marks mostly on the side chains of lysine and arginine residues.
of histones. Methylation on glutamine and histidine residues of histones have also been reported. Unlike acetylation marks, methylation does not neutralise the charge on the positively charged amino acids in histones. Therefore, methylation of histones does not directly impact on the chromatin architecture, instead, the methyl marks are recognised by several other proteins.

![Figure 9.3: Representation of post-translational histone modifications.](image)

### 9.2 Bromodomains

The post-translational modifications (PTMs) of histones are recognised and regulated by the reader proteins. To date, several classes of readers have been identified with conserved domains that detect the modifications on histone.

One of the most important class of readers are bromodomains, which were first identified in the brahma gene of *Drosophila melanogaster* and named after it. Bromodomains are the class of evolutionary conserved protein interaction modules which recognise the ε-N-acetylated lysines. A total of 61 bromodomains in 46 diverse proteins have been identified in the human proteome. They are divided into eight structural classes (Figure 9.4). Proteins containing bromodomains have been implicated in cancer, inflammation and viral diseases which make them one of the most studied targets currently in drug discovery.
**Figure 9.4:** Structure based classification of bromodomains. The eight different classes are represented by Roman numerals.\(^{126}\)

Bromodomains contain an acetylated lysine binding site in a conserved fold containing four \(\alpha\) helices (\(\alpha_Z\), \(\alpha_A\), \(\alpha_B\), \(\alpha_C\)) and two loop regions ZA and BC loops (Figure 9.5). Structural studies with peptides have demonstrated that the acetylated lysine residue lies in the deep hydrophobic pocket where it forms hydrogen bond interactions with the conserved asparagine residue. In addition, there are several conserved water molecules which take part in the network of interactions with the acetylated histone.\(^{141, 146}\)
**Figure 9.5**: X-ray crystal structure of the first bromodomain of human BRD4 in complex with a diacetylated histone 4 peptide (PDB: 3UVW). (A) Helices and loops are highlighted by different colour codes. (B) The charged nature of the surface lining the KAc binding site is also coloured using electrostatic module ranging from -10 to +10 kT/e.\(^{126}\)

### 9.3 Bromodomain Inhibitors

#### 9.3.1 BET-Bromodomain Inhibitors

To date, different classes of bromodomain inhibitors have been identified. The BET family in the subfamily II of the structure-based tree (Figure 9.4) is one of the most studied classes of bromodomains with several known potent small molecule inhibitors.\(^{127, 147}\) BET family comprises of BRD2, BRD3, BRD4 and the testes specific BRDT. BET proteins contain two bromodomains and an extra terminal region.\(^{141}\)

Although, few bromodomain inhibitors were reported in the literature,\(^{148}\) it was in 2010 that two potent and selective inhibitors of BET bromodomains (+)-JQ1 (120)\(^{149}\) and I-BET762 (121)\(^{150}\) were disclosed, and provided the proof-of-principle that bromodomains can be selectively inhibited by small molecules. This sparked the field and led to the development of several potent and selective bromodomain inhibitors.\(^{126, 147, 151}\)
(+)-JQ1 120 is the most widely studied bromodomain inhibitor which results in 179 hits in the scopus search field.\textsuperscript{152} The compound was developed by Filippakopoulos \textit{et al}. after successful optimisation of a benzodiazepine core based on the several patents disclosed between 1998 and 2006.\textsuperscript{149, 153-154} The compound was selective for BET bromodomains over other classes of bromodomains when studied against a panel of 46 human bromodomains.\textsuperscript{149} The first \textit{in vivo} study that demonstrated the effect of bromodomain small-molecule inhibitors in cancer therapy was done with (+)-JQ1 on a mouse xenograft with NUT (Nuclear Protein in Testis) midline carcinoma. At minimal toxicity dose, the compound showed reduction in tumour volume and promoted survival (Figure 9.6).\textsuperscript{149} Similarly, other studies with different BET bromodomain inhibitors have shown reduction in tumour volumes in various mouse models of tumours including acute myeloid leukaemia, gliobastoma, melanoma and prostate cancer.\textsuperscript{155-159}

\textbf{Figure 9.6}: (+)-JQ1 (red line, 50 mg kg\textsuperscript{-1}, daily) on NUT midline carcinoma xenograft model produces (A) decrease in tumour volume, (B) prolonged survival. Vehicle is shown by black line.\textsuperscript{149}
9.3.2 Non-BET bromodomain Inhibitors

After successful development of several small-molecule inhibitors of the BET bromodomains, drug discovery efforts were shifted towards the development of bromodomains outside the BET family. Structural Genomics Consortium (SGC) are aiming to develop a selective inhibitor for each bromodomain.\textsuperscript{160} Bromosporine \textbf{122} was developed at SGC and is a pan-bromodomain inhibitor that inhibits various bromodomains, including BRD2(1), BRD3(1), BRD4(1), BRDT(1), CECR2, TAF1(2), BRD9, and CREBBP.\textsuperscript{161} The compound can be used as a chemical tool for identifying the biological role of the bromodomains for which selective inhibitor have not been developed.

Various selective small molecule inhibitors of non-BET bromodomains have been reported. SGC-CBP30 (\textbf{123}) for CREBBP, GSK2801 (\textbf{124}) for BAZ2A/B, PFI-3 (\textbf{125}) for SMARCA4 and PB1 are few examples of the selective non-BET bromodomain inhibitors.\textsuperscript{162-164}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{images}
\caption{Chemical structures of bromodomain inhibitors.}
\end{figure}

- SGC-CBP30 \textbf{123} for CREBBP, IC = 79 nM
- GSK2801 \textbf{124} for BAZ2A/B, K\textsubscript{D} = 257 nM
- PFI-3 \textbf{125} for SMARCA4, K\textsubscript{D} = 89 nM


9.4 ATAD2 and its Role in Cancer

ATAD2 contains a double AAA+ ATPase domain and a bromodomain. It is also known as ANCAA (AAA nuclear co-regulator cancer-associated protein), and belongs to subfamily IV in the structure-based classification of bromodomains (Figure 9.4). ATAD2 is overexpressed in a wide range of human cancers including breast, lung, prostate, ovarian, liver, osteosarcoma and gastrointestinal carcinomas, and it is present in low levels in normal non-tumour cells. ATAD2 was overexpressed in 23% of 172 tumour specimens analysed by Ciró et al (Table 9.2). In a separate study by Kalashnikova et al, ATAD2 was shown to be overexpressed in around 70% of breast tumours.

Table 9.2: ATAD2 expression in primary tumours.

<table>
<thead>
<tr>
<th>Tumour</th>
<th>No. of Samples</th>
<th>Highly expressed, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast carcinoma</td>
<td>25</td>
<td>9 (36)</td>
</tr>
<tr>
<td>Colon carcinoma</td>
<td>27</td>
<td>10 (37)</td>
</tr>
<tr>
<td>Lung carcinoma</td>
<td>22</td>
<td>5 (23)</td>
</tr>
<tr>
<td>Prostate carcinoma</td>
<td>24</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Stomach carcinoma</td>
<td>21</td>
<td>9 (43)</td>
</tr>
<tr>
<td>Uterus carcinoma</td>
<td>20</td>
<td>4 (20)</td>
</tr>
<tr>
<td>Lymphoma</td>
<td>12</td>
<td>2 (17)</td>
</tr>
<tr>
<td>Melanoma</td>
<td>11</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Sarcoma</td>
<td>10</td>
<td>1 (10)</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>40 (23)</td>
</tr>
</tbody>
</table>

ATAD2 acts as a coactivator of various transcription factors including androgen receptor (AR), estrogen receptor (ER) and MYC. ATAD2 is crucial for the transcription of various androgen genes in prostate cancer. Zou et al. showed that suppression of ATAD2 expression using ATAD2-RNAi inhibited the proliferation of androgen-dependent and androgen-independent or hormone refractory prostate cells (Figure 9.7).
Figure 9.7: Suppression of ATAD2 expression in (A) androgen-dependent LNCaP cell lines (B) androgen-independent cell lines LNCaP-C4-2B, and (C) CW22Rv1. Cells were plated in hormone-deprived medium for 24 h before being infected with adeno-RNAi-ANCCA (ATAD2-RNAi) or adeno-RNAi-GFP control, or mock-infected (for LNCaP, also act as a control).167

Similarly, ATAD2 is also a co-activator of estrogen receptor (ER), thus it is highly expressed in breast cancer.168 ATAD2 is an important cofactor for MYC-dependent transcription. MYC is a critical regulator of cell proliferation, therefore, enhancement of MYC transcriptional activity by ATAD2 contributes to the development of many human cancers.171-172 Various knock-down studies using siRNA and shRNA technologies have confirmed the role of ATAD2 in apoptosis, cell growth and proliferation in several cancer cell lines.170, 172 Altogether, these studies suggest that ATAD2 is an important target for cancer drug discovery, and a small-
molecule inhibitor would provide an insight on the phenotypic response to the inhibition of ATAD2.

### 9.5 Structure and Druggability of ATAD2 Bromodomain

Similar to other bromodomain structures, ATAD2 has four left-handed helices ($\alpha_Z$, $\alpha_A$, $\alpha_B$, $\alpha_C$) and two loops (ZA and BC). The acetyllysine binding pocket is created by two helices ($\alpha_B$, $\alpha_C$), and ZA loop. The amino acids involved in the binding site are Val1008, Val1013, Val1018, Tyr1063 and Ile1074 (Figure 9.8A).

![Figure 9.8](image)

**Figure 9.8:** (A) Crystal structure of ATAD2 in apo form (PDB: 3DAI). The Asn1064 is evolutionarily conserved and is important for the histone recognition. The other highlighted amino acid residues form the acetyllysine binding pocket. (B) Acetyllysine binding surface of ATAD2 bound to H4K5ac (4QUU). The secondary structure of ATAD2 is coloured in blue. The acetyllysine binding surface of ATAD2 is coloured in green. The amino acid residues are coloured based on atoms; carbon: grey, oxygen: red, nitrogen: blue.

The crystal structure of ATAD2 bound to acetyllysine (PDB: 4QSP) shows the key hydrogen bond interaction that anchors the acetyllysine in the binding site (Figure 9.9). The oxygen atom of the acetyl group forms two key hydrogen bond interactions, one with the Asn1064 and
the other with Tyr1021 via a water molecule. ATAD2, like other bromodomains, contains conserved water molecules which are important in the binding of acetyllysine.\textsuperscript{141, 169, 173}

![Diagram of ATAD2 interactions with acetyllysine](image)

**Figure 9.9:** Interactions of ATAD2 with acetyllysine (PDB: 4QSP).\textsuperscript{169} A bound ethylene glycol molecule (highlighted in cyan), water molecules (in pink spheres), Acetyllysine (carbons: yellow, oxygen: red, nitrogen: blue) are shown.

ATAD2 has some flexibility in its loops. Poncet-Montage \textit{et al} studied the flexibility of ATAD2 using different conditions for crystallising ATAD2 in its apo form.\textsuperscript{173} They obtained four crystal structures of apo-ATAD2 and compared with the published structures (PDB: 3DAI and 4QUU). Superimposition of the six structures demonstrates the flexibility in the ZA loop and the BC loop (Figure 9.10).

![Diagram of ATAD2 superimposition](image)

**Figure 9.10:** Superimposition of the 6 crystal structures of ATAD2. Red, yellow and green ZA loop represents the ATAD2 states from wide-open to closed state, respectively.\textsuperscript{173}
The acetyllysine binding site of ATAD2 is polar and shallow compared to several other bromodomains. In Figure 9.11, the acetyllysine binding pocket of the bromodomain of ATAD2 is compared with the bromodomain of BRD4. The ZA loop of ATAD2 which forms a major part of the binding site is polar, whereas, the binding site in BRD4 is mostly hydrophobic.
Figure 9.11: Comparison of the acetyllysine binding pocket of (A) ATAD2 (PDB: 4QUU) and, (B) BRD4 (PDB: 3UVW). The solvent accessible surface (probe radius 1.4) was coloured based on the charge; blue for positive charge and red for negative charge.

Altogether, due to the flexibility of the ZA loop, as well as, the shallow and polar nature of the binding site, ATAD2 bromodomain was grouped in the list of low-druggable bromodomains. However, very recently a low nanomolar inhibitor of ATAD2 was reported by Bamborough et al. which is discussed in detail later in this chapter.
9.6 Reported ATAD2 Bromodomain Inhibitors

9.6.1 Fragments Targeting ATAD2 Bromodomains

In 2014, Chaikuad et al used fragment based approach to identify thymine analogues (126-130) as a chemical starting point for the development of more potent inhibitors of ATAD2 bromodomain-acetylated histone interaction. The optimisation of those fragments was reported as an on-going activity, and the results have not been published yet. Initial attempts to screen a library of acetyllysine mimetics using high-throughput screening methods revealed no potent hits. Therefore, co-crystallisation and NMR chemical shift perturbation experiments were used to identify the fragments. The binding affinities of the fragments were determined by the NMR chemical shift perturbation experiments where the chemical shifts of $^{13}$C-methyl-labelled ATAD2 was monitored. The fragments showed low millimolar binding affinities for ATAD2 bromodomain. As reported with apo-ATAD2, the flexibility of the ZA loop was also observed in the presence of thymine based ligands.

Co-crystal structures of different thymine-based fragments bound to ATAD2 showed that the thymine ring formed the canonical acetyllysine mimetic H-bond with Asn1064 and the water mediated H-bond with Tyr1021 (Figure 9.12).

![Thymine 126](image1)

![Thymidine 127](image2)

![128](image3) $K_D = 21$ mM

![129](image4) $K_D = 17$ mM

![130](image5) $K_D = 18$ mM
Simultaneously, Harner et al. (2014) reported the results of a fragment-based screen using NMR against ATAD2 bromodomain. A total of 65 hits were identified against ATAD2 bromodomain which were clustered into three chemical groups (Table 9.3). Cluster 1 contained the fragments with 5,6 and 6,6-fused ring systems that have been previously identified as acetyllysine mimetics. Cluster 2 contained fragments unique to ATAD2 bromodomains with tricyclic chemotypes. Cluster 3 also contained unique fragments with sulfur containing chemotypes.
<table>
<thead>
<tr>
<th>Cluster 1</th>
<th>$K_d$ (µM)</th>
<th>Cluster 2</th>
<th>$K_d$ (µM)</th>
<th>Cluster 3</th>
<th>$K_d$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="131.png" alt="Image" /></td>
<td>600</td>
<td><img src="135.png" alt="Image" /></td>
<td>350</td>
<td><img src="139.png" alt="Image" /></td>
<td>400</td>
</tr>
<tr>
<td><img src="132.png" alt="Image" /></td>
<td>600</td>
<td><img src="136.png" alt="Image" /></td>
<td>400</td>
<td><img src="140.png" alt="Image" /></td>
<td>450</td>
</tr>
<tr>
<td><img src="133.png" alt="Image" /></td>
<td>650</td>
<td><img src="137.png" alt="Image" /></td>
<td>450</td>
<td><img src="141.png" alt="Image" /></td>
<td>500</td>
</tr>
<tr>
<td><img src="134.png" alt="Image" /></td>
<td>650</td>
<td><img src="138.png" alt="Image" /></td>
<td>600</td>
<td><img src="142.png" alt="Image" /></td>
<td>500</td>
</tr>
</tbody>
</table>

Table 9.3: Representative ATAD2 bromodomain fragment hits identified by Harner et al.$^{176}$

Three fragments from different clusters were used for co-crystallisation studies. Out of the three fragment, two of them displaced conserved water molecules from the KAc binding site. Fragment 135 displaces four conserved water molecules which may account for the higher binding affinity. Fragment 142 displaces one conserved water molecule and the position of the water is occupied by an amine substituent (Figure 9.13).
Figure 9.13: View of Kac binding site in presence of (A) fragment 131, (B) fragment 135, (C) fragment 142. Fragment 131 does not displace any conserved waters, whereas, fragment 135 displaces four and fragment 142 displaces one conserved water.\textsuperscript{176} PDB: 4TYL, 4TZ2, and 4TZ8, respectively.

Poncet-Montage et al (2015) reported two isoxazole based compounds 143 and 144 as ATAD2 bromodomain binders.\textsuperscript{173} The compounds were identified from X-ray crystallography screen of fragments and known acetyllysine mimetic chemical scaffolds.

None of these compounds were potent enough to be used as a chemical tool to study the implications ATAD2 bromodomain inhibition in cells.

\begin{align*}
\text{IC}_{50} &= 96 \mu \text{M} \quad \text{143} \\
\text{IC}_{50} &= 422 \mu \text{M} \quad \text{144}
\end{align*}
9.6.2 Naphthyridone Series

The first, and the only potent and selective small molecule inhibitor of the ATAD2 bromodomain reported to date was developed very recently.\textsuperscript{175,177} The small molecule inhibitor development started from a quinolone fragment 145 inspired from the dihydroquinazoline fragments known to bind the BET bromodomains, as seen in inhibitor PFI-1 146 and its quinolin-2-one analogue 147.\textsuperscript{177}

A crystal structure of fragment 145 bound to ATAD2 revealed that the carbonyl group mimics the acetyllysine binding mode. The carbonyl oxygen forms H-bond with Asn1064 and a water mediated H-bond with Tyr1021. The N-H forms an additional H-bond with the Asn1064. The methyl group points in the same pocket as occupied by the methyl group of the acetyllysine (Figure 9.14).

**Figure 9.14:** Overlay of the crystal structures of ATAD2 bromodomain bound to capped acetylated lysine (grey, PDB: 5a5n) and fragment 145 (magenta, PDB: 5a5o).
A focussed set of compounds based on quinolinone core were screened against ATAD2 bromodomain using time-resolved fluorescence resonance energy transfer (TR-FRET) assay. Compound 148 was considered as a ligand efficient starting point (LE = 0.3), and SAR studies at the C5-position resulted in the identification of a low micromolar inhibitor (149, ATAD2 $IC_{50} = 1.3$ µM, Figure 9.15). Although, the compounds were the most potent inhibitors reported to date, they were not selective for ATAD2 over other BET bromodomains.\textsuperscript{177}

![Chemical Structures]

ATAD2 $IC_{50} = 100$ µM  
ATAD2 $IC_{50} = 1.3$ µM

**Figure 9.15:** Development of the first low-micromolar inhibitor of ATAD2.\textsuperscript{177}

To improve the selectivity and potency of the inhibitor 149, the RVF shelf of ATAD2 was targeted (Figure 9.16B).\textsuperscript{175} The RVF shelf is formed by three amino acids Arg1007, Val1008 and Phe1009. It is in the same area as the WPF shelf (formed by Trp-Pro-Phe) in BET bromodomains. The WPF shelf has been exploited by potent BET inhibitors including (+)-JQ1 as well as I-BET762 (Figure 9.16A). RVF shelf in ATAD2 bromodomain is shallow and polar compared to the WPF shelf in BET bromodomains which was used as an advantage to get selectivity over BET bromodomains.
Figure 9.16: (A) BRD4 BD1 in complex to I-BET762 (PDB: 3p5o); (B) ATAD2 bromodomain in complex to a quinolone compound (PDB: 5a5q). The detailed interactions are on the left and protein surface coloured based on electrostatic charge on the right. A purple arrow head on (B) denotes the area of SAR investigation.\textsuperscript{175}

SAR studies at the 3’-position of the piperidine ring with the aim to target the RVF shelf gave compound \textbf{150} with desired potency and >100-fold selectivity for ATAD2 over other BET bromodomains (Figure 9.17). However, the compound was very hydrophilic with low cell-permeability. Attempts to increase cell permeability by reducing the polar surface area and the number of H-bond acceptors and donors resulted in reduced potency and selectivity. As described by Bamborough \textit{et al}, the further optimisation of the series to obtain a cell permeable, potent and selective ATAD2 small molecule inhibitor is underway and will be published in the near future.\textsuperscript{175}
ATAD2 pIC$_{50}$ (TR-FRET) 6.9
BRD2-4 BD1/2 pIC$_{50}$ (TR-FRET) 4.3-5.3
Chemiluminescent nitrogen detection (CLND) solubility (µM) 179
Artificial membrane permeability (nm/s, pH 7.4) <3

**Figure 9.17**: Summary of properties of compound 150.$^{175}$
Chapter 10. Development of ATAD2 Inhibitors: SAR strategies

10.1 Fragment hits

Seventeen fragment hits bound in the acetylysine binding site of ATAD2, were identified at Astex Pharmaceuticals by X-ray crystallographic focused screening. The fragments were clustered into five different classes based on their structures (Figure 10.1).

Cluster 1 (NMP-like)

Cluster 2 (Triazoles)

Cluster 3 (Acetamides)
Cluster 4 (Isoxazoles)

Cluster 5 (Others)

**Figure 10.1:** Structure-based classification of fragment hits identified at Astex Pharmaceuticals.

The initial strategy was to grow the fragments with subtle modifications including addition of alkyl or aryl groups in the lipophilic region of the binding surface, or adding H-bond donors or acceptors near the polar region of the binding surface. The work described in this thesis includes the SAR studies around four fragments 5, 157, 158 and 160.
10.2 Preliminary SARs around Fragment 157, 158 and 160

10.2.1 Rationale

The crystal structure of ATAD2 in complex with fragment 158 revealed that the amide group mimics the acetyllysine binding mode (Figure 10.2). The oxygen atom of the amide group forms H-bond with Asn1064 and a water mediated H-bond with Tyr1021.

Figure 10.2: Crystal structure of fragment 158 (carbons: grey) bound to ATAD2. The binding surface is coloured based on the charge, positive: blue and negative: red. The ZA-shelf is shown by dark pink coloured circle. The pink arrow denotes the area of potential SAR investigation.

A shelf around the ZA loop (hereafter referred to as ZA-shelf) was identified from the crystal structure (Figure 10.2). Small alkyl substituents at the 3-position of fragment 158 were selected to exploit the ZA-shelf (Figure 10.3).

Figure 10.3: 3-Substituted benzamides to be synthesised.
Similarly, the crystal structure of fragment 160 bound to ATAD2 showed that the oxygen of amide forms H-bond with Asn1064, and a water mediated H-bond with Tyr1021 (Figure 10.4).

**Figure 10.4:** Crystal structure of fragment 160 (carbons: grey) bound to ATAD2. The binding surface is coloured based on the charge, positive: blue and negative: red. The ZA-shelf is shown by pink coloured circle. The pink arrow denotes the area of potential SAR investigation.

It was postulated that substitution at the 2-position of the phenyl ring could access the ZA-shelf. Alkyl and alkoxy substituents were selected for preliminary investigation (Figure 10.5).

**Figure 10.5:** 2-Substituted phenylacetamides to be synthesised.

Crystal structure of triazole fragment 157 bound to ATAD2 showed that the compound adopted a different binding mode compared to other fragments (Figure 10.6). The H-bond with Asn1064 and water mediated H-bond with Tyr1021 was formed with two different nitrogen atoms in the
triazole ring, whereas, the two H-bonds were formed with one oxygen atom in fragments 158 and 160.

![Crystal structure of fragment 157 bound to ATAD2](image)

**Figure 10.6:** Crystal structure of fragment 157 (carbons: grey) bound to ATAD2. The binding surface is coloured based on the charge, positive: blue and negative: red. The ZA-shelf is shown by pink coloured circle. The pink arrow denotes the area of potential SAR investigation.

Interestingly, fragment 157 has a bromine atom on the pyridyl ring that could be utilised as a vector to exploit the ZA-shelf. Therefore, small alkyl substituents were selected for initial investigation (Figure 10.7).

![5-substituted triazolopyridines](image)

**Figure 10.7:** 5-substituted triazolopyridines to be synthesised.

### 10.2.2 Synthesis

Synthesis of compound 172 began with formylation of the commercially available phenol 167 using magnesium chloride and paraformaldehyde (Scheme 10.1). Subsequent Pinnick
oxidation of the aldehyde 168 gave carboxylic acid 169, which was bis-methylated to obtain compound 170. Hydrolysis of ester 170 followed by amide coupling with methylamine provided the target 172 in good yield.

Scheme 10.1: Reagents and conditions: (a) paraformaldehyde, NEt₃, MgCl₂, MeCN, reflux, 3 h, 93%; (b) NaClO₂, sulfamic acid, H₂O, MeCN, r.t., 8 h, 88%; (c) MeI, Cs₂CO₃, DMF, r.t., 18 h, 58%; (d) NaOH, H₂O, MeOH, 60 °C, 18 h, 95%; (e) MeNH₂, DIC, HATU, DMAP, THF, r.t., 18 h, 71%.

Compounds 175 and 178 were prepared following a two-step reaction sequence (Scheme 10.2). Reduction of commercially available nitrophenols 173 and 176 provided the corresponding amines, subsequent acetylation afforded targets 175 and 178.

Scheme 10.2: Reagents and conditions: (a) H₂, 10% Pd/C, MeOH, r.t., 24 h; (b) Ac₂O, AcOH, r.t., 30 min, (175, 80%; 178, 71%, over 2 steps).
To access triazolo[4,3-α]pyridine analogues, two different routes were employed. The first route involved S_N_Ar reaction on commercially available compound 179. 2-Fluoro-5-methylpyridine 179 was resistant to S_N_Ar reaction with acethydrazide 180 at various conditions (Table 10.1).

**Table 10.1:** Reagents and conditions used in step a

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Base</th>
<th>Temp °C</th>
<th>Time h</th>
<th>Result*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>None</td>
<td>100</td>
<td>3</td>
<td>No conversion</td>
</tr>
<tr>
<td>Ethanol</td>
<td>None</td>
<td>100 under µW</td>
<td>3</td>
<td>No conversion</td>
</tr>
<tr>
<td>Ethanol</td>
<td>NEt₃</td>
<td>100</td>
<td>18</td>
<td>No conversion</td>
</tr>
<tr>
<td>Ethanol</td>
<td>DIPEA</td>
<td>100</td>
<td>18</td>
<td>No conversion</td>
</tr>
</tbody>
</table>

*Observations from TLC and LCMS analyses

Failure of the S_N_Ar reaction might be due to the decrease in nucleophility of hydrazine in acethydrazide 180, caused by the electron withdrawing acetyl group. Therefore, the S_N_Ar reaction was performed on commercially available compound 179 with unsubstituted hydrazine to get compound 183 (Scheme 10.3). Subsequent ring closure was performed in the presence of acetic acid and acetic anhydride, and gave target 182 in poor isolated yield.

**Scheme 10.3:** *Reagents and conditions:* (a) hydrazine, EtOH, reflux, 72 h, 43%; (b) Ac₂O, AcOH, reflux, 24 h, 16%.
The second route to access triazolo[4,3-a]pyridine analogues involved Suzuki-Miyaura coupling of compound 157 with alkyl boronic acids. Unfortunately, the coupling reaction with ethyl boronic acid was not successful. Further cross-coupling attempts were therefore performed with vinylboronic acid pinacol ester, followed by reduction (Scheme 10.4). However, the intermediate was found to be unstable with a noticeable colour change from beige to dark brown upon standing. A plausible explanation for the observed degradation is that the ring is very electron rich, therefore, susceptible to oxidation in presence of air. Literature searches of compound 186 and 187 were undertaken using Reaxys® and SciFinder® but with no success as the compounds had not been reported yet. Degradation of compounds with the triazolo[4,3-a]pyridine scaffold was also observed by our bioscientist colleague Dr Matthew Martin whilst performing the HTRF assay. Therefore, the series was not explored further due to the observed degradation of the intermediates 184 and 185.

Scheme 10.4: Reagents and conditions: (a) vinylboronic acid pinacol ester, or isopropenylboronic acid pinacol ester, NaOH, N,N-dicyclohexylmethylamine, Pd(dppf)Cl₂,DCM, THF, 95 °C, 2.5 h, (184, 51%; 185, 62%, over 2 steps); (b) H₂, 10% Pd/C, MeOH.

10.2.3 Biological Evaluation

A homogenous time resolved FRET assay (HTRF) was used to measure the ATAD2 inhibitory activity. GST-tagged ATAD2 was mixed with biotinylated acetylated ligand (Figure 10.8). Streptavidin labelled phycobiliprotein pigment purified from red algae (SA-XL665), 111 which binds to the biotinylated acetylated ligand, and a terbium-labelled anti-GST antibody that binds to GST-tagged ATAD2 was added to the mixture. The terbium label acts as a donor of FRET signal, and SA-XL665 acts as an acceptor of the FRET signal. Upon excitation, there is a
transfer of FRET from donor to acceptor which can be measured. In the absence of an inhibitor, the donor (terbium) and the acceptor (SA-XL665) are in close proximity. The donor terbium absorbs energy and transfers it to SA-XL665. The transfer of energy is measured as a FRET signal. In the presence of an inhibitor, the donor and acceptor are separated which results in a decrease in the amount of FRET. The experimental details of the HTRF assay are discussed in 16.4.1.

**Figure 10.8:** Schematic representation of ATAD2-HTRF assay.

Compounds 172, 175, 178 and 182 did not show measurable inhibition by Homogenous Time Resolved FRET assay (HTRF) at 4 mM concentration. In addition, co-crystallisation experiments of ATAD2 with the compounds had failed. Meanwhile, Dr Duncan Miller had demonstrated that subtle modifications in fragment 5 improved ATAD2 inhibition and the compounds were successfully co-crystallised with ATAD2. Besides, fragment 5 was a novel scaffold which had not been reported to date for bromodomain inhibition. Therefore, most of the medicinal chemistry effort was focussed in SAR studies around fragment 5 which will be discussed in detail in following chapters.

![Fragment 5](attachment:image.png)
Chapter 11. SAR Studies around the N₁-Position of Fragment 5

11.1 Rationale

Crystal structure of fragment 5 bound to ATAD2 showed that the oxygen atom of the pyridone ring formed two key H-bonds, one with Asn1064 and the other water mediated H-bond with Tyr1021 (Figure 11.1).

![Crystal structure of fragment 5](image)

**Figure 11.1**: Crystal structure of fragment 5 (carbons: grey) bound to ATAD2. The binding surface is coloured based on the charge, positive: blue and negative: red. The ZA-shelf is shown by the dark pink coloured circle. The pink arrow denotes the area of potential SAR investigation. Water molecules are shown as red spheres.

The initial strategy was to explore SARs at the N₁-position of the pyrrolidinone ring to access the ZA-shelf (Figure 11.1). A range of polar and lipophilic substituents were selected (Figure 11.2).
Figure 11.2: $N^1$-substituted targets to be synthesised.

11.2 Synthesis

The strategy was to synthesise fragment 5 in bulk quantity, and subsequent alkylation reactions would provide the desired targets. The synthesis started with $N$-Boc protection of commercially available 6-methoxy-4-methylpyridin-3-amine 188 to get compound 189, which was used in a carboxylation reaction to give compound 190 (Scheme 11.1). The ring closure was mediated by acetic anhydride in the presence of catalytic tetrabutylammonium acetate to give compound 191. Subsequent bis-methylation provided compound 192. The $N$-Boc deprotection, as well as formation of pyridone was achieved in a single step by heating in the presence of MeI and catalytic sodium iodide to get compound 5. Formation of pyridone using MeI and sodium iodide is a widely used method to synthesise pyridones from 2-alkoxypyridines.\textsuperscript{179-182} Interestingly, when the reaction was performed in the absence of sodium iodide, quantitative yield of the product was obtained.
Scheme 11.1: Reagents and conditions: (a) Boc₂O, THF, Na₂CO₃, THF, r.t., 42 h, 95%; (b) (i) s-BuLi, THF, -78 °C, 15 min; (ii) CO₂ (dry ice), -78 °C to r.t., 45 min, 74%; (c) Ac₂O, tetrabutylammonium acetate, 65 °C, 1 h, 81%; (d) MeI, Cs₂CO₃, MeCN, 60 °C, 3 h, 78%; (e) MeI, MeCN, 170 °C, μW, 1 h, quant.

Targets 193 and 194 were synthesised following NaH-mediated alkylation reactions (Scheme 11.2).

Scheme 11.2: Reagents and conditions: (a) (i) NaH, DMF, r.t., 15 min; (ii) benzyl bromide or (2-bromoethyl)benzene, r.t., 3 h, 61%; (193), 30% (194).

The isolated yields of NaH mediated alkylation reactions were low to moderate. Besides, isolation of the products was difficult due to close-running impurities. Therefore, in search of alternative alkylation conditions, the stronger base NaH was replaced by caesium carbonate. Caesium carbonate mediated alkylation reactions were faster, and isolation of the product easier (Scheme 11.3).
Scheme 11.3: Reagents and conditions: (a) 1-(2-chloroethyl)pyrrolidine hydrochloride or 4-(2-chloroethyl)morpholine hydrochloride, Cs$_2$CO$_3$, DMF, 100 °C, µW, 30 min, 40% (195), 82% (196).

Target 198 was obtained from a two-step reaction sequence. Alkylation of compound 5 with chloromethyl phenyl sulphide gave sulphide 197, which was subsequently oxidised using Oxone® to the corresponding sulfone 198 (Scheme 11.4).

Scheme 11.4: Reagents and conditions: (a) chloromethyl phenyl sulphide, Cs$_2$CO$_3$, DMF, 100 °C, µW, 30 min, 75%; (b) Oxone®, MeOH, H$_2$O, r.t., 18 h, 52%.

Initial attempt to synthesise target 202 by alkylation of compound 201 using 2-bromoethan-1-ol 199 was unsuccessful. Therefore, an alternate synthetic route was followed to prepare target 202 (Scheme 11.5). The synthesis started with TBS protection of commercially available alcohol 199 to get compound 200, which was used for alkylation of compound 5 to obtain compound 201. Finally, TBS-deprotection provided target 202 in good yield.
Scheme 11.5: Reagents and conditions: (a) TBSCI, NEt₃, DMAP, DCM, r.t., 18 h, quant.; (b) 200, Cs₂CO₃, DMF, 100 °C, µW, 30 min, 55%; (c) TBAF, THF, r.t., 18 h, 90%.

11.3 Biological Evaluation

Diverse substitution at N¹-position of fragment 5 did not increase the ATAD2 inhibitory activity. The compounds did not show ATAD2 inhibition at 2 mM concentration using the HTRF assay (Table 11.1).
Table 11.1: ATAD2 inhibitory activity of derivatives with various substituents at \(N^1\)-position.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>ATAD2 IC\textsubscript{50} (µM)</th>
<th>Compound</th>
<th>R</th>
<th>ATAD2 IC\textsubscript{50} (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>193</td>
<td></td>
<td>&gt;2000</td>
<td>197</td>
<td></td>
<td>&gt;2000</td>
</tr>
<tr>
<td>194</td>
<td></td>
<td>&gt;2000</td>
<td>198</td>
<td></td>
<td>&gt;2000</td>
</tr>
<tr>
<td>195</td>
<td></td>
<td>&gt;2000</td>
<td>202</td>
<td></td>
<td>&gt;2000</td>
</tr>
<tr>
<td>196</td>
<td></td>
<td>&gt;2000</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Therefore, focus was shifted towards the substitution on the other nitrogen \((N^6)\) of the fragment 5, and is discussed in detail in the next chapter.
Chapter 12. SAR Studies around the N\textsuperscript{6}-Position of Fragment 5

12.1 Rationale

SAR studies conducted at the NICR by Dr Duncan Miller identified that methylation at the N\textsuperscript{1}-position of fragment 5 to obtain fragment 203 changed the binding mode of the scaffold. The oxygen atom of the pyridone ring in the original fragment hit 5 forms key H-bonds with Asn1064 and a water-mediated H-bond with Tyr1021 (carbons coloured cyan in Figure 12.1A/B). However, upon N\textsuperscript{1}-methylation, the pyridone ring in fragment 203 (carbons coloured green in Figure 12.1A/B) flips and points towards the ZA-shelf. Besides, the pyridone oxygen is involved in an additional water-mediated H-bond interaction with Asp1014 (Figure 12.1B).

![Figure 12.1](image.png)

Figure 12.1: Overlay of the crystal structures of fragment 203 (green) bound to ATAD2 and the original fragment hit 5 (cyan) showing: (A) solvent accessible surface coloured based on charge, positive: blue, negative: red; (B) key H-bond interactions. Water molecules are shown as red spheres.
The flipped binding mode of fragment 203 encouraged us to explore SAR at the \( N^6 \)-position. Preliminary SAR studies at the \( N^6 \)-position proved that an increase in potency was achievable with the identification of compound 206, which was the most potent in-house inhibitor to date (Table 12.1).

**Table 12.1:** ATAD2 inhibitory activities of \( N^6 \)-substituted analogues.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structure</th>
<th>ATAD2 IC\textsubscript{50} (( \mu \text{M} ))\textsuperscript{a}</th>
<th>L.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>204</td>
<td><img src="image" alt="Structure 204" /></td>
<td>3000</td>
<td>0.17</td>
</tr>
<tr>
<td>205</td>
<td><img src="image" alt="Structure 205" /></td>
<td>716\textsuperscript{b}</td>
<td>0.21</td>
</tr>
<tr>
<td>206</td>
<td><img src="image" alt="Structure 206" /></td>
<td>247\textsuperscript{c}</td>
<td>0.21</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Determinations (\( n = 1 \) unless otherwise stated); \textsuperscript{b} \( n = 2 \); \textsuperscript{c} \( n = 4 \)

The crystal structure of compound 205 bound to ATAD2 suggested that H-bond donors or acceptors in the phenyl ring could interact with polar amino acids in the ZA loop including Arg1007 and Glu1017 (Figure 12.2).
**Figure 12.2**: Crystal structure of ATAD2 in complex with compound 205 (green) showing (A) solvent accessible surface coloured based on the charge, positive: blue, negative: red; (B) Key H-bond interactions. Water molecules are shown as red spheres.

Various in-house crystal structures of ATAD2 in complex with different compounds suggested that the side chains of Glu1017 and Arg1008 were flexible, increasing the complexity of target design. SAR studies were carried out around the phenyl ring of compound 205 to obtain H-bond interactions with the polar residues in the ZA-shelf. It was decided to introduce a range of substituents including H-bond donors, acceptors, lipophilic groups, and halogens at the ortho-, meta- or para-positions of the phenyl ring of compound 205 (Figure 12.3).

**Figure 12.3**: $N^\text{6}$-substituted analogues to be synthesised. SAR studies to be carried out around the phenyl ring highlighted by a green circle.
12.2 Synthesis

Intermediate 192 was synthesised in large quantity (Scheme 11.1) and used for the synthesis of various targets. Formation of pyridone using various alkylating agents, followed by \(N^1\)-methylation gave 10 targets (Scheme 12.1).

\[
\begin{align*}
\text{O} & \text{N} \\
\text{O} & \text{O} \\
\text{N} & \text{N} \\
\text{O} & \text{O} \\
192 & \text{a} \rightarrow \text{O} \\
\text{R} & \text{N} \\
\text{O} & \text{O} \\
\text{b} & \rightarrow \text{N} \\
\text{R} & \text{N} \\ 
\end{align*}
\]

**Scheme 12.1**: Reagents and conditions: (a) R-Br, MeCN, 170°C, \(\mu\)W, 45 min; (b) MeI, Cs\(_2\)CO\(_3\), DMF, 100 °C, \(\mu\)W, 30 min.

The isolated yields obtained in step a and b for various targets are shown in Table 12.2. The pyridone formation reaction (step a, Scheme 12.1) provided the desired products in high yields except for compound 222. The low yield for compound 222 might be due to the steric hindrance caused by the methyl ester in the ortho-position. The final methylation step provided the desired targets in moderate to good yields.
Table 12.2: Isolated yields of step a and step b in Scheme 12.1.

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>Step a</th>
<th>Step b</th>
</tr>
</thead>
<tbody>
<tr>
<td>208</td>
<td></td>
<td>207, 63%</td>
<td>95%</td>
</tr>
<tr>
<td>210</td>
<td></td>
<td>209, 65%</td>
<td>91%</td>
</tr>
<tr>
<td>212</td>
<td>MeO₂S</td>
<td>211, 53%</td>
<td>86%</td>
</tr>
<tr>
<td>6</td>
<td>Cl</td>
<td>213, 80%</td>
<td>90%</td>
</tr>
<tr>
<td>215</td>
<td>Cl</td>
<td>214, 82%</td>
<td>92%</td>
</tr>
<tr>
<td>217</td>
<td>Br</td>
<td>216, 78%</td>
<td>95%</td>
</tr>
<tr>
<td>219</td>
<td>F₃C</td>
<td>218, 71%</td>
<td>92%</td>
</tr>
<tr>
<td>221</td>
<td></td>
<td>220, 85%</td>
<td>92%</td>
</tr>
<tr>
<td>223</td>
<td></td>
<td>222, 22%</td>
<td>74%</td>
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<tr>
<td>225</td>
<td>MeOOC</td>
<td>224, 66%</td>
<td>80%</td>
</tr>
<tr>
<td>229</td>
<td></td>
<td>228, 93%</td>
<td>94%</td>
</tr>
</tbody>
</table>

Nitrile 208 was hydrolysed to get compound 226.
Scheme 12.2: Reagents and conditions: (a) NaOH, EtOH, H₂O, 100 °C, 23 h, 32%.

Similarly compound 227 was obtained via ester-hydrolysis of compound 225.

Scheme 12.3: Reagents and conditions: (a) NaOH, MeOH, H₂O, 70 °C, 2 h, 96%.

12.3 SAR and Biological Evaluation

A small library of compounds with different substituents on the phenyl ring were tested against ATAD2 (Table 12.3). Compounds 6 and 217 with a halogen at the para-position retained ATAD2 inhibitory activity. Compound 219 with a para-substituted trifluoromethyl group was also tolerated. However, compound 221 with a para-substituted methyl group showed a reduction in ATAD2 inhibitory activity by around 4-fold. The para-substituted methyl sulfone derivative 212, which was synthesised to study the effect of an H-bond acceptor sulfone group in the proximity of polar amino acids in ZA-shelf, showed at least 2-fold reduction in potency. Substitution at the ortho-position resulted in a significant loss in ATAD2 inhibition as seen with compounds 208, 215, 223 and 226. Compound 227 with a methoxy group at the ortho-position retained some activity, probably due to the presence of the carboxylic acid at the para-position. Compound 229 with an additional chloro substituent at the meta-position resulted in at least 2-fold loss in potency.
Table 12.3: ATAD2 inhibitory activity of $N^6$-substituted analogues.

![Chemical Structure](image)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>ATAD2 IC₅₀ (µM)ᵃ</th>
<th>Compound</th>
<th>R</th>
<th>ATAD2 IC₅₀ (µM)ᵃ</th>
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<tbody>
<tr>
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<td></td>
<td>&gt;2000</td>
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<td>210</td>
<td></td>
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<td>219</td>
<td></td>
<td>354ᵈ</td>
</tr>
<tr>
<td>212</td>
<td></td>
<td>565</td>
<td>221</td>
<td></td>
<td>815</td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>209ᶜ</td>
<td>223</td>
<td></td>
<td>&gt;2000</td>
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<td>226</td>
<td></td>
<td>&gt;2000</td>
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<td></td>
</tr>
</tbody>
</table>

ᵃ Determinations (n = 1 unless otherwise stated);ᵇ n = 2;ᶜ n = 3;ᵈ n = 6

Compound 6 with a chloro group at the para-position was the most potent inhibitor identified in this series. The crystal structure of compound 6 bound to ATAD2 showed that an additional water-mediated H-bond with the flexible side-chain of Glu1017 was formed (Figure 12.4).
binding mode was very similar to the parent compound 205 where pyrrolidinone oxygen forms H-bond with Asn1064 and water mediated H-bond with Tyr1021.

**Figure 12.4**: Crystal structure of ATAD2 in complex with compound 6 (magenta); (A) front view of the compound; (B) side view of the compound to highlight two water-mediated H-bonds with Asp1014 and Glu1017. Water molecules are shown as red spheres.

The crystal-structure of compound 226 showed that the ortho-substituted carboxylic acid twisted the phenyl ring, probably due to the intramolecular clash of the carboxylic acid with the pyridone oxygen atom, leading to reduced activity (Figure 12.5). In addition, the water-mediated H-bond with Asp1014 was not observed, due to the movement of the scaffold (Figure 12.5B).
As mentioned earlier the methyl sulfone substituted derivative 212 was synthesised to test the effect of 2 H-bond acceptors in the ZA-self. Although, the two oxygens of the sulfone group were in close proximity to Arg1008, H-bond interactions were not formed (Figure 12.6). The binding mode of compound 212 was same as that of compound 6.

Various analogues bearing amines at the para-position were synthesised by other colleagues, with the hypothesis that H-bond donors might interact with the flexible side chain of Glu1017.
leading to improvement in the ATAD2 inhibition. However, the compounds did not improve the activity, making the para-chloro substituted derivative 6, the most potent compound to date. Therefore, the para-chlorobenzyl group was chosen as the optimum group to access the ZA-shelf and focus was shifted towards SAR studies at other positions of compound 6.
Chapter 13. Accessing the Region Occupied by Acetylated Histone

13.1 Rationale

After identifying the para-chlorobenzyl as the optimum substituent at $N^6$-position to access the ZA-shelf, new regions of the ATAD2 binding site were explored. A region around the BC loop was identified from the crystal structure of acetylated histone bound to ATAD2 (PDB: 4QUU, Figure 13.1). The acetylated histone occupies a region between the BC-loop and the ZA-loop. The lead compound 6 has two methyl groups pointing towards the same region (Figure 13.1).

![Figure 13.1](image)

**Figure 13.1**: Overlay of crystal structures of ATAD2 in complex with compound 6 (magenta) and acetylated histone peptide (green, PDB: 4QUU). The binding surface is coloured based on the charge, positive: blue and negative: red. The region of interest is highlighted by a green circle.

It was decided to explore this region by using one of the methyl substituents as a vector for expansion. A series of 3-substituted pyrrolidinone derivatives were proposed (Figure 13.2).
Figure 13.2: 3-substituted pyrrolidinone derivatives to be synthesised.

13.2 Synthesis

13.2.1 Mono-alkylation at the 3-Position

In the initial synthetic route to prepare intermediate 5 (Scheme 13.1), step d involved a bis-methylation at the 3-position. This route had to be modified to enable a mono-alkylation in step d.

Scheme 13.1: Reagents and conditions: (a) Boc₂O, THF, Na₂CO₃, THF, r.t., 42 h, 95%; (b) (i) s-BuLi, THF, -78 °C, 15 min; (ii) CO₂ (dry ice), -78 °C to r.t., 45 min, 74%; (c) Ac₂O, tetrabutylammonium acetate, 65 °C, 1 h, 81%; (d) MeI, Cs₂CO₃, MeCN, 60 °C, 3 h, 78%; (e) MeI, MeCN, 170 °C, µW, 1 h, quant.

The mono-alkylation would provide a versatile intermediate which could be used to introduce a range of substituents at the 3-position (Figure 13.3).
Figure 13.3: Proposed synthetic scheme to prepare a mono-alkylated intermediate.

As there were no literature precedent for mono-alkylating the 3-position of our scaffold, mono-alkylation at the 3-position of oxindoles was searched. There are numerous examples describing the mono-alkylation of the 3-position of oxindoles.\textsuperscript{183-186} The strategy was to deprotonate at the 3-position and use 1 equivalent of an electrophile to obtain the mono-alkylated product. However, various electrophiles and bases at different temperatures did not lead to mono-alkylated product. A significant amount of time was engaged to develop a chemistry that would allow mono-alkylation at the 3-position by three chemists within the group. Few examples are highlighted in Table 13.1.

Table 13.1: Summary of different conditions used for mono-alkylation at the 3-position.

<table>
<thead>
<tr>
<th>Electrophiles RX\textsuperscript{a}</th>
<th>Bases\textsuperscript{a}</th>
<th>Solvent</th>
<th>Temperature °C</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH\textsubscript{3}I</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>MeCN</td>
<td>60</td>
<td>Significant bis-methylation</td>
</tr>
<tr>
<td>CH\textsubscript{3}CH\textsubscript{2}I</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}</td>
<td>MeCN</td>
<td>60</td>
<td>Complex mixture, significant bis-ethylation</td>
</tr>
<tr>
<td>CH\textsubscript{3}CH\textsubscript{2}I</td>
<td>Cs\textsubscript{2}CO\textsubscript{3}\textsuperscript{b}</td>
<td>MeCN</td>
<td>r.t.</td>
<td>Complex mixture, significant bis-ethylation</td>
</tr>
<tr>
<td>CH\textsubscript{3}CH\textsubscript{2}I</td>
<td>LiHMDS</td>
<td>THF</td>
<td>-50</td>
<td>Starting material only</td>
</tr>
</tbody>
</table>
The mono-alkylation reaction worked with very low isolated yield when LHMDS was used as a base and allyl bromide as an electrophile (Scheme 13.2). The product was not stable in air, therefore, it had to be stored under nitrogen. Although, the reaction worked, the yield was poor, the reaction was not reproducible, and there were at least 4 additional steps towards the desired targets. Moreover, the scheme would not allow further functionalisation at the 3-position, therefore, the scheme was discarded.

Scheme 13.2: Reagents and conditions: (a) (i) LHMDS, THF, -78 °C, 45 min; (ii) allyl bromide, -78 °C, 2 h, then, r.t., 20 h, 26%.

13.2.2 Variation at the 3-Position using Knoevenagel Condensation

Whilst attempting various routes for mono-alkylating the 3-position, Knoevenagel condensation of compound 191 with acetaldehyde and formaldehyde was attempted, without any success. However, major breakthrough came from the Knoevenagel condensation of benzaldehyde with compound 191 which provided the desired compound 231 in high yield (Scheme 13.3). Subsequent reduction of the double bond, followed by methylation provided compound 235. Treatment of pyridine 235 with 4-chlorobenzyl bromide at high temperature
provided the target pyridone 237 in moderate yield. Similarly, compound 238 was synthesised via Knoevenagel condensation of compound 191 and picolinaldehyde.

Scheme 13.3: Reagents and conditions: (a) benzaldehyde or picolinaldehyde, piperidine, THF, 100 °C, 30 min, 88% (X = C), 50% (X = N); (b) H₂, 10% Pd/C, THF, MeOH, r.t., 2 h; (c) MeI, Cs₂CO₃, DMF, 50 °C, 1.5 h, 55% (X = C), 56% (X = N) over 2 steps; (d) 4-chlorobenzyl bromide, MeCN, 170 °C μW, 45 min, 64% (X = C), 50% (X = N).

It was decided to utilise the synthetic route via Knoevenagel condensation to synthesise more derivatives. The chemistry was performed with a variety of aldehydes. However, the condensation worked only in the absence of an enolisable proton at the α-position to the aldehyde carbon. Various aldehydes with different ring systems were used in the Knoevenagel condensation to explore SAR at the 3-position. Synthesis of compound 244 started with the oxidation of commercially available alcohol 239 to aldehyde 240. The Knoevenagel condensation of compound 191 with aldehyde 240 provided compound 241. Then, following the chemistry described before, target 244 was obtained.
**Scheme 13.4:** *Reagents and conditions:* (a) DMP, DCM, 0 °C-r.t., 3 h, 78%; (b) 240, piperidine, THF, 100 °C, 30 min, 50%; (c) H₂, 10% Pd/C, THF, MeOH, r.t., 2 h; (d) MeI, Cs₂CO₃, DMF, 50 °C, 1.5 h, 42% over 2 steps; (e) 4-chlorobenzyl bromide, MeCN, 170 °C µW, 45 min, 83%.

### 13.3 Biological Evaluation

A small library of compounds were synthesised using the Knoevenagel condensation chemistry by three different chemists within the group. Compounds with different ring systems at 3-position were tested against ATAD2 inhibition (Table 13.2). The compounds with 6-membered aromatic rings at the 3-position were not tolerated. The 5-membered thiazole ring was tolerated, however, thiophene ring was detrimental to the ATAD2 inhibitory activity. Compounds with smaller substituents 244, 247 and 251 retained activity.
Table 13.2: ATAD2 inhibitory activity of derivatives with various substituents at the 3-position.

![Chemical structure](attachment:image.png)

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>ATAD2 IC₅₀ (µM)ᵃ</th>
<th>Compound</th>
<th>R</th>
<th>ATAD2 IC₅₀ (µM)ᵃ</th>
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<tbody>
<tr>
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<td><img src="" alt="Chemical structure" /></td>
<td>&gt;2000</td>
<td>248&lt;sup&gt;e&lt;/sup&gt;</td>
<td><img src="" alt="Chemical structure" /></td>
<td>352&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>238</td>
<td><img src="" alt="Chemical structure" /></td>
<td>1284</td>
<td>249&lt;sup&gt;e&lt;/sup&gt;</td>
<td><img src="" alt="Chemical structure" /></td>
<td>1633&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>245&lt;sup&gt;e&lt;/sup&gt;</td>
<td><img src="" alt="Chemical structure" /></td>
<td>&gt;2000</td>
<td>244</td>
<td><img src="" alt="Chemical structure" /></td>
<td>214&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>246&lt;sup&gt;e&lt;/sup&gt;</td>
<td><img src="" alt="Chemical structure" /></td>
<td>&gt;2000</td>
<td>250&lt;sup&gt;f&lt;/sup&gt;</td>
<td><img src="" alt="Chemical structure" /></td>
<td>1291&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>247&lt;sup&gt;f&lt;/sup&gt;</td>
<td><img src="" alt="Chemical structure" /></td>
<td>486&lt;sup&gt;b&lt;/sup&gt;</td>
<td>251&lt;sup&gt;f&lt;/sup&gt;</td>
<td><img src="" alt="Chemical structure" /></td>
<td>319&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determinations (n = 1 unless otherwise stated); <sup>b</sup> n = 2; <sup>c</sup> n = 3; <sup>d</sup> n = 4; <sup>e</sup> synthesised by Dr Stephen Hobson; <sup>f</sup> synthesised by Dr Duncan Miller.

The crystal structure of ATAD2 in complex with compound 238 showed that the 6-membered aromatic ring pointed towards solvent, rather than pointing towards the area where the acetylated histone binds, between the ZA and BC loops (Figure 13.4). The overall binding mode of the molecule was similar to the lead compound 6.
Figure 13.4: Crystal structure of ATAD2 in complex with compound 238 (magenta); the solvent accessible surface is coloured based on charge positive: blue, negative: red. Water molecules are shown as red spheres.

Similarly, the crystal structure of ATAD2 in complex with compound 244 showed that the 4-membered oxetane ring pointed towards solvent (Figure 13.5). The oxetane derivative 244 was at least 5-fold more potent than the pyridyl derivative 238, suggesting that the 4-membered ring was tolerated in the binding site.

Figure 13.5: Crystal structure of ATAD2 in complex with compound 244 (magenta); the solvent accessible surface is coloured based on charge positive: blue, negative: red. Water molecules are shown as red spheres.
Chapter 14. Accessing the Bidentate Interaction with Asn1064

14.1 Rationale

It has been reported that the bidentate H-bond interaction with the conserved asparagine residue in bromodomains provided an increase in potency. The crystal structure of compound 244 with ATAD2 revealed that the oxetane ring was in close proximity to Asn1064 (Figure 14.1). The carbon atom at the α-position to the oxygen atom of the oxetane ring was 3.2 Å away from the oxygen of Asn1064. It was hypothesised that an H-bond donor in the region near Asn1064 would form the additional H-bond interaction with Asn1064, improving the potency against ATAD2.

![Figure 14.1: Crystal structure of compound 244 (magenta) bound to ATAD2 showing key H-bond interaction. The distance between the oxetane ring and Asn1064 is highlighted by a double headed black arrow.](image)

The synthetic route used so far did not allow the introduction of desired H-bond donors including alcohols, amines and amides with variable linker lengths at the 3-position (Figure 14.2).
Therefore, a different strategy was taken to form the bidentate interaction with Asn1064. It was decided to synthesise spirocycles at the 3-position to introduce H-bond donors (Figure 14.3). Compound 253 with the pyrrolidine spirocycle was inspired from a natural product Horsfiline 254, which was first isolated in 1991 from Horsfieldia superba, a tree whose extracts are used in local medicines in Malaysia.189

![Figure 14.2: Compounds that were not accessible by the previously described synthetic route.](image)

![Figure 14.3: Proposed pyrrolidinone targets bearing a spirocycle at the 3-position.](image)

### 14.2 Synthesis of Compound 252

Synthesis of compound 252 started with bis-allylation of intermediate 191 at the 3-position (Scheme 14.1). Olefin metathesis of compound 255 using Grubbs catalyst 2nd generation,190-191 gave the desired spirocyclopropene 256 in 90% isolated yield. Compound 256 was converted to the pyridone 257 by heating with 4-chlorobenzyl bromide at 170 °C under microwave irradiation. Subsequent methylation gave compound 258, which was subjected to osmium tetroxide-mediated bis-hydroxylation to get the desired target 252 in 59% yield and the diastereoisomer 259 in 18% yield.
Scheme 14.1: Reagents and conditions: (a) allyl bromide, Cs$_2$CO$_3$, MeCN, 40 °C, 2.5 h, 55%; (b) Grubbs catalyst 2$^{\text{nd}}$ generation, DCM, 80 °C µW, 20 min, 90%; (c) 4-chlorobenzyl bromide, MeCN, 170 °C µW, 45 min, 89%; (d) MeI, Cs$_2$CO$_3$, DMF, 100 °C µW, 30 min, 87%; (e) OsO$_4$, NMO, THF, H$_2$O, r.t., 18 h, 59% (252), 18% (259).

The structures of the two diastereoisomers 252 and 259 were confirmed by NOE experiments. In case of compound 252, the enhancement of both H$_2$ and H$_3$ signals were observed (Figure 14.4A). Enhancement of H$_3$ proton signal suggests that the H$_3$ protons are pointing upwards and the OH groups are pointing downwards.
Figure 14.4: NOE difference spectrometry for compound 252; (A) shows enhancement of H$_2$ and H$_3$ protons on irradiation of H$_1$-proton; (B) shows the $^1$H-NMR spectrum of compound 252.

In the case of compound 259, when H$_1$ proton was irradiated, only the H$_2$ proton signal was enhanced, confirming that the H$_3$ protons face downwards (Figure 14.5).
Figure 14.5: NOE difference spectrometry for compound 259; (A) shows enhancement of the H$_2$ protons on irradiation of the H$_1$-proton; (B) shows the $^1$H-NMR spectrum of compound 259.

14.3 Synthesis of Compound 253

The synthetic route of compound 253 was designed based on the total synthesis of Horsfiline reported by Fischer et al. (Scheme 14.2).$^{192}$ The synthesis started with the alkylation of intermediate 191 using 1,2-dibromoethane to get the spirocyclopropane 260. Ring expansion of cyclopropane 260 using MgI$_2$ and triazinane 261 gave spiropyrrolidine 262 in moderate yield. However, the pyridone formation at 170 °C under microwave irradiation did not work, resulting in a complex mixture of products.
Scheme 14.2: Reagents and conditions: (a) 1,2-dibromoethane, Cs$_2$CO$_3$, MeCN, 40 °C, 3.5 h, 55%; (b) MgI$_2$ (10 mol%), THF, 50 °C, 26 h, 64%; (c) 4-chlorobenzyl bromide, MeCN, 170 °C µW, 45 min.

The synthetic route was redesigned so that the ring-expansion of spirocyclopropane could be performed at a later stage (Scheme 14.3). The spirocyclopropane 260 was converted to compound 265 via pyridone formation and methylation as described before. Ring expansion of spirocyclopropane 265 was achieved using triazinane 261 and MgI$_2$ to get compound 271.
Scheme 14.3: Reagents and conditions: (a) 1,2-dibromoethane, Cs$_2$CO$_3$, MeCN, 40 °C, 3.5 h, 55%; (b) 4-chlorobenzyl bromide, MeCN, 150 °C µW, 45 min, 68%; (c) MeI, Cs$_2$CO$_3$, DMF, 100 °C µW, 30 min, 53%; (d) MgI$_2$ (10 mol%), THF, 50 °C, 13 h, 90 °C, 20 h, 53%.

Fischer et al. proposed a mechanism for the ring expansion of spirocyclopropane, involving the formation of an intermediate enolate 267 (Scheme 14.4). Nucleophilic substitution of iodine by the triazinane 261 forms intermediate 268, which upon fragmentation gives 269. The enolate attack on the iminium ion provides the target pyrolidine 270. Fischer et al. also proposed a different mechanism in which the triazinane 261 undergoes fragmentation to form N-methylimine before attacking the electrophilic centre of C-I bond of the enolate 267.
Scheme 14.4: Proposed mechanism of ring expansion of spirocyclopropane 266 to spiropyrolidine 270.\textsuperscript{192}

The final step to get compound 253 was $N$-demethylation of compound 271. However, various methods of $N$-demethylation using ACE-chloride and benzyl chloroformate did not provide the desired target 253. Therefore, it was decided to incorporate a different group at the pyrrolidine nitrogen using the spirocyclopropane ring expansion approach. A benzyl derivative 272 or an allyl derivative 273 (Figure 14.6) were the first choice of targets because the corresponding triazinanes were either commercially available or the synthesis was reported.\textsuperscript{193}

Figure 14.6: Compounds to be synthesised.

Ring expansion of spirocyclopropane 265 using commercially available triazinane 274 produced a mixture of unidentified products (Scheme 14.5).
Scheme 14.5: **Reagents and conditions:** (a) MgI2 (10 mol%), THF, 50 °C, 13 h, 90 °C, 20 h.

Ring expansion of compound 265 with triazinane 276 provided the desired product 273 (Scheme 14.6). The triazinane 276 was prepared using allylamine 275 and paraformaldehyde following a procedure described by Jasiński et al.\textsuperscript{193}

Scheme 14.6: **Reagents and conditions:** (a) paraformaldehyde, MeOH, 0 °C-rt., 20 h, quant.; (b) MgI2 (10 mol%), THF, 90 °C, 20 h; (c) Pd(PPh\textsubscript{3})\textsubscript{4}, N,N-dimethylbarbituric acid, DCM, 35 °C, 16 h, 22% over two steps.

The compound 273 was not pure enough by \textsuperscript{1}H-NMR, containing unidentified impurities, and a very small amount (16 mg) was remaining. Therefore, the compound was taken forward without further purification. Removal of the \textit{N}-allyl group using Pd(PPh\textsubscript{3})\textsubscript{4} and \textit{N,N}-...
dimethylbarbituric acid following a protocol described by Garro-Helion et al (Scheme 14.6) to afforded desired target **253** in poor yield.\textsuperscript{194}

### 14.4 Biological Evaluation

The ATAD2 inhibitory activity of compounds **252** and **253** along with the intermediates obtained during the synthesis were determined (Table 14.1). Spirocyclopropane intermediate **265** was inactive, whereas, spirocyclopentene derivative **258** retained the potency. The *bis-*hydroxylated compounds **252** and **259** showed no ATAD2 inhibitory activity. The horsfiline analogue **271** showed a loss in potency by at least 7 fold compared to lead compound **6**. Removal of the *N*-methyl group was tolerated but did not improve the potency, although the desired bidentate H-bond interaction with Asn1064 was formed (Figure 14.7). Compound **253** was tested as a racemic mixture, therefore, the correct enantiomer might provide an increase in potency. In conclusion, the spirocycles with H-bond donors did not improve the activity against ATAD2, contradicting the initial hypothesis. A crystal structure of ATAD2 in complex with the *bis-*hydroxylated derivatives **252** and **259** would be beneficial to explain the loss in activity, despite having H-bond donors capable of forming the bidentate interaction with Asn1064.
Table 14.1: ATAD2 inhibitory activity of lead compound 6 compared with analogues bearing a spirocycle at the 3-position.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structures</th>
<th>ATAD2 IC&lt;sub&gt;50&lt;/sub&gt; (µM)&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td><img src="image" alt="Structure 6" /></td>
<td>209&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>265</td>
<td><img src="image" alt="Structure 265" /></td>
<td>&gt;2000</td>
</tr>
<tr>
<td>271</td>
<td><img src="image" alt="Structure 271" /></td>
<td>1571&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>253</td>
<td><img src="image" alt="Structure 253" /></td>
<td>234</td>
</tr>
<tr>
<td>258</td>
<td><img src="image" alt="Structure 258" /></td>
<td>339&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>259</td>
<td><img src="image" alt="Structure 259" /></td>
<td>1467&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>252</td>
<td><img src="image" alt="Structure 252" /></td>
<td>&gt;2000&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup> Determinations (n = 1 unless otherwise stated);
<sup>b</sup> n = 3;  <sup>c</sup>n = 4

A crystal structure of ATAD2 in complex with compound 253 was solved (Figure 14.7). The binding mode of compound 253 was very similar to that observed with the lead compound 6.
The N-H of spiropyrrolidine ring forms a H-bond with Asn1064, making the bidentate interaction with Asn1064.

| Figure 14.7: Crystal structure of ATAD2 in complex with compound 253 (magenta), showing the key H-bond interactions. Water molecules are shown as red spheres. The bidentate interaction with Asn1064 is shown as green dotted lines. |

14.5 Investigation of Scaffold Hopping Approach

The bidentate interaction made by the spiropyrrolidine derivative 253 was not beneficial for increasing ATAD2 inhibition. The chemistry used so far did not allow the exploration of SAR at the 3-position with a range of H-bond donor substituents. Therefore, a scaffold hopping approach was used, changing the scaffold to oxindole, which was chosen because of its structural similarity to the current scaffold. In addition, the chemistry to introduce a range of substituents at 3-position of oxindoles has been reported.\textsuperscript{185, 195-196} Compound 277 with an amide at 3-position of the oxindole was modelled in the binding site of ATAD2 (Figure 14.8). The modelling suggested that the methylene linked amide group at 3-position would form an additional H-bond with Asn1064, providing the desired bidentate interaction.
Figure 14.8: Modelling of compound 277 (green) on the ATAD2 binding site using Accelrys Discovery Studio.

Therefore, to examine the effect of a flexible H-bond donor, compound 277 was chosen as a target (Figure 14.9). Compound 278 was chosen to examine if scaffold hopping to oxindole retains the ATAD2 inhibition (Figure 14.9). The work load was shared with Dr Stephen Hobson, who undertook the synthesis of compound 278.

![Chemical structures](image)

Figure 14.9: Targets to be synthesised based on scaffold hopping approach.

14.5.1 Synthesis of Compound 277

It was decided to synthesise intermediate 282, which could be used with a range of electrophiles to explore the 3-position. Compound 281 was prepared following a literature procedure by Loreto et al.\textsuperscript{197} Synthesis of compound 281 started with the Peterson olefination of N-
methylisatin 279 to give compound 281 (Scheme 14.7). Due to the reported instability, compound 281 was reduced immediately to get intermediate 282 with a mono-methyl group at the 3-position. Nucleophilic attack of the enolate of compound 282 on 2-bromoacetamide gave the desired product in poor yield, but sufficient for co-crystallisation studies.

Scheme 14.7: Reagents and conditions: (a) (trimethylsilyl)methylmagnesium chloride (1M in Et2O), THF, -78 °C to r.t., 1.5 h, 71%; (b) BF3.Et2O, DCM, -78 °C, 1 h; (c) H2, 10% Pd/C, MeOH, Et2O, r.t., 1 h, 50% over 2 steps; (d) 2-bromoacetamide, Cs2CO3, DMF, 50 °C, 2 h, 12%.

14.5.2 Biological Evaluation

Compounds 277 and 278 did not show ATAD2 inhibitory activity at 4 mM concentration (Table 14.2). The inhibitory activity of compound 277 was expected to be low because it is a very small fragment, and it was intended for co-crystallisation studies. Compound 278 did not exhibit ATAD2 inhibition. These results suggested that the pyridone-pyrrolidinone scaffold was important for the ATAD2 inhibition.
Table 14.2: ATAD2 inhibitory activity of compounds 277 and 278 compared with compound 205.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Structures</th>
<th>ATAD2 IC₅₀ (µM)ᵃ</th>
</tr>
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</tr>
<tr>
<td>278ᶜ</td>
<td><img src="image2" alt="Structure 278" /></td>
<td>&gt;4000</td>
</tr>
<tr>
<td>277</td>
<td><img src="image3" alt="Structure 277" /></td>
<td>&gt;4000</td>
</tr>
</tbody>
</table>

ᵃ Determinations (n = 1 unless otherwise stated);
ᵇ n = 2; ᶜ synthesised by Dr Stephen Hobson

A crystal structure of compound 277 bound to ATAD2 was solved (Figure 14.10). The N-methylpyrrolidinone ring mimics the acetyllysine, forming a key H-bond interaction with Asn1064 and a water-mediated H-bond with Tyr1021. The amide group flips away from the Asn1064 and did not form a H-bond interaction, contradicting the hypothesis based on modelling studies. Altogether, the compounds with an oxindole scaffold did not retain ATAD2 inhibitory activity, therefore, the series was not pursued further. However, the crystal structure of compound 277 bound to ATAD2 was helpful to demonstrate that a flexible methylene linked H-bond donor amide group at the 3-position would not be beneficial to get the bidentate interaction with Asn1064 (Figure 14.10).
Figure 14.10: Crystal structure of ATAD2 in complex with compound 277 (green) showing the key H-bond interactions.
14.6 Introduction of a H-bond Donor Heterocycle at 3-Position

The crystal structure of compound 248 bound to ATAD2 showed that the 5-membered thiazole ring was close to the Asn1064 (Figure 14.11). It was hypothesised that a 5-membered heterocycle with a H-bond donor would interact with Asn1064.

**Figure 14.11**: Crystal structure of ATAD2 in complex with compound 248 (green) showing the H-bond interactions.

In order to form the bidentate interaction with Asn1064, an imidazole derivative 283 was designed.
14.6.1 Synthesis

Previous work within the research group have suggested that the Knoevenagel condensation of unprotected heterocycles resulted in a complex mixture of products. Therefore, it was decided to protect 1H-imidazole-4-carbaldehyde 284. The protecting group should withstand high temperatures up to 170 °C, mild acidic, and basic conditions. After an extensive literature search, N,N-dimethylsulfonic amide was selected as a protecting group as it was reported to withstand high temperatures, as well as, mild acidic and basic conditions. The protecting group could be cleaved by either strongly acidic or basic conditions. The synthesis started with the protection of 1H-imidazole-4-carbaldehyde 284 to get compound 285 (Scheme 14.8). Knoevenagel condensation of intermediate 191 and aldehyde 285 provided compound 286. Surprisingly, the Boc group was not cleaved, unlike previous synthetic schemes. Compound 286 was then reduced, followed by methylation to get methoxypyridine 288. The subsequent pyridone formation at 170 °C produced a mixture of complex products.
Scheme 14.8: Reagents and conditions: (a) N,N-dimethylsulfamoyl chloride, NEt₃, DCM, r.t., 24 h, 85%; (b) 285, piperidine, THF, 50 °C, 1 h, 30%; (c) H₂, 10% Pd/C, THF, MeOH, r.t., 2 h; (d) MeI, Cs₂CO₃, DMF, 50 °C, 1.5 h, 69% over 2 steps; (e) 4-chlorobenzyl bromide, MeCN, 170 °C µW, 45 min.

Concurrently, the synthesis of pyrrole derivative 296 was also undertaken, based on the same rationale as the imidazole derivative 283. Synthesis of compound 296 started with a SEM-protection of 1H-pyrrole-2-carbaldehyde 291 to get compound 292 (Scheme 14.9). The
reduction of compound 293, followed by bis-methylation gave compound 295. The pyridone formation did not work as observed with the imidazole derivative 288 in Scheme 14.8.

Scheme 14.9: Reagents and conditions: (a) (i) NaH, THF, 0 °C, 30 min; (ii) SEMCl, r.t., 2.5 h, 85%; (b) 292, piperidine, THF, 60 °C, 1 h, 79%; (c) H2, 10% Pd/C, THF, MeOH, r.t., 2 h; (d) MeI, Cs2CO3, DMF, r.t., 1 h, 67% over 2 steps; (e) 4-chlorobenzyl bromide, MeCN, 170 °C microwave, 45 min.

The pyridone formation reaction was performed at a very high temperature under microwave. Therefore, various milder conditions were attempted for the pyridone formation, however, a complex mixture of products were obtained in each case (Table 14.3).
Table 14.3: Reagents and conditions attempted for pyridone formation.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Reagents and conditions</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4M HCl, Dioxane, 80 °C, 18 h</td>
<td>Complex mixture of products</td>
</tr>
<tr>
<td>2</td>
<td>BB₃, DCM, -78 °C, 2 h</td>
<td>Complex mixture of products</td>
</tr>
<tr>
<td>3</td>
<td>TMSI, MeCN, r.t., 1 h</td>
<td>Complex mixture of products</td>
</tr>
</tbody>
</table>

Due to the failure of several attempts of pyridone formation, it was decided to submit pyridine 298 for biological evaluation. The pyridine 298 would provide an answer to the design question, that is, whether an H-bond donor heterocycle would form an H-bond with Asn1064. The pyridine 298 was synthesised by TBAF-mediated removal of the SEM group from compound 295 (Scheme 14.10).

Scheme 14.10: Reagents and conditions: (a) TBAF, THF, 50 °C, 48 h, 51%.

14.6.2 Biological Evaluation

As expected, compound 298 did not show any ATAD2 inhibition at 2 mM in HTRF assay. A crystal structure of the compound bound to ATAD2 would be beneficial to understand if the
bidentate interaction with Asn1064 was achievable with H-bond donor heterocycles at 3-position. However, several attempts of co-crystallisation of the compound with ATAD2 have failed.
Chapter 15. Conclusion and Future Directions

15.1 MDMX

Based on the docking and modelling studies, a substituted-pyrrole series was identified. Introduction of an acidic group led to the identification of lead compound 2.

A novel route to the synthesis of RO-2443 3 and RO-5963 4 was identified. The compounds were used to validate the in-house biochemical assay ELISA. There were at least 1000 fold discrepancies between the IC\textsubscript{50} values obtained from in-house ELISA and the published HTRF assay. Therefore, a structural biology placement was undertaken.

An HTRF assay was developed, which provided reasonable dose-response curves for published small molecules as well as peptides. The Newcastle small molecule inhibitors with logD < 3 yielded reasonable dose-response curves, but the lipophilic compounds with logD > 3 were completely inactive in the HTRF assay. The HTRF assay results for Newcastle small molecule inhibitors were not in line with the values obtained from the ELISA. The MDMX construct used in the HTRF assay does not include an autoinhibitory sequence. In contrast, a full length MDMX construct that includes the autoinhibitory sequence was used in the ELISA. This difference might be a major factor responsible for the discrepancies between the results obtained from the ELISA and HTRF assays. Therefore, development of HTRF assay with a full-length MDMX would be of interest.

Several MDMX and MDM2 constructs were expressed and purified. Co-crystallisation trials of MDM2 and MDMX with both small molecule inhibitors and autoinhibitory domains were
attempted. Around 20,000 different conditions were screened for co-crystallisation studies with MDMX but without any success. Future work will include the identification of a MDMX surface-entropy reduction mutant that could enhance crystallogenesis and might be used for the co-crystallisation of MDMX with small molecules, as reported for MDM2.\textsuperscript{200}

15.2 ATAD2

The ATAD2 project is in the hit to lead phase. Subtle modifications were undertaken around the fragment hits, obtained from Astex pharmaceuticals, with the aim to improve the ATAD2 inhibitory activity. Extensive SARs around fragment 5 led to the identification of compound 6.

5, ATAD2 IC\textsubscript{50} > 4000 \mu M  
6, ATAD2 IC\textsubscript{50} = 209 \pm 26 \mu M

The SAR studies presented in this thesis have shown that substitution at ortho- or meta-position of the phenyl ring reduced the ATAD2 inhibition. SAR studies with different linkers between the phenyl ring and the N\textsuperscript{6}-position would be of interest.

Based on the crystal structure of compound 6 bound to ATAD2, extensive SARs at the 3-position were performed. Chemistry to allow variations at the 3-position was challenging. The synthetic route involving a Knoevenagel condensation allowed rapid exploration of SARs around the 3-position. Compounds with methylene-linked 6-membered ring at the 3-position showed significant reduction in ATAD2 inhibition. Interestingly, methylene linked small sized rings, such as 244, retained the potency.

244, ATAD2 IC\textsubscript{50} = 214 \pm 36 \mu M
In an attempt to form a bidentate interaction with Asn1064, several strategies were employed, including introduction of H-bond donor spirocycles, and flexible H-bond donor amides or heterocycles at the 3-position. Compound 253, which was submitted as a racemic mixture, formed the desired bidentate interaction with Asn1064, and retained potency against ATAD2. The crystal structure of compound 253 bound to ATAD2 suggests that the substitution at the 2-position of the spiropyrrolidine ring would access the small RVF shelf, which was explored by Bamborough et al. to develop a potent ATAD2 inhibitor. Therefore, SAR studies around spirocycles at the 3-position might provide the desired bidentate interaction, as well as access to the RVF shelf (Figure 15.1).

![Figure 15.1: Possible future SAR studies around the spiropyrrolidine at the 3-position of compound 253 to access the RVF shelf.](image)

253, ATAD2 IC₅₀ = 234 ± 107 μM

Thus, the work presented in this thesis has enhanced our understanding of the binding site of ATAD2 bromodomain. Fragment based drug discovery and structure based drug design led to the identification of sub-millimolar inhibitors of ATAD2. The key SAR studies are summarised in Figure 15.2.
Figure 15.2: Summary of SARs around the pyridone-pyrrolidinone series.
Chapter 16. Experimental

16.1 Summary of Generic Reactions, Analytical and Chromatographic Conditions

16.1.1 Solvents and Reagents

All chemicals and reagents were purchased from Sigma Aldrich, Acros, Alfa Aesar, Apollo Scientific, Fisher Scientific, Fluorochem, Tokyo Chemical Industry or Strem Chemicals. The chemicals were of the highest available purity. Anhydrous solvents were stored in Aldrich Sure/Seal bottles and stored under an atmosphere of nitrogen. Petrol refers to the fraction with a boiling point between 40 and 60 °C.

16.1.2 Chromatography

Thin layer chromatography utilised to monitor reaction progress was conducted on plates pre-coated with silica gel (Merck 60F254) or NH2F254S. The eluent was as stated (where this consisted of more than one solvent, the ratio is stated as volume:volume) and visualisation was either by short wave (254 nm) ultraviolet light, or by treatment with the visualisation reagent stated followed by heating. ‘Flash’ medium pressure liquid chromatography (MPLC) was carried out either on a Biotage SP4 automated purification system or a Varian 971-FP automated purification system, using pre-packed Varian or Grace silica or amino-bonded silica cartridges. Compounds were first loaded onto Biotage Isolute HM-N.

16.1.3 Microwave Reactions

All reactions carried out in a microwave were performed in a Biotage Initiator with Sixty robot.

16.1.4 Analytical Techniques

Melting points were determined using a VWR Stuart SMP40 apparatus and are uncorrected.
\(^1\)H, \(^{13}\)C, \(^{19}\)F and \(^2\)D nuclear magnetic resonance (NMR) spectra were obtained as either CDCl\(_3\), CD\(_3\)OD or DMSO-\(d_6\) solutions and recorded at 500 MHz, 125 MHz, 470 MHz and 76 MHz respectively, on a Bruker Avance III 500 spectrometer. Chemical shifts are quoted in parts per million (δ) referenced to the appropriate deuterated solvent employed. Multiplicities are indicated by s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad), ap (apparent) or combinations thereof. Coupling constant values are given in Hz. Homonuclear and heteronuclear two dimensional NMR experiments were used where appropriate to facilitate assignment of chemical shifts.

LC-MS was carried out on a Waters Acquity UPLC system with PDA and ELSD employing positive or negative electrospray modes as appropriate to the individual compound. High resolution mass spectrometry was performed by the EPSRC UK National Mass Spectrometry Facility, University of Wales Swansea, Singleton Park, Swansea, SA2 8PP.

FTIR spectra were recorded on either a Bio-Rad FTS 3000MX diamond ATR or an Agilent Cary 630 FTIR as a neat sample.

UV spectra were obtained using a U-2001 Hitachi Spectrophotometer with the sample dissolved in ethanol.

Data were compared with literature data for compounds which had been previously reported.

16.2 Synthesis of MDMX Inhibitors-Experimental Procedures

16.2.1 MDMX Biology Procedures

ELISA

To evaluate IC\(_{50}\) values of compounds, ELISA assay was undertaken by Dr Yan Zhao at the Northern Institute for Cancer Research (details taken from Hardcastle \textit{et al.}).\(^{201}\) The 96-well black and white high binding luminometry isoplates (Wallac, Cat N0 140-155) were coated by overnight incubation at 35 °C with 200 µL per well of 5 µg mL\(^{-1}\) streptavidin (Chemicon International) in coating buffer (0.1 M Na\(_2\)HPO\(_4\)·2H\(_2\)O; 0.1 M citric acid; pH 5.0). The plates were washed five times in 1× dissociation enhanced lanthanide fluorescence immunoassay (DELFIA) buffer (Wallac) and then incubated for 3 h at room temperature with saturation buffer (0.3 M D-sorbitol; 50 mM Tris; 150 mM NaCl; 0.1% BSA; 0.05% sodium azide; pH 7.0) to block nonspecific protein binding sites on the plate. After removal of the buffer from the plates, they were allowed to dry in a sterile laminar air flow hood at room temperature before incubation for 1 h at 4 °C with 200 µL per well of 100 µg mL\(^{-1}\) biotinylated IP3 peptide (b-
IP3: Ac-Met-Pro-Arg-Phe19-Met-Asp-Tyr-Trp-Glu-Gly-Leu26-Asn-NH2)17 dissolved in 0.05% DMSO-PBS, pH 7.4 buffer. After washing the wells three times with PBS, the plates were ready to use for MDM2 binding.

For initial testing, the compounds and controls were plated out in triplicate into clear 96-well plates (Nunc) in 10-µL aliquots to give final concentrations of 500 µM, 100 µM, and 20 µM in the assay. Control samples consisted of 5% DMSO carrier alone as a negative control and 100 nM active peptide (AP-B: Ac-Phe19-MetAib-Pmp-6-Cl-Trp-Glu-Ac3-Leu26-NH2) as a positive control peptide antagonist of the MDM2-p53 interaction (IC50 5 nM).18 Compounds and controls aliquoted in 96-well plates were preincubated at 20 °C for 20 min with 190 µL aliquots of optimized concentrations of in vitro translated MDM2, before transfer of the MDM2-compound mixture to the biotin streptavidin plates, and incubation at 4 °C for 90 min. After washing three times with PBS to remove unbound MDM2, each well was incubated at 20 °C for 1 h with a TBS-Tween (50 mM Tris pH 7.5; 150 mM NaCl; 0.05% Tween 20 nonionic detergent) buffered solution of primary mouse monoclonal anti-MDM2 antibody (Ab-5, Calbiochem, used at a 1/200 dilution), then washed three times with TBS-Tween before incubation for 45 min at 20 °C with a goat-anti-mouse horseradish peroxidase (HRP) conjugated secondary antibody (Dako, used at 1/2000). The unbound secondary antibody was removed by washing three times with TBS-Tween. The bound HRP activity was measured by enhanced chemiluminescence (ECL, Amersham Biosciences) using the oxidation of the diacylhydrazide substrate, luminol, to generate a quantifiable light signal. The luminol substrate together with enhancer was automatically injected into each well and the relative luminescence units (RLU) measured over a 30 s interval using a Berthold MicroLumat-Plus LB 96 V microplate luminometer. The percentage MDM2 inhibition at a given concentration is calculated as the (RLU detected in the compound treated sample ÷ RLU of DMSO controls) × 100. The IC50 was calculated using a plot of % MDM2 inhibition versus concentration and is the average of three independent experiments.

The same procedure was used for measuring MDMX inhibition.

16.2.2 Synthesis of MDMX Inhibitors: General Procedures

Except where water was included in the reaction mixture, all reactions were carried out under strict anhydrous conditions with glassware oven-dried and cooled under nitrogen. Temperatures quoted refer to bath temperatures.
General Procedure A: Grignard reaction

The relevant aldehyde (1 eq.) was dissolved in THF (1.2 mL/mmol) and cooled to -78 °C. A 1.6 M solution of vinylmagnesium chloride in THF (5 eq.) was added dropwise and the reaction mixture was stirred at -78 °C for 30 min and at r.t. until the starting material was completely consumed. The reaction was quenched with saturated aqueous NH₄Cl (20 mL), and extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, washed with brine, dried over MgSO₄, and the solvent removed in vacuo.

General procedure B: Synthesis of β-keto ester

A solution of Meldrum’s acid (1 eq.) in DCM (1.15 mL/mmol) was cooled to 0 °C and pyridine (2 eq.) was added dropwise followed by addition of the relevant acid chloride (1 eq.). The reaction mixture was stirred at 0 °C for 1 h and at r.t. for 2.5 h. The reaction mixture was poured into a mixture of ice and 2 M HCl (10 mL) and extracted with DCM (3 × 50 mL). The organic layers were combined, washed with brine (20 mL) and water (20 mL), dried over MgSO₄ and evaporated in vacuo to get an orange solid. The residue was suspended in ethanol, refluxed for 2 h and stirred at r.t. for 16 h. The mixture was cooled and the solvent was removed in vacuo.

General Procedure C: Synthesis of 1, 4-diketone

To a solution of the relevant β-keto ester (1 eq.) in THF (0.6 ml/mmol) was added NaH (60% in mineral oil, 1.3 eq) and stirred for 20 min at 0 °C. A solution of the relevant 2-bromoacetophenone (1.1 eq.) in THF (0.5 mL/mmol) was added dropwise and the mixture was stirred at r.t. for 2.5 h. The reaction was quenched with water (20 mL) and extracted with EtOAc (3 × 30 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo.

General Procedure D: Paal-Knorr pyrrole synthesis

A mixture of the relevant 1,4-diketone (1 eq.), the relevant amine (5 eq.) and acetic acid (3 mL/mmol) was heated under microwave irradiation to 170 °C for 10 min. The reaction was allowed to cool, neutralised with saturated aqueous NaHCO₃ and extracted with EtOAc (3 × 30 ml). The organic layers were combined, washed with brine, dried over MgSO₄ and the solvent removed in vacuo.
General Procedure E: Hydrolysis of esters

To a solution of the relevant ester (1 eq.), in MeOH:water (2:1) was added NaOH (30-50 eq.) and the mixture was stirred at 65 °C for 18 h. MeOH was added to the resulting suspension and stirred at 65 °C for 6 h. The reaction mixture was cooled and solvents removed in vacuo. The residue was dissolved in water (20 mL), acidified with 2M HCl and extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo.

General procedure F: DIBAL reduction of esters to alcohols

The relevant pyrrole-ester (1 eq.) was dissolved in THF (1.6 mL/mmol) and cooled to 0 °C. 1M DIBAL in cyclohexane (2.5 eq.) was added dropwise and stirred at 0 °C until the starting material was completely consumed. The reaction mixture was warmed to r.t., MeOH (1.8 mL/mmol) was added and stirred for 10 min. A saturated aqueous solution of Rochelle’s salt (6 mL/mmol) was added and stirred for 10 min. Water (20 mL), brine (10 mL) and EtOAc (30 mL) were added and the resulting mixture was stirred for 30 min. The aqueous layer was extracted with EtOAc (3 × 30 mL), organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo.

General Procedure G: Oxidation of alcohols to aldehydes using Dess-Martin periodinane

The relevant alcohol (1 eq.) was dissolved in DCM (4 mL/mmol) and cooled to 0 °C. Dess-Martin periodinane (1.7 eq.) in DCM (6 mL/mmol) was added dropwise and the resulting mixture was stirred at 0 °C for 15 minutes, followed by stirring at r.t. until the starting material was completely consumed. Saturated aqueous NaHCO₃ (20 mL) and saturated aqueous Na₂S₂O₃ (25 mL) were added and stirred vigorously for 20 min. The aqueous layer was extracted with DCM (4 × 30 mL), dried over MgSO₄, and the solvent removed in vacuo.

General procedure H: Formylation of pyrroles

The relevant pyrrole (1 eq.) was dissolved in DMF (5 mL/mmol) and cooled to 0 °C. Phosphorous oxychloride (5 eq.) was added dropwise, warmed to 70 °C and stirred until the starting material was completely consumed. NaOH (1M in H₂O, 10 ml) and ice cubes were
added to the reaction mixture and heated at 100 °C for 45 min. The reaction mixture was cooled to r.t. and extracted with EtOAc (3 × 30 mL). The organic layers were combined, washed with brine, dried over MgSO₄ and the solvent removed in vacuo.

**General procedure I: Pinnick Oxidation**

To a solution of the relevant pyrrole (1 eq.) in MeCN (11.8 mL/mmol), was added a solution of sodium chlorite (1.4 eq.) in water (1.12 mL/mmol of sodium chlorite). A solution of sulfamic acid (1.4 eq.) in water (1.12 mL/mmol of sodium chlorite) was added dropwise and the mixture was stirred at r.t. until the starting material was consumed. The solvent was removed in vacuo and water (30 mL) was added to the residue. The aqueous layer was extracted with EtOAc (3 × 30 mL), dried over MgSO₄ and the solvent removed in vacuo.
16.2.3 MDMX Inhibitors: Synthetic Procedures

1-Phenylbut-3-en-2-ol (32)

![Chemical structure of 1-Phenylbut-3-en-2-ol (32)]

Prepared according to general procedure A using phenylacetaldehyde (1.4 mL, 12.48 mmol), 1.6 M solution of vinylmagnesium chloride in THF (11.7 mL, 18.72 mmol) and THF (15 mL). Purification by MPLC on SiO<sub>2</sub> (Petrol:EtOAc, 0-17%) gave a yellow liquid (1.12 g, 61%).

R<sub>f</sub> = 0.39 (15% EtOAc/Petrol); IR ν<sub>max</sub>/cm<sup>-1</sup> 3279 (OH), 3045, 2921, 2868; <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ H 1.64 (1H, brs, OH), 2.81 (1H, dd, J = 13.6 and 8.0 Hz, ArCH<sub>2</sub>), 2.88 (1H, dd, J = 13.6 and 5.4 Hz, 2 × ArCH<sub>2</sub>), 4.33-4.37 (1H, m, CH<sub>2</sub>OH), 5.15 (1H, app dt, J = 10.5 and 1.4 Hz, CH=C<sub> cis </sub>H), 5.27 (1H, app dt, J = 17.2 and 1.4 Hz, CH=C<sub> trans </sub>H), 5.95 (1H, ddd, J = 17.2, 10.5, 5.8 Hz, CH=CH<sub>2</sub>), 7.24-7.27 (3H, m, 3 × ArH), 7.32-7.35 (2H, m, 2 × ArH); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ C 47.2 (ArCH<sub>2</sub>), 127.0 (CH-Ar), 128.8 (2 × CH-Ar), 129.1 (CH=CH<sub>2</sub>), 129.4 (2 × CH-Ar), 134.1 (C-Ar), 135.6 (CH=CH<sub>2</sub>), 197.7 (C=O).

1-Phenylbut-3-en-2-one (33)

![Chemical structure of 1-Phenylbut-3-en-2-one (33)]

Prepared according to general procedure G using compound 32 (698 mg, 4.71 mmol) in DCM (17 ml) and Dess-Martin periodinane (3.99 g, 9.42 mmol) in DCM (25 mL). The product was used for next step without further purification (685 mg, quant.). R<sub>f</sub> = 0.67 (15% EtOAc/Petrol); λ<sub>max</sub> (EtOH)/nm 210, 256; IR ν<sub>max</sub>/cm<sup>-1</sup> 1670 (C=O); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ H 3.87 (2H, s, 2 × ArCH<sub>2</sub>), 5.82 (1H, dd, J = 10.3 and 1.2 Hz, CH=C<sub><sub> cis </sub></sub>H), 6.30 (1H, dd, J = 17.5 and 1.2 Hz, CH=C<sub><sub> trans </sub></sub>H), 6.40 (1H, dd, J = 17.5 and 10.3 Hz, CH=CH<sub>2</sub>), 7.20-7.22 (2H, m, 2 × ArH), 7.24-7.27 (1H, m, ArH), 7.31-7.35 (2H, m, 2 × ArH); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ C (125 MHz, CDCl<sub>3</sub>) 47.2 (ArCH<sub>2</sub>), 127.0 (CH-Ar), 128.8 (2 × CH-Ar), 129.1 (CH=CH<sub>2</sub>), 129.4 (2 × CH-Ar), 134.1 (C-Ar), 135.6 (CH=CH<sub>2</sub>), 197.7 (C=O).
1,5-Diphenylpentane-1,4-dione (34)

Benzaldehyde (0.14 ml, 1.4 mmol) was dissolved in ethanol (0.8 ml), triethylamine (0.4 ml, 2.8 mmol), compound 33 (225 mg, 1.5 mmol), and 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (94 mg, 0.35 mmol) were added and stirred at 80 °C for 19 h. The solvent was evaporated in vacuo and the residue was treated with 2M HCl (10 ml). The aqueous layer was extracted with DCM (3 × 20 ml). The organic layers were combined, washed with saturated aqueous NaHCO₃, dried over MgSO₄ and evaporated in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-1%) gave a pale brown viscous liquid (230 mg, 65%). Rf = 0.40 (20% EtOAc/Petrol); λmax (EtOH)/nm 240; IR νmax/cm⁻¹ 1709 (C=O), 1686 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 2.89 (2H, t, J = 6.3 Hz, 2 × C₆H₂CO), 3.26 (2H, t, J = 6.3 Hz, 2 × C₆H₂CO), 3.82 (2H, s, 2 × ArC₆H₂), 7.24-7.28 (3H, m, 3 × ArH), 7.32-7.35 (2H, m, 2 × ArH), 7.43-7.46 (2H, m, 2 × ArH) 7.52-7.57 (1H, m, ArH), 7.95-7.97 (2H, m, 2 × ArH); ¹³C NMR (125 MHz, CDCl₃) δC 32.5 (C₆H₂CO), 35.6 (C₆H₂CO), 50.2 (ArCH₂), 127.1 (CH-Ar), 128.1 (2 × CH-Ar), 128.6 (2 × CH-Ar), 128.7 (2 × CH-Ar), 129.5 (2 × CH-Ar), 133.2 (C-Ar), 134.3 (C-Ar), 136.6 (C-Ar), 198.5 (C=O), 207.1 (C=O). LRMS (ES⁺) m/z 253.3 [M+H]⁺.

2-Benzyl-1-(4-chlorophenyl)-5-phenyl-1H-pyrrole (30)

A mixture of compound 34 (70 mg, 0.28 mmol), 4-chloroaniline (40 mg, 0.31 mmol) and acetic acid (2 ml) was refluxed for 1.5 h. After cooling to r.t., water (20 ml) was added and the mixture was neutralised with 1N NaOH. The aqueous layer was extracted with DCM (3 × 20 ml), the organic layers were combined, washed with water (30 ml), dried over MgSO₄ and evaporated in vacuo. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-20%) gave a white solid (29 mg, 30%). Rf = 0.83 (20% EtOAc/Petrol); m.p. 165 °C; λmax (EtOH)/nm 286; IR νmax/cm⁻¹ 3065, 2922, 2658, 1489.; ¹H NMR (500 MHz, CDCl₃) δH 3.73 (2H, s, 2 × CH₂), 6.00 (1H, d, J
= 3.5 Hz, Pyrrole-H), 6.30 (1H, d, J = 3.5 Hz, Pyrrole-H), 6.87-6.89 (2H, m, 2 × ArH), 6.93-6.97 (4H, m, 4 × ArH), 7.00-7.03 (1H, m, ArH), 7.05-7.09 (3H, m, 3 × ArH), 7.11-7.18 (4H, m, 4 × ArH); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta_c\) 33.6 (Ar\(\mathrm{C}\)\(\mathrm{H}\)_2), 109.0 (2 × CH-Pyrrole), 126.0 (C-Ar), 126.1 (C-Ar), 127.9 (2 × C-Ar), 128.1 (2 × C-Ar), 128.3 (2 × C-Ar), 128.6 (2 × C-Ar), 129.1 (2 × C-Ar), 130.0 (2 × C-Ar), 133.0 (C-Ar), 133.4 (C-Ar), 134.8 (C-Ar), 134.8 (C-Ar), 137.7 (C-Ar), 139.4 (C-Ar); LRMS (ES\(^+\)) \(m/z\) 344.3 [M(\(^{35}\)Cl)+H]\(^+\), 346.4 [M(\(^{37}\)Cl)+H]\(^+\).

2-(4-Chlorophenyl)acetaldehyde (36)

Prepared according to general procedure G using 2-(4-chlorophenyl) ethanol (244 mg, 1.56 mmol) in DCM (5 mL) and Dess-Martin periodinane (1 g, 2.35 mmol) in DCM (8 mL). Purification by MPLC on SiO\(_2\) (Petrol:EtOAc, 0-20%) gave a colourless liquid (160 mg, 66%). \(R_f\) = 0.70 (15% EtOAc/Petrol); IR \(\nu_{\text{max}}/\text{cm}^{-1}\) 3030, 2925, 1701 (C=O); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta_H\) 3.68 (2H, d, \(J = 2.1\) Hz, 2 × Ar\(\mathrm{C}\)\(\mathrm{H}\)_2), 7.14-7.16 (2H, m, 2 × ArH), 7.33-7.35 (2H, m, 2 × ArH), 9.74 (t, \(J = 2.1\) Hz, C\(\mathrm{H}\)O); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta_c\) 49.8 (C\(\mathrm{H}\)_2), 129.1 (2 × C-Ar), 130.3 (C-Ar), 130.9 (2 × CH-Ar), 133.5 (C-Ar), 198.6 (C=O).

1-(4-Chlorophenyl)but-3-en-2-ol (37)

Prepared according to general procedure A using compound 36, 1.6 M solution of vinylmagnesium chloride in THF (9.5 mL, 15.16 mmol) and THF (15 mL). Purification by MPLC on SiO\(_2\) (Petrol:EtOAc, 0-20%) gave a colourless liquid (535 mg, 39%). \(R_f\) = 0.38 (15% EtOAc/Petrol); \(\lambda_{\text{max}}/\text{nm}\) 221, IR \(\nu_{\text{max}}/\text{cm}^{-1}\) 3368 (OH), 3081, 2921, 2854, 1490; \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta_H\) 2.76 (1H, dd, \(J = 13.6, 7.6\) Hz, Ar\(\mathrm{C}\)\(\mathrm{H}\)_2), 2.82 (1H, dd, \(J = 13.6, 5.2\) Hz, Ar\(\mathrm{CH}\)_2), 4.29-4.33 (1H, m, CH\(\mathrm{OH}\)), 5.12 (1H, dt, \(J = 10.4\) and 1.2 Hz, CH=CH\(\mathrm{cis}\)\(\mathrm{H}\)), 5.22 (1H, dt, \(J = 17.2\) and 1.2 Hz, CH=CH\(\mathrm{trans}\)\(\mathrm{H}\)), 5.89 (1H, ddd, \(J = 17.2, 10.4, 5.9\) Hz, CH=CH\(\mathrm{cis}\)), 7.14-7.14 (2H, m, 2 × ArH), 7.25-7.28 (2H, m, 2 × ArH); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \(\delta_c\) 43.0 (Ar\(\mathrm{CH}\)_2), 73.5 (CH\(\mathrm{OH}\)), 115.3 (CH=CH\(\mathrm{cis}\)), 128.6 (2 × CH-Ar), 130.9 (2 × CH-Ar), 132.4 (C-
Ar), 136.3 (C-Ar), 139.9 (CH=CH2). LRMS (ES+) \( m/z \) 165.2 [(M\(^{35}\text{Cl})\text{-H}_{2}\text{O})+\text{H}]^+, 167.2 [(M\(^{37}\text{Cl})\text{-H}_{2}\text{O})+\text{H}]^+.

1-(4-Chlorophenyl)but-3-en-2-one (38)

Prepared according to general procedure G using compound 37 (100 mg, 0.547 mmol) in DCM (2 mL) and Dess-Martin periodinane (348 mg, 0.82 mmol) in DCM (4 mL). Purification (SP4, silica, EtOAc/Petrol, 0-10%) gave a colourless liquid; \( R_f = 0.70 \) (EtOAc/Petrol, 15%); \( \lambda_{\text{max}} \) (EtOH)/nm 218; IR \( \nu_{\text{max}} \)/cm\(^{-1}\) 3035, 2921, 1690 (C=O stretch); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 3.85 (2H, s, 2\times ArCH\(_2\)), 5.86 (1H, dd, \( J = 10.3, 1.2 \) Hz, CH=C\(_{\text{cis}}\)H), 6.30 (1H, dd, \( J = 17.5, 1.2 \) Hz, CH=C\(_{\text{trans}}\)H), 6.40 (1H, dd, \( J = 17.5 \) and 10.3 Hz, CH=CH\(_2\)), 7.12-7.14 (2H, m, 2 \times ArH), 7.28-7.31 (2H, m, 2 \times ArH).

5-(4-Chlorophenyl)-1-(4-fluorophenyl)pentane-1,4-dione (39)

To a solution of 4-fluorobenzaldehyde (0.06 ml, 0.497 mmol) in ethanol (0.3 mL), was added triethylamine (0.14 mL, 0.25 mmol), compound 38 (crude product), and 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazolium chloride (33 mg, 0.124 mmol) and stirred at 80 °C for 19 h. The solvent was evaporated in vacuo and the residue was treated with 2 M HCl (10 mL). The aqueous layer was extracted with DCM (3 \times 20 mL), organic layers were combined, washed with saturated aqueous NaHCO\(_3\), dried over MgSO\(_4\) and evaporated in vacuo. Purification by MPLC on SiO\(_2\) (Petrol:DCM, 0-80%) gave a colourless viscous liquid (50 mg, 30% in 2 steps). \( R_f = 0.42 \) (20% EtOAc/Petrol); \( \lambda_{\text{max}} \) (EtOH)/nm 240; IR \( \nu_{\text{max}} \)/cm\(^{-1}\) 3078, 2909, 1710 (C=O), 1689 (C=O); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta \) 2.88 (2H, t, \( J = 6.1 \) Hz, CH\(_2\)CO), 3.23 (2H, t, \( J = 6.1 \) Hz, CH\(_2\)CO), 3.80 (2H, s, ArCH\(_2\)), 7.09-7.14 (2H, m, 2 \times ArH), 7.15-7.18 (2H, m, 2 \times ArH), 7.29-7.31 (2H, m, 2 \times ArH), 7.96-8.00 (2H, m, 2 \times ArH); \(^{13}\)C NMR (125 MHz, CDCl\(_3\)) \( \delta \)c 32.5 (CH\(_2\)CO), 35.8 (CH\(_2\)CO), 49.3 (ArCH\(_2\)), 115.7 (2C, d, \( J = 21.5 \) Hz, 2 \times ArCHCF), 128.8 (2 \times CH-Ar), 130.7 (2C, d, \( J = 9.3 \) Hz, 2 \times ArCHCHCF), 130.9 (2 \times CH-Ar), 132.6 (C-Ar), 133.0 (d, \( J = 2.9 \) Hz, ArCCHCHCF), 133.1 (C-Ar), 165.8 (d, \( J = 254.9 \) Hz, ArCF), 196.8 (C=O), 178
206.4 (C=O); $^{19}$F NMR (470 MHz; CDCl$_3$) $\delta$ -105.0; HRMS calcd for C$_{17}$H$_{15}$ClFO$_2$ [M($^{35}$Cl)+H]$^+$ 305.0720, found 305.0721.

2-(4-Chlorobenzyl)-1-(4-Chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrole (40)

A mixture of compound 39 (35 mg, 0.115 mmol), 4-chloroaniline (15 mg, 0.115 mmol) and acetic acid (0.9 ml) were refluxed for 8 h. After cooling to r.t., water (20 mL) was added and the mixture was neutralised with 1M NaOH. The aqueous layer was extracted with DCM (3 $\times$ 20 mL), the organic layers were combined, washed with water (30 mL), dried over MgSO$_4$ and evaporated in vacuo. Purification by MPLC on SiO$_2$ (Petrol:DCM, 0-15%) gave a white solid (14 mg, 31%) $R_f$ = 0.56 (10% EtOAc/Petrol); m.p. 110-112 °C; $\lambda_{max}$ (EtOH)/nm 247; IR $\nu_{max}$/cm$^{-1}$ 2924, 2251, 1493; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$H 3.68 (2H, s, C$_2$H$_2$), 5.99 (1H, d, $J$ = 3.5 Hz, Pyrrole-H), 6.24 (1H, d, $J$ = 3.5 Hz, Pyrrole-H), 6.75-6.79 (2H, m, 2 $\times$ ArH), 6.83-6.85 (4H, m, 4 $\times$ ArH), 6.90-6.93 (2H, m, 2 $\times$ ArH), 7.08-7.11 (2H, m, 2 $\times$ ArH), 7.16-7.18 (2H, m, 2 $\times$ ArH). $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$C 33.0 (CH$_2$), 108.9 (CH-Pyrrole), 109.1 (CH-Pyrrole), 115.1 (2C, d, $J$ =21.5 Hz, 2 $\times$ ArCHCF), 128.4 (2 $\times$ CH-Ar), 129.1 (d, $J$ = 3.2 Hz, ArCCHCHCF) 129.2 (2 $\times$ CH-Ar), 129.6 (2C, d, $J$ = 7.8 Hz, 2 $\times$ ArCCHCHCF), 129.9 (2 $\times$ CH-Ar), 129.9 (2 $\times$ CH-Ar), 131.9 (C-Ar), 133.7 (C-Ar), 133.9 (C-Ar), 134.0 (C-Ar), 137.4 (C-Ar), 137.8 (C-Ar), 161.4 (d, $J$ = 246.0 Hz, ArCF). $^{19}$F NMR (470 MHz; CDCl$_3$) $\delta$F -116.0; HRMS calcd for C$_{23}$H$_{17}$Cl$_2$FN [M($^{35}$Cl)$_2$+H]$^+$ 396.0526, found 396.0523.
1-(4-Bromophenyl)-2-(4-chlorobenzyl)-5-(4-fluorophenyl)-1H-pyrrole (41)

Prepared according to general procedure D using compound 39 (50 mg, 0.164 mmol), 4-bromoaniline (141 mg, 0.82 mmol) and acetic acid (0.5 mL). Purification by MPLC on SiO\textsubscript{2} (Petrol:EtOAc, 0-20%) gave a white solid (60 mg, 83%) R\textsubscript{f}=0.56 (EtOAc/Petrol, 10%); m.p. 123-125 °C; λ\textsubscript{max} (EtOH)/nm 248; IR ν\textsubscript{max}/cm\textsuperscript{-1} 3053, 2847, 2285, 1590, 1485; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ\textsuperscript{H} 3.68 (2H, s, CH\textsubscript{2}), 5.98 (1H, d, J = 3.5 Hz, Pyrrole-H), 6.24 (1H, d, J = 3.5 Hz, Pyrrole-H), 6.75-6.79 (4H, m, 4 × ArH), 6.84-6.86 (2H, m, 2 × ArH), 6.90-6.93 (2H, m, 2 × ArH); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) δ\textsuperscript{C} 33.0 (CH\textsubscript{2}), 108.9 (CH-Pyrrole), 109.1 (CH-Pyrrole), 115.1 (2C, d, J = 21.5 Hz, 2 × ArCHCF), 121.7 (C-Ar), 128.4 (2 × CH-Ar), 129.1 (d, J = 3.2 Hz, ArCCHCHCF) 129.6 (2C, d, J = 7.9 Hz, 2 × ArCCHCF), 129.9 (2 × CH-Ar), 130.3 (2 × CH-Ar), 132.0 (C-Ar), 132.7 (C-Ar), 133.9 (C-Ar), 134.0 (C-Ar), 134.0 (C-Ar), 137.4 (C-Ar), 137.8 (C-Ar), 137.9 (C-Ar), 161.4 (d, J = 246.1 Hz, ArCF); \textsuperscript{19}F NMR (470 MHz; CDCl\textsubscript{3}) δ\textsuperscript{F} -116.0; HRMS calcd for C\textsubscript{23}H\textsubscript{16}BrClF\textsubscript{N} [M\textsuperscript{+}(79Br\textsuperscript{35}Cl)+H]\textsuperscript{+} 440.0025, found 440.0023.

2-(4-Chlorobenzyl)-1-(3,4-difluorophenyl)-5-(4-fluorophenyl)-1H-pyrrole (42)

Prepared according to general procedure D using compound 39 (23 mg, 0.075 mmol), 3,4-difluoroaniline (0.04 ml, 0.375 mmol) and acetic acid (0.23 mL). Purification by MPLC on SiO\textsubscript{2} (Petrol:EtOAc, 0-20%) gave a white solid (60 mg, 77%). R\textsubscript{f}=0.52 (10% EtOAc/Petrol); m.p. 118-120 °C; λ\textsubscript{max} (EtOH)/nm 277; IR ν\textsubscript{max}/cm\textsuperscript{-1} 2912, 2088, 1519; \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ\textsuperscript{H} 3.69 (2H, s, CH\textsubscript{2}), 5.99 (1H, d, J = 3.5 Hz, Pyrrole-H), 6.23 (1H, d, J = 3.5 Hz,
Pyrrole-H), 6.63-6.66 (1H, m, ArH), 6.73-6.76 (1H, m, ArH), 6.77-6.80 (2H, m, 2 × ArH), 6.83-6.85 (2H, m, 2 × ArH), 6.90-6.93 (2H, m, 2 × ArH), 6.98 (1H, q, J = 18.4, 8.7 Hz, ArH), 7.11 (2H, d, J = 8.3 Hz, 2 × ArH); 13C NMR (125 MHz, CDCl3) δC 33.0 (CH2), 109.0 (CH-Pyrrole), 109.3 (CH-Pyrrole), 115.2 (2C, d, J = 21.5 Hz, 2 × ArCHCF), 117.4 (d, J = 18.2 Hz, ArCHCFCF), 118.1 (d, J = 18.0 Hz, ArCHCFCF), 125.1 (dd, J = 6.1, 3.3 Hz, ArCHCHCFCF), 128.5 (2 × CH-Ar), 128.9 (d, J = 3.3 Hz, ArCCHCHCF), 129.6 (2C, d, J = 7.9 Hz, 2 × ArCHCHCF), 129.8 (2 × CH-Ar), 132.1 (C-Ar), 134.0 (C-Ar), 134.2 (C-Ar), 135.2 (dd, J = 7.4, 3.4 Hz, ArCCHCFCF), 137.6 (C-Ar), 149.8 (dd, J = 250.4, 12.5 Hz, ArCF), 149.9 (dd, J = 246.4 Hz, ArCF), 161.5 (dd, J = 246.4 Hz, ArCF). 19F NMR (470 MHz; CDCl3) δF -137.2 (d, J = 20.8 Hz), -134 (d, J = 20.8 Hz), -115.7; HRMS calcd for C23H16ClF3N [M(35Cl)+H]+ 398.0920, found 398.0918.

6-Chloro-3-nitro-1H-indole (44)

![Chemical Structure](image)

6-chloroindole (305 mg, 2.01 mmol) and silver nitrate (362 mg, 2.13 mmol) were suspended in MeCN (1.9 mL) and cooled to 0 °C. Benzoyl chloride (0.25 mL, 2.13 mmol) was added dropwise and the mixture was stirred at 0 °C for 30 min. The reaction mixture was treated with MeOH (5 mL) and filtered through PL-thiol cartridge. The filtrate was evaporated in vacuo and the residue was purified by MPLC (H2O:MeOH, reversed phase with 0.1% HCOOH modifier, 0-85%) to get a yellow solid (165 mg, 42%). Rf = 0.46 (40% EtOAc/Petrol); m.p. 234-236 °C; IR νmax/cm⁻¹ 3191, 3144, 1456, 1368, 1199; 1H NMR (500 MHz, DMSO-d6) δH 7.37 (1H, dd, J = 8.5 and 1.8 Hz, H5), 7.61 (1H, d, J = 1.8 Hz, H7), 8.05 (1H, d, J = 8.5 Hz, H4), 8.67 (1H, s, H2), 12.74 (1H, br s, NH); 13C NMR (125 MHz, DMSO-d6): δC 113.6 (CH2), 119.1 (C-Ar), 121.3 (CH3), 124.5 (CH3), 128.9 (C-Ar), 129.2 (C-Ar), 131.9 (CH2), 135.9 (C-Ar). LRMS (ES⁻) m/z 195.1 [M(35Cl)-H]⁻, 197.1 [M(37Cl)-H]⁻.
tert-Butyl 6-chloro-1H-indole-1-carboxylate (47)

6-chloroindole (1 g, 6.59 mmol) and DMAP (402 mg, 3.29 mmol) were dissolved in MeCN (5 mL). A solution of di-tert-butyl dicarbonate (2.27 mL, 9.88 mmol) in MeCN (15 mL) was added dropwise and the mixture was stirred at r.t. for 2 h. The solvent was evaporated in vacuo to get brown coloured liquid. Purification by MPLC on SiO$_2$ (Petrol:EtOAc, 0-15%) gave a colourless liquid (1.6 g, 97%). R$_f$ = 0.50 (20% EtOAc/Petrol); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$H 1.67 (9H, s, 3 × CH$_3$), 6.52 (1H, d, $J = 3.3$ Hz, $H^6$), 7.19 (1H, dd, $J = 8.3$ and 1.9 Hz, $H^5$), 7.44 (1H, d, $J = 8.3$ Hz, $H^4$), 7.56 (1H, d, $J = 3.3$ Hz, $H^2$), 8.19 (1H, s, $H^7$); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$C 28.2 (3 × $C_H_3$), 84.2 ($C(CH_3)$), 107.0 ($C(CH_3)$), 115.5 ($CH_3$), 121.6 ($CH_4$), 123.2 ($CH_5$), 126.4 ($CH_3$), 129.0 (C-Ar), 130.2 (C-Ar), 135.6 (C-Ar), 149.4 (C=O). LRMS (ES$^+$) m/z 252.3 [(M($^{35}$Cl)+H)$^+$, 254.4 [(M($^{37}$Cl)+H)$^+$.

tert-Butyl 6-chloro-3-nitro-1H-indole-1-carboxylate (48)

Acetyl nitrate was generated by the dropwise addition of fuming HNO$_3$ (0.15 mL, 3.57 mmol) to Ac$_2$O (2.4 mL) at 0 °C followed by standing at r.t. for 5 min and was used immediately. Compound 47 (300 mg, 1.19 mmol) was dissolved in Ac$_2$O (6 mL) and cooled to -78 °C and a solution of the acetyl nitrate was added dropwise. The mixture was warmed to 0 °C and stirred for 17 h. The reaction mixture was neutralised with saturated aqueous NaHCO$_3$ and stirred for 20 min. The aqueous layer was extracted with EtOAc (4 × 50 mL), the organic layers were combined, dried over MgSO$_4$ and the solvent was removed in vacuo. Purification by MPLC on SiO$_2$ (Petrol:EtOAc, 0-6%) gave a pale yellow solid (180 mg, 51%). R$_f$ = 0.50 (5% EtOAc/Petrol); $\lambda_{max}$(EtOH)/nm 319, 250; IR $\nu_{max}$/cm$^{-1}$3148, 3114, 2974, 2928, 1747 (C=O), 1542, 1493, 1326; $^1$H NMR (500 MHz, CDCl$_3$) $\delta$H 1.70 (9H, s, 3 × CH$_3$), 7.42 (1H, dd, $J = 8.6$ and 1.8 Hz, $H^6$), 8.17 (1H, d, $J = 8.6$ Hz, $H^6$), 8.28 (1H, d, $J = 8.6$ Hz, $H^6$), 8.49 (1H, s, $H^2$); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$C 28.0 (3 × CH$_3$), 87.3 (C-CH$_3$), 115.9 ($CH_3$), 120.0 (C-Ar), 121.6 ($CH_4$), 126.2 ($CH_5$), 128.1 ($CH_3$), 132.3 (C-Ar), 132.9 (C-Ar), 134.6 (C-Ar), 147.8 (C-Ar); HRMS calcd for C$_{13}$H$_{14}$ClN$_2$O$_4$ [M($^{35}$Cl)+H]$^+$ 297.0637, found 297.0638.
Methyl 4-(4-chlorophenyl)-2-(2-(4-fluorophenyl)-2-oxoethyl)-3-oxobutanoate (54)

1M Diethylzinc in hexane (15 mL, 14.76 mmol) was dissolved in DCM (28 mL) and cooled to 0 °C. Diiodomethane (1.2 mL, 14.76 mmol) was added dropwise, and the mixture was stirred for 30 min. After the formation of a white precipitate, methyl 4-fluorobenzoyl acetate (0.57 mL, 3.6 mmol) was added dropwise, and the reaction mixture was stirred for 30 min. Compound 36 (736 mg, 5 mmol) was added and the mixture was stirred at 0 °C for 1.5 h. Silica gel (10 g) was added and the mixture was stirred at r.t. for 30 min. The mixture was filtered and the solvent was evaporated in vacuo. The residue was dissolved in DCM (8 mL), PCC (853 mg, 3.96 mmol) was added, and the mixture was stirred at r.t. for 17 h. Then, additional PCC (776 mg, 3.6 mmol) was added and the reaction mixture was stirred at r.t. for 24 h. The mixture was passed through a short path of silica gel and eluted with DCM and concentrated in vacuo. Purification by MPLC on SiO\textsubscript{2} (Petrol:EtOAc, 0-20%) followed by another purification by MPLC (H\textsubscript{2}O:MeOH, reversed phase with 0.1% HCOOH modifier, 0-100%) gave a pale yellow viscous liquid (436 mg, 33%). R\textsubscript{f} = 0.36 (25% EtOAc/Petrol); \lambda_{max} (EtOH)/nm 244, 225; IR \nu_{max}/cm\textsuperscript{-1} 3070, 2954, 2920, 1742 (C=O), 1719 (C=O), 1680 (C=O); \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) \delta H 3.51 (1H, dd, \textit{J} = 18.3 and 4.8 Hz, 1 \times C\textsubscript{6}H\textsubscript{2}), 3.70-3.75 (1H, m, peaks coincided with OMe singlet, 1 \times C\textsubscript{6}H\textsubscript{2}), 3.75 (3H, s, C\textsubscript{3}H\textsubscript{3}), 4.05 (1H, d, \textit{J} = 16.9 Hz, ArCH\textsubscript{2}), 4.10 (1H, d, \textit{J} = 16.9 Hz, ArCH\textsubscript{2}), 4.30 (2H, dd, \textit{J} = 8.9 and 4.8 Hz, CHCOOMe), 7.11-7.19 (4H, m, 4 \times ArH), 7.29-7.31 (2H, m, 2 \times ArH), 7.97-8.00 (2H, m, 2 \times ArH); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) \delta C 37.7 (C\textsubscript{6}H\textsubscript{2}), 49.1 (ArCH\textsubscript{2}), 52.4 (CHCOOMe), 52.9 (COOCH\textsubscript{3}), 115.9 (2C, d, J = 22.0 Hz, 2 \times ArCHCF), 128.7 (2 \times CH-Ar), 130.9 (2C, d, J = 9.4 Hz, 2 \times ArCHCHCF), 131.2 (2 \times CH-Ar), 131.9 (C-Ar), 132.4 (d, \textit{J} = 2.9 Hz, ArCCHCHCF), 133.1 (C-Ar), 166.1 (d, \textit{J} = 255.5 Hz, ArCF), 169.1 (C=O), 195.5 (C=O), 201.7 (C=O); \textsuperscript{19}F NMR (470 MHz; CDCl\textsubscript{3}) \delta F -104.1; HRMS calcd for C\textsubscript{19}H\textsubscript{17}ClFO\textsubscript{4} [M\textsuperscript{(35Cl)}+H]\textsuperscript{+} 363.0794, found 363.0787.
Methyl 2-(4-chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrole-3-carboxylate (55)

Prepared according to general procedure D using compound 54 (200 mg, 0.55 mmol), 4-chloroaniline (350 mg, 2.75 mmol) and acetic acid (1.6 ml). Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-20%) gave a white solid (121 mg, 48%). R<sub>r</sub>=0.56 (10% EtOAc/Petrol); m.p. 124-126 °C; λ<sub>max</sub> (EtOH)/nm 263; IR ν<sub>max</sub>/cm<sup>-1</sup> 3068, 2946, 1700 (C=O); <sup>1</sup>H NMR (500 MHz, CDCl<sub>3</sub>) δ<sub>H</sub> 3.84 (3H, s, COOC<sub>H</sub>₃), 4.23 (2H, s, 2 × CH₂), 6.78-6.87 (7H, m, 7 × ArH), 6.96-6.99 (2H, d, 2 × ArH), 7.11-7.13 (2H, m, 2 × ArH), 7.23-7.25 (2H, m, 2 × ArH); <sup>13</sup>C NMR (125 MHz, CDCl<sub>3</sub>) δ<sub>C</sub> 30.8 (CH₂), 51.2 (COOCH₃), 110.3 (CH-Pyrrole), 113.8 (C-Ar), 115.3 (2C, d, J = 21.6 Hz, 2 × ArCHCF), 127.9 (d, J = 3.3 Hz, ArCCHCHCF), 128.4 (2 × CH-Ar), 129.4 (2 × CH-Ar), 129.5 (2 × CH-Ar), 130.0 (2 × CH-Ar), 130.1 (2C, d, J=8.05 Hz, 2 × ArCHCHCF), 131.9 (C-Ar), 133.8 (C-Ar), 134.7 (C-Ar), 136.0 (C-Ar), 137.4 (C-Ar), 139.0 (C-Ar), 161.8 (d, J = 247.5 Hz, ArCF), 165.5 (COOME). ¹⁹F NMR (470 MHz; CDCl<sub>3</sub>) δ<sub>F</sub> -114.5; HRMS calcd for C<sub>25</sub>H<sub>19</sub>Cl<sub>2</sub>FNO<sub>2</sub> [M(35Cl<sub>2</sub>)+H]<sup>+</sup> 454.0771, found 454.0763.

2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrole-3-carboxylic acid (2)

Compound 55 (70 mg, 0.15 mmol) was dissolved in THF (1 mL), 4 M LiOH in H₂O monohydrate (1 mL), and the mixture was stirred at r.t. for 15 h. To the resulting suspension, MeOH (1 mL) was added and the mixture was stirred at r.t. for 72 h. Then, the reaction mixture
was neutralised with 2 M HCl and extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over MgSO₄, and evaporated in vacuo. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-20%) gave a white solid (60 mg, 91%). The reaction repeated with 50 mg of the ester starting material to have enough material for the next step. Rₐ = 0.62 (2% MeOH/DCM); m.p. 184-186 °C; λₘₐₓ (EtOH)/nm 255; IR νₘₐₓ/cm⁻¹ 2300-3100 (very broad, O-H overlap with C-H); ¹H NMR (500 MHz, CDCl₃) δ ≈ 4.24 (2H, s, CH₂), 6.79-6.88 (7H, m, 7 × ArH), 6.97-7.00 (2H, m, 2 × ArH), 7.12 (2H, d, J = 7.9 Hz, 2 × ArH), 7.25 (2H, d, coincided with CDCl₃ peak, 2 × ArH); ¹³C NMR (125 MHz, CDCl₃) δC 30.8 (CH₂), 110.8 (ArCHCF), 115.3 (2C, d, J = 21.6 Hz, 2 × ArCHCF), 127.7 (d, J = 3.4 Hz, ArCCHCHCF), 128.4 (2 × CH-Ar), 129.4 (2 × CH-Ar), 129.5 (2 × CH-Ar), 130.0 (2 × CH-Ar), 130.1 (2C, d, J = 8.1 Hz, 2 × ArCHCHCF), 131.9 (C-Ar), 134.2 (C-Ar), 134.8 (C-Ar), 135.9 (C-Ar), 137.1 (C-Ar), 140.1 (C-Ar), 161.9 (d, J = 247.5 Hz, ArCF), 170.0 (COOH). ¹⁹F NMR (470 MHz; CDCl₃) δF -114.3; HRMS calcd for C₂₄H₁₇Cl₂FNO₂ [M(³⁵Cl₂)+H]⁺ 440.0615, found 440.0610.

2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-N-(2,2-dimethyl-1,3-dioxan-5-yl)-5-(4-fluorophenyl)-1H-pyrrole-3-carboxamide (57)

Compound 2 (98 mg, 0.22 mmol), amine 115 (34 mg, 0.26 mmol) and DMAP (2.5 mg, 0.02 mmol) were dissolved in DCM (2 mL) and the reaction mixture was stirred at 0 °C for 5 min. DIC (0.04 ml, 0.26 mmol) was added and the resulting solution was stirred at 0 °C for 15 min and at r.t. for 16 h. The reaction mixture was quenched with water (10 mL) and extracted with DCM (3 × 20 mL). The organic layers were combined, dried over MgSO₄, and concentrated in vacuo. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-50%) gave a white solid (85 mg, 70%). Rₐ = 0.17 (33% EtOAc/Petrol); m.p. 152-154°C; λₘₐₓ (EtOH)/nm 240; IR νₘₐₓ/cm⁻¹ 3302 (NH), 2968, 2939, 2876, 1616 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 1.43 (3H, s, CH₃), 1.51 (3H, s,
2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-N-(1,3-dihydroxypropan-2-yl)-5-(4-fluorophenyl)-1H-pyrrole-3-carboxamide (58)

Compound 57 (60 mg, 0.108 mmol) was dissolved in THF (1.7 mL) and 2 M HCl (0.57 mL) was added dropwise and stirred at r.t. for 3 h. 2 M HCl (0.29 mL) was added again and the reaction was stirred for 15 min. The solvent was evaporated in vacuo and the residue was neutralised with 1 M NaOH and extracted with EtOAc (3 × 20 mL). The organic layers were combined, washed with H₂O, dried over MgSO₄ and evaporated in vacuo to get a white solid (52 mg, 94%). Rₜ=0.34 (5% MeOH/DCM); m.p. 210-212 °C; λₘₐₓ (EtOH)/nm 239; IR νₘₐₓ/cm⁻¹ 3344 (broad, Amide N-H and O-Hs overlapped), 2922, 2852, 1600 (C=O stretch); ¹H NMR (500 MHz, DMSO-d₆) δH 3.52 (4H, t, J = 5.7 Hz, 2 × CH₂OC), 3.93-3.99 (1H, m, CONHCH₂), 4.29 (2H, s, 2 × ArCH₂), 4.65 (2H, t, J = 5.7 Hz, 2 × CH₂OH), 6.78-6.80 (2H, m, 2 × ArH), 6.99 (1H, s, Pyrrole-H₁), 7.01-7.03 (2H, m, 2 × ArH), 7.07 (4H, d, J = 7.2 Hz, 4 × ArH), 7.15-7.17 (2H, m, 2 × ArH), 7.46 (1H, d, J = 8.5 Hz, CONH); ¹³C NMR (125 MHz, DMSO-d₆) δC 29.6 (CH₂), 52.9 (CONHCH₂), 60.5 (2C, 2 × CH₂OC), 108.4 (CH-Pyrrole), 186
115.2 (2C, d, \( J = 21.6 \) Hz, 2 × ArCHCF), 116.7 (C-Ar), 127.8 (2 × CH-Ar), 128.4 (d, \( J = 3.3 \) Hz, ArCCHCHCF), 129.2 (2 × CH-Ar), 129.7 (2 × CH-Ar), 129.8 (C-Ar), 130.3 (C-Ar), 130.5 (2 × CH-Ar), 132.2 (C-Ar), 133.0 (C-Ar), 136.1 (C-Ar), 136.5 (C-Ar), 138.3 (C-Ar), 160.9 (d, \( J = 244.7 \) Hz, ArCF), 164.3 (CONH). 19F NMR (470 MHz; DMSO-\( d_6 \)) δF -115.2; HRMS calcd for C27H22Cl2F2O3 (M+H)+ 511.0997, found 511.0981.

**Ethyl 4-(4-chlorophenyl)-3-oxobutanoate (72)**

![Chemical Structure of 72](image)

Prepared according to general procedure B using Meldrum’s acid (1 g, 6.94 mmol), DCM (6 mL), pyridine (1.1 mL), 4-chlorophenylacetyl chloride (1 mL, 6.94 mmol) and ethanol (22 mL). Purification by MPLC on SiO2 (Petrol:EtOAc, 0-20%) gave a colourless liquid (1.3 g, 78%).

\(^1\)H NMR (500 MHz, CDCl3) \( \delta \)H 1.26 (3H, t, \( J = 7.2 \) Hz, COOCH2C6H3), 3.45 (2H, s, C6H2), 3.81 (2H, s, CH2), 4.17 (2H, q, \( J = 7.2 \) Hz, COOC6H3CH3), 7.12-7.14 (2H, m, 2 × ArH), 7.30-7.32 (2H, m, 2 × ArH) \(^{13}\)C NMR (125 MHz, CDCl3) \( \delta \)C 14.1 (COOCH2C6H3), 48.5 (CH2), 49.0 (CH2), 61.6 (COOCH2C6H3), 129.0 (2 × CH-Ar), 131.0 (2 × CH-Ar), 131.6 (C-Ar), 133.4 (C-Ar), 167.0 (C=O), 199.9 (C=O).\(^{205}\)

**Ethyl 4-(4-chlorophenyl)-2-(2-(4-fluorophenyl)-2-oxoethyl)-3-oxobutanoate (74)**

![Chemical Structure of 74](image)

Prepared according to general procedure C using compound 72 (500 mg, 2.07 mmol) and NaH (60% in mineral oil, 107.6 mg, 2.69 mmol) in THF (1.3 ml) and 2-bromo-4’-fluoroacetophenone (492 mg, 2.27 mmol) in THF (1 ml). Purification by MPLC on SiO2 (Petrol:EtOAc, 0-20%) gave a colourless viscous liquid which solidifies upon standing (709 mg, 91%). Rf = 0.43 (20% EtOAc/Petrol); \( \lambda \)max (EtOH)/nm 244, 225; IR \( \nu \)max/cm\(^{-1}\) 3072, 2954, 1740 (C=O), 1718 (C=O), 1680 (C=O); \(^1\)H NMR (500 MHz, CDCl3) \( \delta \)H 1.28 (3H, t, \( J = 7.1 \) Hz, COOCH2C6H3), 3.50 (1H, dd, \( J = 18.3 \) and 4.8 Hz, 1 × CH2), 3.72 (1H, dd, \( J = 18.3 \) and 9.0 Hz, 1 × CH2).
1 × CH₂), 4.08 (1H, d, J = 17.1 Hz, ArCH₂), 4.11 (1H, d, J = 17.1 Hz, ArCH₂), 4.20 (2H, q, J = 7.1 Hz, COOCH₂CH₃), 4.29 (2H, dd, J = 9.0 and 4.8 Hz, CHCOOEt), 7.11-7.18 (4H, m, 4 × ArH), 7.29-7.31 (2H, m, 2 × ArH), 7.97-8.00 (2H, m, 2 × ArH); ¹³C NMR (125 MHz, CDCl₃) δC 14.1 (COOCH₂CH₃), 37.7 (CH₂), 49.2 (ArCH₂), 52.6 (CHCOOMe), 62.0 (COOCH₂CH₃), 115.8 (2C, d, J = 22.0 Hz, 2 × ArCHCF), 128.7 (2 × CH-Ar), 130.9 (2C, d, J = 9.4 Hz, 2 × ArCHCHCF), 131.2 (2 × CH-Ar), 132.0 (C- Ar), 132.4 (d, J = 3.0 Hz, ArCCHCHCF), 133.1 (C- Ar), 166.0 (d, J = 255.5 Hz, ArCF), 168.6 (C=O), 195.6 (C=O), 201.9 (C=O); ¹⁹F NMR (470 MHz; CDCl₃) δF -104.2.

**Ethyl 2-(4-chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrole-3-carboxylate (75)**

![Image of the compound](image_url)

Prepared according to general procedure D using 74 (350 mg, 0.93 mmol), 4-chloroaniline (593 mg, 4.65 mmol) and acetic acid (2.8 mL). Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-10%) gave a white solid (290 mg, 67%). The reaction was repeated to get enough material for next steps. Rf = 0.45 (10% EtOAc/Petrol); m.p. 132-134 °C; IR v max/cm⁻¹ 2985, 2927, 1702 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 1.25 (3H, t, J = 7.1 Hz, COOCH₂CH₃), 4.15 (2H, s, ArCH₂), 4.24 (2H, q, J = 7.1 Hz, COOCH₂CH₃), 6.72-6.80 (6H, m, 6 × ArH), 6.90-6.93 (2H, m, 2 × ArH), 7.04-7.06 (2H, m, 2 × ArH), 7.15-7.18 (2H, m, 2 × ArH); ¹⁹F NMR (470 MHz, CDCl₃) δF -114.61; HRMS calcd for C₂₆H₂₁Cl₂NO₂F [M(³⁵Cl₂)+H]+ 468.0928, found 468.0915.
(2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrol-3-yl)methanol (76)

Prepared according to general procedure F using compound 75 (300 mg, 0.64 mmol), THF (1 mL), 1 M DIBAL in cyclohexane (1.53 mL, 1.53 mmol) and MeOH (1 mL). Purification by MPLC on SiO2 (Petrol:EtOAc, 0-50%) gave a white solid (259 mg, 95%). Rf = 0.20 (25% EtOAc/Petrol); m.p. 124-126 °C; λmax (EtOH)/nm 277; IR νmax/cm⁻¹ 3279 (O-H), 3045, 2921, 2868, 1525, 1488.; ¹H NMR (500 MHz, CDCl₃) δH 1.46 (1H, s, O-H), 3.86 (2H, s, Ar-CH₂), 4.63 (2H, s, CH₂OH), 6.44 (1H, s, Pyrrole-H), 6.80-6.86 (6H, m, 6 × ArH), 6.96-6.99 (2H, m, 2 × ArH), 7.13-7.14 (2H, m, 2 × ArH), 7.20-7.21 (2H, m, 2 × ArH); ¹³C NMR (125 MHz, CDCl₃) δC 30.1 (Ar-CH₂), 57.9 (CH₂OH), 109.6 (CH-Pyrrole), 115.2 (2C, d, J = 21.5 Hz, 2 × ArCHCF), 121.9 (C-Ar), 128.5 (2 × CH-Ar), 128.7 (d, J = 3.3 Hz, ArCCHCHCF), 129.2 (2 × CH-Ar), 129.3 (2 × CH-Ar), 129.7 (2C, d, J = 7.9 Hz, 2 × ArCHCHCF), 130.0 (2 × CH-Ar), 131.0 (C-Ar), 131.9 (C-Ar), 133.6 (C-Ar), 133.9 (C-Ar), 136.1 (C-Ar), 138.0 (C-Ar), 161.5 (d, J = 246.6 Hz, ArCF). ¹⁹F NMR (470 MHz; CDCl₃) δF -115.6; HRMS calcd for C₂₄H₁₉Cl₂FNO [M(³⁵Cl₂)+H]^⁺ 426.0822, found 426.0817.

2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrole-3-carbaldehyde (77)

Prepared according to general procedure G using compound 76 (135 mg, 0.316 mmol) in DCM (3 ml) and Dess-Martin periodinane (268 mg, 0.632 mmol) in DCM (4 mL). Purification by MPLC on SiO2 (Petrol:EtOAc, 0-50%) gave an off-white viscous liquid (82 mg, 61%). Rf = 0.35 (20% EtOAc/Petrol); λmax (EtOH)/nm 247; IR νmax/cm⁻¹ 3057, 2922, 2852, 2731, 1664
(C=O); 1H NMR (500 MHz, CDCl₃) δH 4.17 (2H, s, Ar-CH₂), 6.78-6.89 (7H, m, 7 × ArH), 6.98-7.01 (2H, m, 2 × ArH), 7.14-7.15 (2H, m, 2 × ArH), 7.27-7.28 (1H, m, ArH), 10.01 (1H, s, CHO); 13C NMR (125 MHz, CDCl₃) δC 30.3 (Ar-CH₂), 109.3 (CH-Pyrrole), 115.4 (2C, d, J = 21.6 Hz, 2 × ArCHCF), 123.5 (C-Ar), 127.4 (d, J = 3.2 Hz, ArCCHCHCF), 128.7 (2 × CH-Ar), 129.4 (2 × CH-Ar), 129.5 (2 × CH-Ar), 129.8 (2 × CH-Ar), 130.2 (2C, d, J = 8.1 Hz, 2 × ArCCHCHCF), 132.4 (C-Ar), 135.0 (C-Ar), 135.4 (C-Ar), 135.4 (C-Ar), 136.5 (C-Ar), 140.2 (C-Ar), 162.0 (d, J = 248.3 Hz, ArCF), 186.0 (C=O). 19F NMR (470 MHz; CDCl₃) δF -113.9.


5-((2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrol-3-yl)methylene)pyrimidine-2,4,6(1H,3H,5H)-trione (63)

A mixture of compound 77 (48 mg, 0.11 mmol), barbituric acid (15 mg, 0.12 mmol) and acetic acid (1.2 mL) was refluxed for 2.5 h. The product precipitates on cooling which was filtered and washed with EtOAc to get yellow solid (28 mg, 48%). Rf = 0.33 (2% MeOH/DCM); m.p. 276 °C (degraded); λmax (EtOH)/nm 398; IR νmax/cm⁻¹ 3198, 3054, 2924, 2848, 1737 (C=O), 1690 (C=O), 1652 (C=O); 1H NMR (500 MHz, DMSO-d₆) δH 4.17 (2H, s, ArCH₂), 6.84-6.85 (2H, m, 2 × ArH), 7.10-7.16 (4H, m, 4 × ArH), 7.19-7.20 (2H, m, 2 × ArH), 7.24-7.26 (2H, m, 2 × ArH), 7.45-7.47 (2H, m, 2 × ArH), 7.95 (1H, s, Pyrrole-H), 8.35 (1H, s, HC=C(CO)₂), 11.05 (1H, s, NH), 11.15 (1H, s, NH); 13C NMR (125 MHz, DMSO-d₆) δC 29.4 (ArCH₂), 110.8 (C-Ar), 113.1 (CH-Pyrrole), 115.4 (2C, d, J = 21.7 Hz, 2 × ArCHCF), 118.2 (C-Ar), 128.5 (2 × CH-Ar), 129.4 (2 × CH-Ar), 129.6 (2 × CH-Ar), 130.1 (2 × CH-Ar), 130.5 (2C, d, J = 8.2 Hz, 2 × ArCHCHCF), 131.1 (C-Ar), 133.7 (C-Ar), 135.2 (C-Ar), 135.5 (C-Ar), 136.8 (C-Ar), 145.8 (C-Ar), 146.5 (C-Ar), 150.3 (C-Ar), 160.4 (C=O), 161.4 (d, J = 240.7 Hz, ArCF), 162.5 (C=O), 164.4 (C=O). 19F NMR (470 MHz; DMSO-d₆) δF -114.0; HRMS calcd for C₂₈H₁₉Cl₂F₃N₃O₃ [M(³Cl₂)+H]+ 534.0782, found 534.0776.
1,2-\textit{Bis}(4-chlorobenzyl)-5-(4-fluorophenyl)-1\textit{H}-pyrrole-3-carboxylic acid (69)

A mixture of 79 (38 mg, 0.078 mmol), NaOH (75 mg, 1.75 mmol), MeOH (3.5 mL) and water (1.5 mL) was heated at 75 °C for 18 h. The solvent was evaporated \textit{in vacuo} and the remaining residue was acidified with 1 M HCl. The aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layers were combined, dried over MgSO₄ and evaporated \textit{in vacuo}. Purification by MPLC on SiO₂ (DCM:MeOH, 0-2%) gave a white solid (25 mg, 70%). \( R_f = 0.56 \) (2% MeOH/DCM); m.p. 215-217 °C; \( \lambda_{\text{max}} \) (EtOH)/nm 398; IR \( \nu_{\text{max}} \)/cm⁻¹ 2922, 2851, 2765, 2668, 2596, 1665 (C=O); \(^1\)H NMR (500 MHz, DMSO-\textit{d}_6) \( \delta_{\text{H}} \) 4.29 (2H, s, ArC\( \text{H}_2 \)), 5.06 (2H, s, ArCH₂-N), 6.59 (1H, s, Pyrrole-\( \text{H} \)), 6.64-6.66 (2H, m, 2 × Ar\( \text{H} \)), 7.06-7.07 (2H, m, 2 × Ar\( \text{H} \)), 7.14-7.18 (2H, m, 2 × Ar\( \text{H} \)), 7.20-7.22 (4H, m, 4 × Ar\( \text{H} \)), 7.28-7.31 (2H, m, 2 × Ar\( \text{H} \)), 12.10 (1H, br s, COOH); \(^13\)C NMR (125 MHz, DMSO-\textit{d}_6) \( \delta_{\text{C}} \) 29.5 (ArC\( \text{H}_2 \)), 46.5 (ArCH₂-N), 110.5 (CH-Pyrrole), 113.6 (C-Ar), 115.5 (2C, d, \( J = 21.4 \text{ Hz} \), 2 × ArCHCF), 127.2 (2 × CH-Ar), 128.2 (2 × CH-Ar), 128.3 (d, \( J = 4.1 \text{ Hz} \), ArCCHCHCF), 128.4 (2 × CH-Ar), 129.8 (2 × CH-Ar), 130.7 (C-Ar), 130.9 (2C, d, \( J = 8.2 \text{ Hz} \), 2 × ArCHCHCF), 131.5 (C-Ar), 132.8 (C-Ar), 136.4 (C-Ar), 137.3 (C-Ar), 137.6 (C-Ar), 161.6 (d, \( J = 245.4 \text{ Hz} \), ArCF), 165.9 (COOH). \(^19\)F NMR (470 MHz; DMSO-\textit{d}_6) \( \delta_{\text{F}} \) -113.3; HRMS calcd for \( \text{C}_{25}\text{H}_{19}\text{Cl}_2\text{FNO}_2 \) [M\( ^{35}\text{Cl}_2 \)+\text{H}]⁺ 454.0771, found 454.0772.
Ethyl 2-(4-chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-4-formyl-1H-pyrrole-3-carboxylate (80)

Prepared according to general procedure H using 75 (100 mg, 0.213 mmol), DMF (3 mL) phosphorus oxychloride (0.06 mL, 0.639 mmol) heated under microwave irradiation to 70 °C for 4 h. Purification by MPLC on SiO$_2$ (Petrol:EtOAc, 0-20%) gave a white solid (75 mg, 71%). $R_f = 0.22$ (20% EtOAc/Petrol); m.p. 132-134 °C; $\lambda_{\text{max}}$ (EtOH)/nm 228; IR $\nu_{\text{max}}$/cm$^{-1}$ 3050, 2962, 2849, 1679 (C=O); $^1$H NMR (500 MHz, CDCl$_3$) $\delta_H$ 1.34 (3H, t, $J = 7.1$ Hz, COOCH$_2$C$_3$H$_3$), 4.14 (2H, s, ArC$_2$H$_2$), 4.38 (2H, q, $J = 7.1$ Hz, COOCH$_2$C$_3$H$_3$), 6.68-6.70 (2H, m, 2 × ArH), 6.77-6.79 (2H, m, 2 × ArH), 6.88-6.91 (2H, m, 2 × ArH), 7.06-7.09 (2H, m, 2 × ArH), 7.13-7.14 (2H, m, 2 × ArH), 7.17-7.19 (2H, m, 2 × ArH), 10.31 (1H, s, CHO); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta_C$ 14.3 (COOCH$_2$C$_3$H$_3$), 30.9 (ArCH$_2$), 60.9 (COOCH$_2$C$_3$H$_3$), 114.3 (C-Ar), 115.2 (2C, d, $J = 21.7$ Hz, 2 × ArCHCF), 121.4 (C-Ar), 128.5 (2 × CH-Ar), 129.4 (2 × CH-Ar), 129.5 (2 × CH-Ar), 129.9 (2 × CH-Ar), 132.3 (C-Ar), 132.8 (2C, d, $J = 8.4$ Hz, 2 × ArCHCHCF), 134.3 (C-Ar), 135.3 (C-Ar), 136.3 (C-Ar), 138.8 (C-Ar), 139.1 (C-Ar), 164.7 (C=O), 187.7 (C=O), ArCCHCHCF and ArCF was not seen. $^{19}$F NMR (470 MHz; CDCl$_3$) $\delta_F$ -111.52; HRMS calcd for C$_{27}$H$_{21}$Cl$_2$FNO$_3$ [M$^{35}$Cl$_2$]+ 496.0877, found 496.0878.
5-(4-Chlorobenzyl)-1-(4-chlorophenyl)-4-(ethoxycarbonyl)-2-(4-fluorophenyl)-1H-pyrrole-3-carboxylic acid (81)

Prepared according to general procedure I using 80 (65 mg, 0.131 mmol), MeCN (2 mL), a solution of sodium chlorite (17 mg, 0.183 mmol) in H₂O (0.2 mL), a solution of sulfamic acid (18 mg, 0.183 mmol) in H₂O (0.2 mL). EtOAc extraction gave an off-white solid (68 mg, quant.) which did not require further purification; Rᵣ = 0.56 (2% MeOH/DCM); m.p. 172-174 °C; λₘₐₓ (EtOH)/nm 223; IR νₘₐₓ/cm⁻¹ 2919, 2850, 2639, 1680 (broad, 2×C=O overlapped); ¹H NMR (500 MHz, DMSO-d₆) δH 1.18 (3H, t, J = 7.1 Hz, COOCH₂C₃H₃), 4.05 (2H, s, ArCH₂), 4.18 (2H, q, J = 7.1 Hz, COOCH₂CH₃), 6.81-6.83 (2H, m, 2 × ArH), 7.03-7.09 (4H, m, 4 × ArH), 7.18-7.22 (4H, m, 4 × ArH), 7.33-7.35 (2H, m, 2 × ArH), 12.39 (1H, s, COOH); ¹³C NMR (125 MHz, DMSO-d₆) δC 13.9 (COOCH₂CH₃), 29.9 (ArCH₂), 59.9 (COOCH₂CH₃), 113.0 (C-Ar), 114.7 (2C, d, J = 21.6 Hz, 2 × ArCH₂CF), 116.6 (C-Ar), 126.4 (d, J = 3.3 Hz, ArCH₂CF), 128.1 (2 × CH-Ar), 129.0 (2 × CH-Ar), 129.6 (2 × CH-Ar), 130.7 (C-Ar), 130.7 (2 × CH-Ar), 132.8 (2C, d, J = 8.4 Hz, 2 × ArCH₂CF), 133.5 (C-Ar), 133.9 (C-Ar), 134.9 (C-Ar), 136.1 (C-Ar), 137.0 (C-Ar), 161.6 (d, J = 245.4 Hz, ArC=O), 164.2 (C=O), 165.8 (C=O); ¹⁹F NMR (470 MHz; DMSO-d₆) δF -113.3; HRMS calcd for C₂₇H₂₁Cl₂FNO₄ [M(³⁵Cl₂)+H]⁺ 512.0826, found 512.0820.
2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrole-3,4-dicarboxylic acid (64)

A mixture of 81 (55 mg, 0.107 mmol), NaOH (185 mg, 4.625 mmol), MeOH (3 mL), water (1.5 mL) was refluxed for 4 h. The solvent was evaporated in vacuo and the remaining residue was acidified with 2 M HCl. The aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over MgSO₄ and evaporated in vacuo to obtain an off-white solid (50 mg, 97%) which did not require further purification. Rᵣ = 0.47 (7.5% DCM/MeOH); m.p. 143-145 °C; λmax (EtOH)/nm 224; IR νmax/cm⁻¹ 3506, 3048, 2919, 2849, 1687 (C=O), 1607 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 4.16 (2H, s, 2 × ArCH₂), 6.81-6.83 (2H, m, 2 × ArH), 7.03-7.06 (4H, m, 4 × ArH), 7.19-7.21 (4H, m, 4 × ArH), 7.31-7.33 (2H, m, 2 × ArH), 13.2 (1H, br s, COOH), other COOH could not be seen.; ¹³C NMR (125 MHz, DMSO-d₆) δC 30.0 (ArCH₂), 114.5 (2C, d, J = 21.4 Hz, 2 × ArCH₂CF), 115.7 (2 × CH-Ar), 127.1 (d, J = 4.0 Hz, ArCH₂CHCF), 128.0 (2 × CH-Ar), 129.9 (2 × CH-Ar), 129.6 (2 × CH-Ar), 130.6 (C-Ar), 130.7 (2 × CH-Ar), 132.8 (2C, d, J = 8.3 Hz, 2 × ArCH₂CHCF), 133.4 (C-Ar), 134.9 (C-Ar), 135.5 (C-Ar), 137.3 (C-Ar), 137.5 (C-Ar), 161.6 (d, J = 245.7 Hz, ArCF), 165.7 (COOH), 166.8 (COOH); ¹⁹F NMR (470 MHz; DMSO-d₆) δF -113.5; HRMS calcd for C₂₅H₁₇Cl₂FNO₄ [M(³⁵Cl₂)+H]⁺ 484.0507, found 484.0508.
(E)-3-(2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-(4-fluorophenyl)-1H-pyrrol-3-yl)acrylic acid (65)

A mixture of aldehyde 77 (70 mg, 0.16 mmol), malonic acid (26 mg, 0.247 mmol), piperidine (1 drop), and pyridine (0.5 mL) was refluxed for 2.5 h. The reaction was acidified to pH 2 with 2M HCl and extracted with EtOAc (3 × 25 mL). The organic layers were combined, dried over MgSO₄, and evaporated in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-10%) gave a yellow solid (58 mg, 71%); Rᵣ = 0.24 (2% MeOH/DCM); m.p. 242-244 °C; λᵤₚₑₚₑₑ (EtOH)/nm 264; IR νₑₓₓₑₓₑ/cm⁻¹ 3061, 2920, 2851, 1674 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δₜ 4.01 (2H, s, CH₂), 6.24 (1H, d, J = 15.5 Hz, alkene-H), 6.79-6.81 (2H, m, 2 × ArH), 6.91 (1H, s, Pyrrole-H), 7.06-7.13 (6H, m, 6 × ArH), 7.23-7.24 (2H, m, 2 × ArH), 7.30-7.41 (2H, m, 2 × ArH), 7.59 (1H, d, J = 15.5 Hz, alkene-H); ¹³C NMR (125 MHz, DMSO-d₆) δₜ 28.9 (ArCH₂), 107.0 (Pyrrole-CH), 114.9 (Alkene-CH), 115.2 (2C, d, J = 21.4 Hz, 2 × ArCHCF), 118.6 (C-Ar), 128.2 (d, J = 3.5 Hz, ArCCHCHCF), 128.3 (2 × CH-Ar), 129.2 (2 × CH-Ar), 129.4 (2 × CH-Ar), 130.0 (2C, d, J = 8.1 Hz, 2 × ArCHCHCF), 130.3 (2 × CH-Ar), 130.7 (C-Ar), 133.0 (C-Ar), 134.6 (C-Ar), 135.8 (C-Ar), 136.2 (C-Ar), 136.5 (C-Ar), 137.7 (Alkene-CH), 161.1 (d, J = 244.6 Hz, ArCF), 168.2 (COOH); ¹⁹F NMR (470 MHz; DMSO-d₆) δₑ -115.0; LRMS (ES⁺) m/z 466.3 [M(³⁵Cl₂)+H]+, 468.3 [M(³⁵Cl³⁷Cl)+H]+.
Ethyl 4-(4-chlorophenyl)-3-oxo-2-(2-oxo-2-phenylethyl)butanoate, (96)

\[
\begin{array}{c}
\text{Cl} \\
\text{CH}_2 \\
\text{CH}_2 \\
\text{O}
\end{array}
\]

Prepared according to general procedure C using compound 72 (2.01 g, 8.35 mmol) and NaH (60% in mineral oil, 433 mg, 10.85 mmol) in THF (6 mL) followed by addition of 2-bromo-1-phenylethan-1-one (1.83 mg, 9.18 mmol) in THF (4 mL). The residue was purified by MPLC on SiO\textsubscript{2} (Petrol:EtOAc, 0-20%) to give a white solid (2.36 g, 79%). R\textsubscript{f} = 0.22 (10% EtOAc/Petrol); m.p. 68-70°C; \lambdamax (EtOH)/nm 241.8, 225.6; IR ν\textsubscript{max}/cm\textsuperscript{-1} 3064, 3036, 2973, 2928, 2898, 1711 (C=O stretch), 1684 (C=O); \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ \text{H} 1.28 (3H, t, J = 7.1 Hz, COOCH\textsubscript{2}CH\textsubscript{3}), 3.55 (1H, dd, J = 18.4, 4.8 Hz, 1 × CH\textsubscript{2}), 3.76 (1H, dd, J = 18.4, 9.1 Hz, 1 × CH\textsubscript{2}), 4.07 (1H, d, J = 17.1 Hz, ArCH\textsubscript{2}), 4.12 (1H, d, J = 17.1 Hz, ArCH\textsubscript{2}), 4.20 (2H, q, J = 7.1 Hz, COOCH\textsubscript{2}CH\textsubscript{3}), 4.29 (1H, dd, J = 9.1 and 4.8 Hz, CHCOOEt), 7.18-7.20 (2H, m, 2 × ArH), 7.29-7.31 (2H, m, 2 × ArH), 7.45-7.48 (2H, m, 2 × ArH), 7.56-7.59 (1H, m, 1 × ArH), 7.95-7.97 (2H, m, 2 × ArH); \textsuperscript{13}C NMR (125 MHz, CDCl\textsubscript{3}) δ\text{C} 14.1 (COOCH\textsubscript{2}CH\textsubscript{3}), 37.8 (CH\textsubscript{2}), 49.2 (ArCH\textsubscript{2}), 52.6 (CHCOOEt), 62.0 (COOCH\textsubscript{2}CH\textsubscript{3}), 128.2 (2 × C-Ar), 128.7 (2 × C-Ar), 131.3 (2 × C-Ar), 132.0 (C-Ar), 133.1 (C-Ar), 133.6 (C-Ar), 135.9 (C-Ar), 168.7 (C=O), 197.2 (C=O), 202.0 (C=O); HRMS calcd for C\textsubscript{20}H\textsubscript{18}ClO\textsubscript{4} [M(\textsuperscript{35}Cl\textsubscript{2})-H]\textsuperscript{+} 357.0899, found 357.0890.

Ethyl 2-(4-chlorobenzyl)-1-(4-chlorophenyl)-5-phenyl-1\textit{H}-pyrrole-3-carboxylate (82)

\[
\begin{array}{c}
\text{Cl} \\
\text{O}
\end{array}
\]

Prepared according to general procedure D using compound 96 (1.4 g, 3.90 mmol), 4-chloroaniline (2.5 g, 19.5 mmol) and acetic acid (11 mL). Purification by MPLC on SiO\textsubscript{2} (Petrol:EtOAc, 0-10%) gave a white solid (1.28 g, 73%). R\textsubscript{f} = 0.52 (10% EtOAc/Petrol); m.p. 127-129°C; IR ν\textsubscript{max}/cm\textsuperscript{-1} 2985, 2927, 2657, 1702 (C=O); \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}) δ\text{H} 1.33
(3H, t, J = 7.1 Hz, COOCH₂CH₃), 4.23 (2H, s, 2 × ArCH₂), 4.31 (2H, q, J = 7.1 Hz, COOCH₂CH₃), 6.80-6.86 (5H, m, 5 × ArH), 7.01-7.14 (2H, m, 2 × ArH), 7.11-7.18 (5H, m, 5 × ArH), 7.22-7.26 (2H, m, 2 × ArH); ¹³C NMR (125 MHz, CDCl₃) δc 14.5 (COOCH₂CH₃), 30.8 (ArCH₂), 59.8 (COOCH₂CH₃), 110.5 (C-Ar), 111.3 (C-Ar), 113.0 (C-Ar), 127.0 (C-Ar), 128.2 (2 × C-Ar), 128.3 (2 × C-Ar), 128.4 (2 × C-Ar), 129.4 (2 × C-Ar), 129.5 (2 × C-Ar), 130.6 (2 × C-Ar), 131.8 (C-Ar), 131.8 (C-Ar), 134.5 (C-Ar), 134.7 (C-Ar), 135.3 (C-Ar), 137.4 (C-Ar), 138.8 (C-Ar), 165.1 (C=O); HRMS calcd for C₂₆H₂₂Cl₂NO₂ [M⁺(³⁵Cl₂)+H]⁺ 450.1022, found 450.1012.

2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-phenyl-1H-pyrrole-3-carboxylic acid (93)

Prepared according to general procedure E using compound 82 (45 mg, 0.099 mmol), NaOH (143 mg, 3.56 mmol) in MeOH (2 ml) and water (1.5 ml) followed by addition of more MeOH (1 ml) after 18 h. Compound 93 was obtained as a white solid (41 mg, 98%) which did not require further purification. Rᵣ = 0.55 (2% MeOH/DCM); m.p. 217-219 °C; λ_max (EthOH)/nm 264.6; IR ν_max/cm⁻¹ 3300-2200 (broad COOH peak), 2936, 2851, 2591, 1665 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 4.24 (2H, s, 2 × ArCH₂), 6.76 (1H, s, Pyrrole-H), 6.79 (2H, d, J = 8.5 Hz, 2 × ArH), 7.04-7.07 (4H, m, 4 × ArH), 7.13-7.21 (5H, m, 5 × ArH), 7.40 (2H, d, J = 8.5 Hz, 2 × ArH), 12.15 (1H, s, COOH); ¹³C NMR (125 MHz, DMSO-d₆) δc 29.8 (ArCH₂), 110.3 (C-Ar), 114.0 (C-Ar), 126.9 (C-Ar), 128.0 (2 × C-Ar), 128.1 (2 × C-Ar), 128.2 (2 × C-Ar), 129.2 (2 × C-Ar), 129.5 (2 × C-Ar), 130.5 (2 × C-Ar), 131.5 (C-Ar), 133.2 (C-Ar), 133.9 (C-Ar), 136.1 (C-Ar), 137.7 (C-Ar), 138.5 (C-Ar), 165.8 (C=O); LRMS (ES⁺) m/z 422.2 [M(³⁵Cl₂)+H]⁺, 424.2 [M(³⁵Cl³⁷Cl)+H]⁺.
**Ethyl 4-(4-methoxyphenyl)-3-oxobutanoate (98)**

![Ethyl 4-(4-methoxyphenyl)-3-oxobutanoate](image)

Prepared according to the procedure B using Meldrum’s acid (1 g, 6.93 mmol), 4-methoxyphenylacetyl chloride (0.93 mL, 6.06 mmol), pyridine (1.1 ml, 13.21 mmol), DCM (6 mL), EtOH (21 mL). Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-20%) gave a yellow oil (1.03 g, 72%). ¹H NMR (500 MHz, CDCl₃) δH 1.25 (3H, t, J = 7.2 Hz, COOCH₂C₃H₇), 3.45 (2H, s, CH₂), 3.75 (2H, s, CH₂Ar), 3.79 (3H, s, OCH₃), 4.16 (2H, q, J = 7.2 Hz, COOC₂H₅CH₃), 6.86-6.88 (2H, m, 2 × ArH), 7.11-7.13 (2H, m, 2 × ArH); ¹³C NMR (125 MHz, CDCl₃) δC 14.1 (COOCH₂C₃H₇), 48.2 (CH₂), 49.2 (CH₂Ar), 55.3 (OCH₃), 61.4 (COOC₂H₅CH₃), 114.3 (2 × CH-Ar), 125.2 (C-Ar), 130.6 (2 × CH-Ar), 131.6 (C-Ar), 158.9 (C-Ar), 167.2 (C=O), 201.0 (C=O).

**Ethyl 4-(4-methoxyphenyl)-3-oxo-2-(2-oxo-2-phenylethyl)butanoate (99)**

![Ethyl 4-(4-methoxyphenyl)-3-oxo-2-(2-oxo-2-phenylethyl)butanoate](image)

Prepared according to general procedure C using compound 98 (629 mg, 2.66 mmol) and NaH (60% in mineral oil, 138 mg, 3.45 mmol) in THF (1.7 mL) followed by addition of 2-bromo-1-phenylethan-1-one (582 mg, 2.92 mmol) in THF (1.3 mL). The residue was purified by MPLC on SiO₂ (Petrol:EtOAc, 0-20%) to give a yellow oil (715 mg, 76%). Rf = 0.33 (20% EtOAc/Petrol); λmax (EtOH)/nm 241.2; IR νmax/cm⁻¹ 2979, 2836, 1738 (C=O stretch), 1715 (C=O), 1681 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 1.28 (3H, t, J = 7.1 Hz, COOCH₂C₃H₇), 3.54 (1H, dd, J = 18.3 and 5.5 Hz, 1 × CH₂), 3.69 (1H, dd, J = 18.3 and 8.5 Hz, 1 × CH₂), 3.79 (3H, s, OCH₃), 4.00 (1H, d, J = 16.6 Hz, ArCH₂), 4.04 (1H, d, J = 16.6 Hz, ArCH₂), 4.19 (2H, q, J = 7.1 Hz, COOCH₂C₃H₇), 4.31 (1H, dd, J = 8.5 and 5.5 Hz, CHCOOEt), 6.86-6.88 (2H, m, 2 × ArH), 7.16-7.18 (2H, m, 2 × ArH), 7.44-7.47 (2H, m, 2 × ArH), 7.55-7.59 (1H, m, 1 × ArH), 7.95-7.97 (2H, m, 2 × ArH); ¹³C NMR (125 MHz, CDCl₃) δC 14.1 (COOCH₂C₃H₇), 37.8 (CH₂), 49.0 (ArCH₂), 52.5 (CHCOOEt), 55.3 (CH₃), 61.8 (COOCH₂C₃H₇), 114.1 (2 × C-Ar), 125.6 (C-Ar), 128.2 (2 × C-Ar), 128.7 (2 × C-Ar), 130.9 (2 × C-Ar), 133.5 (C-Ar), 136.1 (C-Ar), 158.7 (C-Ar), 168.9 (C=O), 197.2 (C=O), 202.6 (C=O); HRMS calcd for C₂₁H₂₃O₅ [M+H]⁺ 355.1540, found 355.1536.
Ethyl 1-(4-chlorophenyl)-2-(4-methoxybenzyl)-5-phenyl-1H-pyrrole-3-carboxylate (100)

Prepared according to general procedure D using compound 99 (319 mg, 0.90 mmol), 4-chloroaniline (574 mg, 4.50 mmol) and acetic acid (2.7 mL). Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-15%) gave a white solid (300 mg, 75%). R_f = 0.56 (20% EtOAc/Petrol); λ_{max} (EtOH)/nm 274.8, 224.0; IR ν_{max}/cm⁻¹ 3110, 2996, 2936, 1701 (C=O); ¹H NMR (500 MHz, CDCl₃) δ H 1.33 (3H, t, J = 7.1 Hz, COOCH₂C₂H₅), 3.74 (3H, s, OC₆H₃), 4.20 (2H, s, 2 × ArC₆H₂), 4.32 (2H, q, J = 7.1 Hz, COOC₂H₂CH₃), 6.68-6.71 (2H, m, 2 × ArH), 6.77-6.79 (2H, m, 2 × ArH), 6.84-6.86 (3H, m, 3 × ArH), 7.01-7.03 (2H, m, 2 × ArH), 7.13-7.17 (3H, m, 3 × ArH), 7.21-7.23 (2H, m, 2 × ArH); ¹³C NMR (125 MHz, CDCl₃) δC 14.5 (COOCH₂C₂H₅), 30.6 (ArCH₂), 55.2 (OCH₃), 59.8 (COOCH₂C₂H₅), 110.5 (C-Ar), 113.6 (C-Ar), 114.0 (C-Ar), 126.8 (2 × C-Ar), 128.2 (2 × C-Ar), 128.3 (2 × C-Ar), 129.1 (2 × C-Ar), 129.1 (2 × C-Ar), 130.1 (2 × C-Ar), 131.1 (C-Ar), 131.9 (C-Ar), 134.3 (C-Ar), 134.5 (C-Ar), 136.5 (C-Ar), 136.5 (C-Ar), 140.0 (C-Ar), 157.9 (C-Ar), 165.3 (C=O); HRMS calcd for C_{27}H_{25}ClNO₃ [M^{(35)Cl+}+H]^+ 446.1517, found 446.1511.

1-(4-Chlorophenyl)-2-(4-methoxybenzyl)-5-phenyl-1H-pyrrole-3-carboxylic acid (101)

Prepared according to general procedure E using compound 100 (31 mg, 0.069 mmol), NaOH (140 mg, 3.5 mmol) in MeOH (3 ml) and water (1.5 ml) followed by addition of more MeOH (1 ml) after 18 h. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-100%) gave a white solid (28 mg, quant.). R_f = 0.40 (2% MeOH/DCM); m.p. 214-216 °C; λ_{max} (EtOH)/nm 275.4; IR
\( \nu_{\text{max}} /\text{cm}^{-1} \) 3005, 2996, 2922, 2828, 2576, 1654 (C=O); \(^1\)H NMR (500 MHz, DMSO-\( d_6 \)) \( \delta_H \) 3.66 (3H, s, OCH\(_3\)), 4.19 (2H, s, 2 \times \text{ArCH}_2\), 6.67-6.71 (4H, m, 4 \times \text{ArH}), 6.74 (1H, s, 1 \times \text{ArH}), 7.01-7.06 (4H, m, 4 \times \text{ArH}), 7.14-7.22 (3H, m, 3 \times \text{ArH}), 7.40-7.42 (2H, m, 2 \times \text{ArH}), 12.13 (1H, br s, COO\(_\text{H}\)). \(^{13}\)C NMR (125 MHz, DMSO-\( d_6 \)) \( \delta_C \) 29.5 (Ar\( \text{CCH}_2\)), 54.9 (OCH\(_3\)), 110.3 (C-Ar), 113.5 (C-Ar), 113.8 (C-Ar), 126.8 (2 \times C-Ar), 127.9 (2 \times C-Ar), 128.2 (2 \times C-Ar), 128.7 (2 \times C-Ar), 129.1 (2 \times C-Ar), 130.5 (2 \times C-Ar), 130.5 (C-Ar), 131.6 (C-Ar), 133.0 (C-Ar), 133.6 (C-Ar), 136.2 (C-Ar), 139.6 (C-Ar), 157.4 (C-Ar), 165.9 (C=O); LRMS (ES\(^+\)) \( m/z \) 416.2 [M\(^{(35}\text{Cl})-\text{H}\)], 418.3 [M\(^{(37}\text{Cl})-\text{H}\)].

1-(4-Chlorophenyl)-2-(4-hydroxybenzyl)-5-phenyl-1\(H\)-pyrrole-3-carboxylic acid (94)

![Chemical Structure](image)

Compound **101** (20 mg, 0.048 mmol) was dissolved in DCM (2 mL) and cooled to 0 °C. Boron tribromide (1 M in DCM, 70 \( \mu \)L, 0.072 mmol) was added dropwise and the reaction mixture was stirred at 0 °C for 1 h. The mixture was quenched with water (20 mL) and extracted with EtOAc (3x30 mL). The organic layers were combined, washed with brine, dried over MgSO\(_4\) and the solvent removed \( \text{in vacuo} \). Purification by MPLC (H\(_2\)O:MeOH, reversed phase with 0.1% HCOOH modifier, 30-100%) gave a white solid (17 mg, 88%). \( R_t = 0.52 \) (8% MeOH/DCM); m.p. 230°C (degraded); IR \( \nu_{\text{max}} /\text{cm}^{-1} \) 3567 (OH), 3328-2325 (COOH), 3025, 2923, 2854, 1665 (C=O); \(^1\)H NMR (500 MHz, DMSO-\( d_6 \)) \( \delta_H \) 4.19 (2H, s, 2 \times \text{ArCH}_2\), 6.48-6.49 (2H, m, 2 \times \text{ArH}), 6.54-6.56 (2H, m, 2 \times \text{ArH}), 6.70 (1H, s, Pyrrole-H), 6.99-7.02 (4H, m, 4 \times \text{ArH}), 7.10-7.13 (1H, m, 1 \times \text{ArH}), 7.16-7.19 (2H, m, 2 \times \text{ArH}), 7.37-7.39 (2H, m, 2 \times \text{ArH}); \(^{13}\)C NMR was not be obtained; LRMS (ES\(^+\)) \( m/z \) 402.2 [M\(^{(35}\text{Cl})-\text{H}\)], 404.2 [M\(^{(37}\text{Cl})-\text{H}\)].
Ethyl 2-(4-chlorobenzyl)-1-(4-chlorophenyl)-4-formyl-5-phenyl-1H-pyrrole-3-carboxylate (83)

Prepared according to general procedure H using compound 82 (940 mg, 2.08), phosphorous oxychloride (0.97 mL, 10.4 mmol) and DMF (10.5 mL). Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-30%) gave a white solid (980 mg, quant.). Rₙ = 0.38 (20% EtOAc/Petrol); m.p. 162-165 °C; IR νmax/cm⁻¹ 3060, 2972, 2928, 2846, 2767, 1697 (C=O), 1665 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.22 (3H, t, J = 7.1 Hz, COOCH₂CH₃), 4.11 (2H, s, 2 × ArCH₂), 4.26 (2H, q, J = 7.1 Hz, COOC₂H₅CH₃), 6.85-6.87 (2H, m, 2 × ArH), 7.09-7.11 (2H, m, 2 × ArH), 7.20-7.26 (7H, m, 7 × ArH), 7.33-7.35 (2H, m, 2 × ArH) 10.11 (1H, s, CHO); ¹³C NMR (125 MHz, DMSO-d₆) δC 14.5 (COOCH₂CH₃), 30.6 (ArCH₂), 60.7 (COOCH₂CH₃), 113.8 (C-Ar), 121.2 (C-Ar), 128.2 (2×C-Ar), 128.6 (2×C-Ar), 129.0 (C-Ar), 129.4 (2×C-Ar), 129.7 (C-Ar), 130.1 (2×C-Ar), 131.2 (2×C-Ar), 131.3 (C-Ar), 131.5 (2×C-Ar), 134.2 (C-Ar), 134.9 (C-Ar), 137.3 (C-Ar), 138.5 (C-Ar), 140.6 (C-Ar), 164.7 (C=O) 187.1 (C≡O); HRMS calcd for C₂₇H₂₂Cl₂NO₃ [M(Cl₂)+H]⁺ 478.0971, found 478.0967.

5-(4-Chlorobenzyl)-1-(4-chlorophenyl)-4-(ethoxycarbonyl)-2-phenyl-1H-pyrrole-3-carboxylic acid (84)

Prepared according to general procedure I using compound 83 (1.21 g, 2.52 mmol) in MeCN (30 ml), sodium chlorite (320 mg, 3.54 mmol) in water (4 mL), sulfamic acid (344 mg, 3.54 mmol) in water (4 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a white solid
(780 mg, 62%). Rf = 0.44 (2% MeOH/DCM); m.p. 210-212 °C; λmax (EtOH)/nm 398; IR νmax/cm⁻¹ 3031, 2984, 2921, 2903, 2628, 1709 (C=O), 1683 (C=O); ¹H NMR (500 MHz, DMSO-d⁶) δH 1.20 (3H, t, J = 7.1 Hz, COOCH₂CH₃), 4.08 (2H, s, 2 × ArCH₂), 4.19 (2H, q, J = 7.1 Hz, COOCH₂CH₃), 6.82-6.84 (2H, m, 2 × ArH), 7.06-7.08 (2H, m, 2 × ArH), 7.14-7.16 (2H, m, 2 × ArH), 7.19-7.21 (4H, m, 4 × ArH), 7.33-7.34 (2H, m, 2 × ArH), 12.39 (1H, s, COOH); ¹³C NMR (125 MHz, DMSO-d⁶) δC 14.4 (COOCH₂CH₃), 30.4 (ArCH₂), 60.4 (COOCH₂CH₃), 113.4 (C-Ar), 117.2 (C-Ar), 128.2 (2 × C-Ar), 128.4 (C-Ar), 128.6 (2 × C-Ar), 129.4 (2 × C-Ar), 130.1 (2 × C-Ar), 130.5 (C-Ar), 130.9 (2 × C-Ar), 131.2 (C-Ar), 131.2 (2 × C-Ar), 133.9 (C-Ar), 135.1 (C-Ar), 135.5 (C-Ar), 136.7 (C-Ar), 137.6 (C-Ar), 164.6 (C=O), 166.5 (C=O); HRMS calcd for C₂₇H₂₂Cl₂NO₄ [M(Cl₂)+H]⁺ 494.0920, found 494.0908.

2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-phenyl-1H-pyrrole-3,4-dicarboxylic acid (67)

Compound 84 (735 mg, 1.48 mmol) was dissolved in MeOH (5 mL) and THF (5 mL) and 2 M NaOH (5 mL) was added. The reaction mixture was stirred at 80 °C for 3 h. After cooling to r.t., the solvent was removed in vacuo, residue was acidified with 2 M HCl and extracted with EtOAc (3×30 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-20%) gave a beige solid (690 mg, quant.). Rf=0.13 (8% MeOH/DCM); m.p. 184-188 °C; λmax (EtOH)/nm 270.0, 224.4; IR νmax/cm⁻¹ 3031, 2984, 2921, 2903, 2767, 1709 (C=O), 1683 (C=O); ¹H NMR (500 MHz, DMSO-d⁶) δH 4.21 (2H, s, 2 × ArCH₂), 6.82 (2H, d, J = 8.4 Hz, 2 × ArH), 7.01 (2H, d, J = 8.4 Hz, 2 × ArH), 7.12-7.18 (5H, m, 5 × ArH), 7.20 (2H, d, J = 8.4 Hz, 4 × ArH), 7.30 (2H, d, J = 8.4 Hz, 2 × ArH); ¹³C NMR (125 MHz, DMSO-d⁶) δC 30.5 (ArCH₂), 114.4 (C-Ar), 116.4 (C-Ar), 127.9 (2 × C-Ar), 128.1 (C-Ar), 128.5 (2 × C-Ar), 129.2 (2 × C-Ar), 130.1 (2 × C-Ar), 130.9 (C-Ar), 131.1 (2 × C-Ar), 131.3 (2 × C-Ar), 131.6 (C-Ar), 133.7 (C-Ar), 135.7 (C-Ar), 137.2 (C-Ar), 137.9 (C-Ar), 138.1 (C-Ar), 166.5 (C=O), 167.3 (C=O); HRMS calcd for C₂₅H₂₁Cl₂NO₄ [M(Cl₂)+H]⁺ 466.0607, found 466.0598.
4-(4-Chlorobenzyl)-5-(4-chlorophenyl)-6-phenyl-1H-furo[3,4-c]pyrrole-1,3(5H)-dione (85)

![Chemical Structure]

Compound 67 (520 mg, 1.11 mmol) was dissolved in acetic anhydride (8 mL) and refluxed for 2 h. After cooling to r.t., acetic anhydride was removed by filtration. The residue was washed with water (3×20mL) and petrol (3×20mL) and dried in vacuum oven at 40 °C to get an off-white solid (410 mg, 82%). Rf = 0.42 (20% EtOAc/Petrol); m.p. 233-235 °C; λmax (EtOH)/nm 275.4; IR νmax/cm⁻¹ 3092, 2649, 2376, 1816 (C=O), 1756 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 4.02 (2H, s, 2 × ArC₆H₂), 6.96 (2H, d, J = 8.3 Hz, 2 × ArH), 7.25-7.27 (4H, m, 4 × ArH), 7.30-7.34 (5H, m, 5 × ArH), 7.53 (2H, d, J = 8.3 Hz, 2 × ArH); ¹³C NMR (125 MHz, DMSO-d₆) δC 31.0 (ArC₆H₂), 116.1 (C-Ar), 117.3 (C-Ar), 127.7 (C-Ar), 128.9 (2 × C-Ar), 129.0 (2 × C-Ar), 129.6 (2 × C-Ar), 129.9 (C-Ar), 130.2 (2 × C-Ar), 130.6 (2 × C-Ar), 130.8 (2 × C-Ar), 131.9 (C-Ar), 134.9 (C-Ar), 135.1 (C-Ar), 135.3 (C-Ar), 135.7 (C-Ar), 136.0 (C-Ar), 159.6 (C=O), 159.7 (C=O); HRMS calcd for C₂₅H₁₆Cl₂NO₃ [M(²Cl₂)+H]⁺ 448.0502, found 448.0496.

4-(4-Chlorobenzyl)-5-(4-chlorophenyl)-6-phenylpyrrolo[3,4-c]pyrrole-1,3(2H,5H)-dione (68)

![Chemical Structure]

Compound 85 (65 mg, 0.14 mmol) was suspended in acetic acid (1 mL) and 0.5 M NH₃ in dioxane (4.2 mL, 2.1 mmol) was added. The mixture was heated at 120 °C under microwave irradiation for 30 min. After cooling to r.t., the solvent was removed in vacuo. The residue was
suspended in THF (3 mL) and thionyl chloride was added (70 µL). The resulting solution was stirred at r.t. for 1.5 h and refluxed for 1.5 h. After cooling to r.t., the solvent was removed in vacuo. The residue was purified by MPLC on SiO2 (Petrol:EtOAc, 0-50%) to give a beige solid (39 mg, 62%). Rf = 0.52 (2% MeOH/DCM); m.p. 274-276 °C; λmax (EtOH)/nm 322.8; IR νmax/cm\(^{-1}\) 3191 (NH), 3061, 2922, 2851, 2753, 1742 (C=O), 1701 (C=O); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) δ\(_H\) 3.97 (2H, s, 2 × ArC\(H_2\)), 6.88-6.90 (2H, m, 2 × ArH), 7.22-7.29 (9H, m, 9 × ArH), 7.48-7.50 (2H, m, 2 × ArH), 10.59 (1H, s, NH); \(^13\)C NMR (125 MHz, DMSO-\(d_6\)) δ\(C\) 30.6 (ArC\(H_2\)), 107.5 (C-Ar), 118.5 (C-Ar), 119.7 (C-Ar), 128.7 (2 × C-Ar), 128.8 (2 × C-Ar), 129.1 (C-Ar), 129.5 (2 × C-Ar), 130.0 (2 × C-Ar), 130.4 (2 × C-Ar), 131.0 (2 × C-Ar), 131.6 (C-Ar), 132.6 (C-Ar), 132.8 (C-Ar), 134.5 (C-Ar), 135.5 (C-Ar), 136.6 (C-Ar), 165.5 (C=O), 165.9 (C=O); HRMS calcd for C\(_{25}\)H\(_{17}\)Cl\(_2\)N\(_2\)O\(_2\) [M+(3Cl\(_2\))+H\(^+\)] \(^4\)47.0662, found 447.0655.

4-(4-Chlorobenzyl)-2,5-bis(4-chlorophenyl)-6-phenylpyrrolo[3,4-c]pyrrole-1,3(2H,5H)-dione (86)

![Chemical Structure](image_url)

Compound 85 (65 mg, 0.14 mmol) and 4-chloroaniline (90 mg, 0.70 mmol) were suspended in acetic acid (1.5 mL). The mixture was heated at 120 °C under microwave irradiation for 20 min. After cooling to r.t., the solvent was removed in vacuo. The residue was purified by MPLC on SiO2 (DCM:MeOH, 0-10%) to give an off-white solid. The product was suspended in THF (5 mL) and thionyl chloride was added (200 µL). The resulting solution was stirred at r.t. for 18 h. After cooling to r.t., the solvent was removed in vacuo. The residue was purified by MPLC on SiO2 (DCM:MeOH, 0-10%) to give a beige solid (52 mg, 64%). Rf = 0.52 (20% EtOAc/Petrol); m.p. 256 °C (degraded); λmax (EtOH)/nm 246.8; IR νmax/cm\(^{-1}\) 3055, 2922, 2850, 1752 (C=O), 1700 (C=O); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) δ\(_H\) 4.05 (2H, s, 2 × ArCH\(_2\)), 6.94-6.96 (2H, m, 2 × ArH), 7.24-7.27 (4H, m, 4 × ArH), 7.30 (5H, s, 5 × ArH), 7.44-7.46 (2H, m, 2 × ArH), 7.51-7.53 (2H, m, 2 × ArH), 7.57-7.59 (2H, m, 2 × ArH); \(^13\)C NMR (125 MHz,
DMSO-\textit{d}_6 \delta_C 30.8 (Ar\text{CH}_2), 116.9 (C-Ar), 118.1 (C-Ar), 128.5 (C-Ar), 128.8 (2 × C-Ar), 128.8 (2 × C-Ar), 129.2 (2 × C-Ar), 129.4 (C-Ar), 129.6 (2×C-Ar), 129.7 (2×C-Ar), 130.1 (2 × C-Ar), 130.6 (2 × C-Ar), 131.0 (2 × C-Ar), 131.7 (C-Ar), 132.1 (C-Ar), 132.5 (C-Ar), 133.9 (C-Ar), 134.2 (C-Ar), 134.7 (C-Ar), 135.3 (C-Ar), 136.3 (C-Ar), 163.0 (C=O), 163.3 (C=O); HRMS calcd for C\textsubscript{31}H\textsubscript{20}Cl\textsubscript{3}N\textsubscript{2}O\textsubscript{2} [M(35Cl\textsubscript{3})+H\textsuperscript{+}] 557.0585, found 557.0585.

(2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-phenyl-1\textit{H}-pyrrolo-3-yl)methanol (308)

Prepared according to general procedure F using compound 82 (1.2 g, 2.66 mmol), 1 M DIBAL in THF (6.7 mL, 6.70 mmol) and THF (5 mL). Purification by MPLC on SiO\textsubscript{2} (Petrol:EtOAc, 0-30%) gave an off-white solid (1.06 g, 97%). R\textsubscript{f} = 0.25 (25% EtOAc/Petrol); m.p. 137-140 °C; \lambda\textsubscript{max} (EtOH)/nm 283.4; IR \nu\textsubscript{max}/cm\textsuperscript{-1} 3319 (OH), 3089, 2918, 2877, 2224; \textit{^1}H NMR (500 MHz, DMSO-\textit{d}_6) \delta_H 3.88 (2H, s, Ar\text{CH}_2), 4.44 (2H, d, J = 5.3 Hz, Ar\text{CH}_2\text{OH}), 4.84 (1H, t, J = 5.3 Hz, OH), 6.44 (1H, s, Pyrrole-H), 6.83-6.86 (2H, m, 2 × Ar\textit{H}), 6.95-6.98 (2H, m, 2 × Ar\textit{H}), 6.99-7.01 (2H, m, 2 × Ar\textit{H}), 7.07-7.11 (1H, m, 1 × Ar\textit{H}), 7.15-7.19 (4H, m, 4 × Ar\textit{H}), 7.34-7.37 (2H, m, 2 × Ar\textit{H}); \textit{^13}C NMR (125 MHz, DMSO-\textit{d}_6) \delta_C 29.8 (Ar\text{CH}_2), 56.4 (CH\textsubscript{2}OH), 110.6 (C-Ar), 123.5 (C-Ar), 126.5 (C-Ar), 127.8 (2 × C-Ar), 128.4 (2 × C-Ar), 128.7 (2 × C-Ar), 129.5 (2 × C-Ar), 130.2 (2 × C-Ar), 130.8 (C-Ar), 130.9 (2×C-Ar), 130.9 (C-Ar), 132.7 (C-Ar), 133.0 (C-Ar), 133.4 (C-Ar), 137.9 (C-Ar), 139.0 (C-Ar).
2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-phenyl-1H-pyrrole-3-carbaldehyde (90)

Prepared according to general procedure G using compound 308 (1.05 g, 2.57 mmol) in DCM (10 mL) and Dess-Martin periodinane (1.185 g, 4.37 mmol) in DCM (15 mL). Purification by MPLC on SiO$_2$ (Petrol:EtOAc, 0-30%) gave an off-white viscous liquid (680 mg, 65%). $R_f = 0.50$ (25% EtOAc/Petrol); m.p. 132-135 °C; $\lambda_{max}$ (EtOH)/nm 250.8; IR $\nu_{max}$/cm$^{-1}$ 3066, 2985, 2920, 2819, 2714, 1662 (C=O); $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 4.18 (2H, s, 2 × ArCH$_2$), 6.79-6.80 (2H, m, 2 × ArH), 6.84-6.86 (3H, m, 3 × ArH), 7.01-7.03 (2H, m, 2 × ArH), 7.14-7.16 (2H, m, 2 × ArH), 7.17-7.19 (3H, m, 3 × ArH), 7.25-7.27 (2H, m, 2 × ArH), 10.01 (1H, s, CHO); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$C 30.3 (ArCH$_2$), 109.4 (C-Ar), 123.5 (C-Ar), 127.4 (C-Ar), 128.3 (2 × C-Ar), 128.4 (2 × C-Ar), 128.6 (2 × C-Ar), 129.4 (2 × C-Ar), 129.4 (2 × C-Ar), 129.8 (2 × C-Ar), 131.3 (C-Ar), 132.4 (C-Ar), 134.9 (C-Ar), 135.6 (C-Ar), 136.5 (C-Ar), 136.6 (C-Ar), 140.2 (C-Ar), 186.1 (C=O); LRMS (ES$^+$) m/z 406.2 [M($^{35}$Cl$_2$)+H]$^+$, 408.2 [M($^{35}$Cl $^{37}$Cl)+H]$^+$.

2,2,2-Trichloro-1-(2-(4-chlorobenzyl)-1-(4-chlorophenyl)-5-phenyl-1H-pyrrol-3-yl)ethanol (91)

Compound 90 (400 mg, 0.98 mmol) was dissolved in DMF (0.6 mL) and cooled to -10 °C. CHCl$_3$ (190 µL, 2.35 mmol) was added dropwise and the mixture was stirred at -10 °C for 15 min. KOH (44 mg, 0.78 mmol) in MeOH (0.12 mL) was added and the mixture was stirred at -10 °C for 3 h. The reaction was quenched with 1 M HCl (2 mL) and toluene (2 mL) and stirred at -10 °C for 1 h. After warming to r.t., the aqueous layer was extracted with EtOAc (3×30 mL).
The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-20%) gave an off-white solid (330 mg, 64%). Rₜ=0.44 (20% EtOAc/Petrol); m.p. 84-87 °C; λₘₐₓ (EtOH)/nm 277.6; IR νₘₐₓ/cm⁻¹ 3550, 3035, 2920, 2850, 2656; ¹H NMR (500 MHz, CDCl₃) δH 3.88 (1H, d, J = 17.1 Hz, ArCH₂), 4.07 (1H, d, J = 17.1 Hz, ArCH₂), 5.20 (1H, s, CHOH), 6.69 (1H, br s, 1 × ArH), 6.75 (1H, s, 1 × ArH), 6.81-6.83 (2H, m, 2 × ArH), 6.98-7.05 (3H, m, 3 × ArH), 7.10-7.17 (6H, m, 6 × ArH), 7.24 (1H, br s, 1 × ArH); ¹³C NMR (125 MHz, CDCl₃) δC 30.6 (ArCH₂), 79.6 (CHOH), 104.0 (C-Ar), 108.0 (C-Ar), 117.5 (C-Ar), 126.6 (2 × C-Ar), 128.1 (2 × C-Ar), 128.5 (2 × C-Ar), 129.1 (2 × C-Ar), 129.3 (2 × C-Ar), 130.0 (2 × C-Ar), 132.0 (C-Ar), 132.1 (C-Ar), 133.0 (C-Ar), 134.0 (C-Ar), 135.0 (C-Ar), 137.0 (C-Ar), 137.2 (C-Ar); LRMS (ES⁺) m/z 524.0 [M(³⁵Cl₂)+H]+, 526.1 [M(³⁵Cl₂³⁷Cl)+H]+, 528.1 [M(³⁵Cl³⁷Cl₂)+H]+.

2-(2-(4-Chlorobenzyl)-1-(4-chlorophenyl)-5-phenyl-1H-pyrrol-3-yl)acetic acid (92)

Diphenyl diselenide (76 mg, 0.24 mmol) was dissolved in ethanol (1 mL) and NaBH₄ (18 mg, 0.48 mmol) was added and the mixture was stirred at r.t. for 30 min. Compound 91 (122 mg, 0.23 mmol) was added, followed by the addition of NaOH (55 mg, 1.38 mmol) and the mixture was stirred at r.t. for 24 h. After cooling to r.t., the solvent was removed in vacuo. EtOAc (5 mL) and H₂O (5 mL) was added to the residue and acidified to pH 1 with 1 M HCl. The aqueous layer was extracted with EtOAc (3×30 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a beige solid (90 mg, 90%). Rₜ = 0.47 (5% MeOH/DCM); m.p. 145-148 °C; λₘₐₓ (EtOH)/nm 252.8; IR νₘₐₓ/cm⁻¹ 3067, 2919, 2850, 2727, 2621, 1696 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 3.44 (2H, s, CH₂COOH), 3.86 (2H, s, 2 × ArCH₂), 6.38 (1H, s, Pyrrole-H), 6.82-6.84 (2H, m, 2 × ArH), 6.98-7.02 (4H, m, 4 × ArH), 7.08-7.11 (1H, m, 1 × ArH), 7.16-7.19 (4H, m, 4 × ArH), 7.32-7.36 (2H, m, 2 × ArH), 12.22 (1H, s, COOH); ¹³C NMR (125 MHz, DMSO-d₆) δC 29.7 (ArCH₂), 32.5 (CH₂COOH), 111.3 (C-Ar), 115.8 (C-Ar), 126.5 (C-Ar), 127.8 (2 × C-Ar), 128.4 (2 × C-Ar), 128.7 (2 × C-Ar), 129.4 (2 × C-Ar), 130.1 (2 × C-Ar), 207.
130.8 (2 × C-Ar), 130.8 (C-Ar), 131.3 (C-Ar), 132.7 (C-Ar), 132.9 (C-Ar), 133.5 (C-Ar), 138.0 (C-Ar), 138.8 (C-Ar), 173.5 (C=O); HRMS calcd for C<sub>25</sub>H<sub>20</sub>Cl<sub>2</sub>NO<sub>2</sub> [M<sup>(35)Cl<sub>2</sub>)]+ 436.0866, found 436.0857.

16.2.4 Synthesis of RO-2443 (3) and RO-5963 (4)

6-chloro-7-methyl-1H-indole (103)

![6-chloro-7-methyl-1H-indole](image)

2-Chloro-6-nitrotoluene (618 mg, 3.6 mmol) was dissolved in THF (25 mL) and cooled to -40 °C. 1 M vinylmagnesium bromide in THF (10.8 mL, 10.8 mmol) was added dropwise and the reaction mixture was stirred at -40 °C for 1 h. Saturated aqueous NH<sub>4</sub>Cl (30 mL) was added and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The organic layers were combined, dried over MgSO<sub>4</sub>, and evaporated in vacuo. Purification by MPLC on SiO<sub>2</sub> (Petrol:EtOAc, 0-20%) gave a dark brown viscous liquid (260 mg, 43%). R<sub>f</sub> = 0.33 (20% EtOAc:Petrol); m.p. 116-119 °C. UV λ<sub>max</sub> (EtOH)/nm 271, 220; IR ν<sub>max</sub>/cm<sup>-1</sup> 3413 (NH), 1732, 1606; <sup>1</sup>H NMR (500 MHz; CDCl<sub>3</sub>) δ<sub>H</sub> 2.45 (3H, s, CH<sub>3</sub>), 6.46 (1H, dd, J = 2.2 and 3.1 Hz, ArH), 7.05 (1H, d, J = 8.4 Hz, ArH), 7.13 (1H, dd, J = 2.2 and 3.1 Hz, ArH), 7.33 (1H, d, J = 8.4 Hz, ArH), 8.01 (1H, br s, NH); <sup>13</sup>C NMR (125 MHz; CDCl<sub>3</sub>) δ<sub>C</sub> 13.9 (CH<sub>3</sub>), 103.4 (CH-Ar), 118.1 (C-Ar), 118.9 (CH-Ar), 121.3 (CH-Ar), 124.5 (CH-Ar), 126.0 (C-Ar), 127.5 (C-Ar), 136.0 (C-Ar).

6-Chloro-7-methyl-1H-indole-3-carbaldehyde (104)

![6-Chloro-7-methyl-1H-indole-3-carbaldehyde](image)

Prepared according to general procedure H using compound 103 (160 mg, 0.96 mmol), DMF (12 mL) and phosphorus oxychloride (0.25 mL, 2.89 mmol) heated under microwave irradiation at 70 °C for 10 min. EtOAc extraction gave a light brown solid (175 mg, 93%) which did not
require further purification, Rf = 0.35 (2% MeOH/DCM); m.p. 239-241 °C; λmax (EtOH)/nm 263; IR νmax/cm⁻¹ 1632 (C=O). 1H NMR (500 MHz; DMSO-d6) δH 2.54 (3H, s, CH3), 7.26 (1H, d, J = 8.5 Hz, ArH), 7.91 (1H, d, J = 8.5 Hz, ArH), 8.36 (1H, s, 1×ArH), 9.94 (1H, s, CHO), 12.33 (1H, br s, NH). 13C NMR (125 MHz; DMSO-d6) δC 14.0 (CH3), 118.4 (C-Ar), 119.3 (C-Ar), 119.8 (CH-Ar), 122.7 (CH-Ar), 123.1 (C-Ar), 127.8 (C-Ar), 137.1 (CH-Ar), 138.9 (C-Ar), 185.1 (C=O). HRMS calcd for C10H7ClNO [M(H-Cl)]⁺ 192.0222, 192.0213.

3-(3,4-difluorobenzyl)imidazolidine-2,4-dione (107)

Hydantoin (500 mg, 5 mmol), 3,4-difluorobenzyl bromide (0.64 mL, 5 mmol) and K2CO3 (700 mg, 5 mmol) were suspended in acetonitrile (12 mL). The reaction mixture was refluxed for 6 hours and stirred at 50 °C for 16 h. The reaction was cooled to r.t, quenched with ice and extracted with EtOAc (3x25 mL). The organic layers were combined, washed with brine, dried over MgSO4 and evaporated in vacuo. Purification by MPLC on SiO2 (Petrol:EtOAc, 0-20%) gave a white solid (643 mg, 57%). Rf = 0.37 (2% MeOH/DCM); m.p. 134-137 °C; λmax (EtOH)/nm 339, 268, 263; IR νmax/cm⁻¹ 3259 (NH), 1771 (C=O), 1712 (C=O); 1H NMR (500 MHz, CDCl3) δH 3.92 (2H, s, CH2), 4.54 (2H, s, CH2), 6.15 (1H, br s, NH), 7.00-7.08 (2H, m, 2×Ar-H), 7.15-7.20 (1H, m, Ar-H); 13C NMR (125 MHz, CDCl3) δC 41.3 (CH2), 46.5 (CH2), 117.5 (d, J = 17.3, CH-Ar), 118.0 (d, J = 17.9, CH-Ar), 125.0 (dd, J = 3.7 and 6.2 Hz, CH-Ar), 150.2 (dd, J = 12.7 and 249.3 Hz, ArCF), 150.2 (dd, J = 12.7 and 248.8 Hz, ArCF), 157.7 (C=O), 170.7 (C=O). 19F NMR (470 MHz; CDCl3) δF -138.5 (d, J = 21.0 Hz), -136.9 (d, J =21.0 Hz). LRMS (ES⁺) m/z 227.2 [M + H]⁺.
(Z)-5-((6-chloro-7-methyl-1H-indol-3-yl)methylene)-3-(3,4-difluorobenzyl)imidazolidine-2,4-dione (3)

A mixture of compound 104 (50 mg, 0.26 mmol), 107 (68 mg, 0.30 mmol) and piperidine (0.08 mL) was heated at 130 °C for 30 min. The reaction mixture was cooled to 60 °C, water (2 mL) was added and the mixture was stirred for 1 hour and additional water (20 mL) was added, extracted with ethyl acetate (2 × 25 mL). The organic layers were combined, dried over MgSO₄ and evaporated in vacuo to get a yellow solid (96 mg, 92 %). Rf = 0.39 (4% MeOH/DCM); m.p. 278-280 °C (decomposed); λ max (EtOH)/nm 368, 230. IR ν max/cm⁻¹ 3475 (NH), 3216, 3151, 3054, 1743 (C=O), 1699 (C=O). ¹H NMR (500 MHz, THF-d₈) δ H 2.52 (3H, s, CH₃), 4.67 (2H, s, benzyl-CH₂), 6.92 (1H, s, CH=Hydantoin), 7.14 (1H, d, J = 8.5 Hz, Indole-H), 7.16-7.22 (2H, m, 2 × Phenyl-H), 7.31-7.35 (1H, m, Phenyl-H), 7.59 (1H, d, J = 8.5, indole-H), 7.82 (1H, d, J = 2.4 Hz, Indole-H₂), 9.27 (1H, br s, NH), 10.97 (1H, s, NH). ¹³C NMR (125 MHz, THF-d₈) δ C 13.9 (CH₃), 40.4 (d, J = 0.9 Hz, Benzyl-CH₂), 103.5 (CH=Hydantoin), 111.2 (C-CO), 117.8 (CH-Indole), 118.0 (d, J = 17.6, Phenyl-CHCF), 118.4 (d, J = 17.6, Phenyl-CHCF), 119.7 (C-Ar), 122.5 (CH-Indole), 124.6 (C-Ar), 125.9 (dd, J = 3.6 and 6.5 Hz, Phenyl-CH), 126.8 (C-Ar), 126.9 (CH-Indole), 128.9 (C-Ar), 135.7 (dd, J = 3.9, 5.5 Hz, C-Ar), 137.4 (C-Ar), 150.8 (dd, J = 30.8 and 247.2 Hz, ArCF), 150.9 (dd, J = 31.2 and 247.2 Hz, ArCF), 155.2 (C=O), 164.3 (C=O). ¹⁹F NMR (470 MHz; THF-d₈) δ F -141.4 (d, J = 22.5 Hz), -139.4 (d, J = 20.7 Hz). HRMS calcd for C₂₀H₁₃ClF₂N₃O₂ [M⁺] 400.0670, found 400.0655.

(Z)-5-((6-chloro-7-methyl-1H-indol-3-yl)methylene)imidazolidine-2,4-dione (118)

A mixture of compound 104 (100 mg, 0.52 mmol), hydantoin (47 mg, 0.47 mmol) and piperidine (0.16 mL) was heated at 130 °C for 1 h. The reaction mixture was cooled to 60 °C, 210
water (2 mL) was added and the mixture was stirred for 1 hour and additional water (20 mL) was added, extracted with ethyl acetate (2×25 mL). The organic layers were combined, dried over MgSO₄ and evaporated in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-10%) gave a yellow solid (112 mg, 86 %). Rᵣ = 0.29 (4% MeOH/DCM); m.p. 330-331 °C (decomposed); λₘₐₓ (EtOH)/nm 362, 267, 231; IR νₘₐₓ/cm⁻¹ 3047 (N-H), 3193, 2980, 2919, 1745 (C=O), 1703 (C=O). ¹H NMR (500 MHz, DMSO-d₆) δ/H 2.52 (3H, s, CH₃), 6.70 (1H, s, CH=C=CO), 7.14 (1H, d, J = 8.4 Hz, indole-H), 7.62 (1H, d, J = 8.4 Hz, indole-H), 8.16 (1H, d, J = 2.1 Hz, indole-H₂), 10.17 (1H, s, NH), 11.00 (1H, br s, NH), 11.94 (1H, s, NH). ¹³C NMR (125 MHz, DMSO-d₆) δ/C 13.9 (CH₃), 101.1 (CH=C=CO), 109.0 (indole-C), 116.8 (indole-CH), 119.0 (indole-C), 121.1 (indole-CH), 124.1, 125.5, 126.8 (indole-C), 127.2 (indole-CH), 135.9 (indole-C), 155.2 (C=O), 165.2 (C=O). HRMS calcd for C₁₁H₉ClN₃O₂ [M(³⁵Cl)-H]⁻ 274.0389, found 274.0382.

2-Bromo-2-(3,4-difluorophenyl)acetic acid (109)

2-amino-2-(3,4-difluorophenyl)acetic acid (580 mg, 3.10 mmol) was added into a solution of 48% hydrobromic acid (2.8 mL, 24.8 mmol), KBr (1.48 g, 12.4 mmol) and water (6 mL). The mixture was stirred at 0°C for 15 min, then, a solution of NaNO₂ (684 mg, 9.92 mmol) in water (4 mL) was added dropwise and the mixture was stirred at r.t. for 3 hours. The aqueous layer was extracted with DCM (3×30 ml). The organic layers were combined, washed with water (30 mL), brine (20 mL), dried over MgSO₄, and evaporated in vacuo. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-100%, 0.5% Acetic acid) gave a white solid (281 mg, 36%). Rᵣ = 0.66 (50% EtOAc/Petrol, 5 % AcOH); m.p. 89-90 °C; λₘₐₓ (EtOH)/nm 398; IR νₘₐₓ/cm⁻¹ 3060, 2920, 2669, 1728 (C=O); ¹H NMR (500 MHz, CDCl₃) δ/H 5.22 (1H, s, benzyl-CH), 7.06-7.11 (1H, m, Ar-H), 7.14-7.21 (1H, m, Ar-H), 7.37-7.41 (1H, m, Ar-H), 8.87 (1H, br s, COOH). ¹³C NMR (125 MHz, CDCl₃) δ/C 44.0 (benzyl-CH), 117.6 (d, J = 17.7 Hz, ArCH), 118.3 (d, J = 18.6 Hz, ArCH), 125.1 (dd, J = 3.7 and 6.6 Hz, ArC₆H), 131.8 (dd, J = 4.0, 5.8 Hz, ArC₆), 150.3 (dd, J = 11.6 and 249.0 Hz, ArCF), 151.0 (dd, J = 6.2 and 246.0 Hz, ArCF), 173.0 (COOH). ¹⁹F NMR (470 MHz; CDCl₃) δ/F -135.5 (d, J = 20.9 Hz), -131.5 (d, J =20.9 Hz). LRMS (ES⁻) m/z 205.0 [(M(⁷⁹Br)-COOH)-H]⁻, 207.0 [(M(⁸¹Br)-COOH)-H]⁻.
**N-(1,3-dihydroxypropan-2-yl)-2,2,2-trifluoroacetamide (113)**

![Chemical structure](image)

A mixture of 2-aminopropane-1,3-diol (562 mg, 6.17 mmol), ethyl trifluoroacetate (6 mL) and THF (2 mL) was stirred at r.t. for 48 h. The solvent was evaporated *in vacuo* and the residue was purified by filtering through silica to get a white solid (1.12 g, 98%). $R_f = 0.57$ (10% MeOH/DCM); m.p. 61-62 °C; IR $\nu_{\text{max}}$/cm$^{-1}$ 3289 (O-H), 1703 (C=O); $^1$H NMR (500 MHz, CD$_3$OD) $\delta$H 3.52 (2H, dd, $J = 6.2$ and 11.3 Hz, CH$_2$), 3.58 (2H, dd, $J = 5.4$ and 11.3 Hz, CH$_2$), 3.91 (1H, m, NHCH); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$C 55.6 (NHCH), 61.5 (CH$_2$), 117.5 (d, $J = 286.7$ Hz, CF$_3$), 159.2 (d, $J = 36.8$ Hz, C=O). $^{19}$F NMR (470 MHz, CD$_3$OD) $\delta_F$ -77.2 (s).

**N-(2,2-dimethyl-1,3-dioxan-5-yl)-2,2,2-trifluoroacetamide (114)**

![Chemical structure](image)

Compound 113 (721 mg, 3.9 mmol) was dissolved in DCM (7 mL), camphor-10-sulfonic acid (91 mg, 0.39 mmol) and 2,2-dimethoxypropane (3.84 mL, 31.2 mmol) were added, respectively. The reaction mixture was stirred at r.t. for 96 h. Saturated aqueous NaHCO$_3$ (20 mL) was added and stirred for 1 h. The aqueous layer was extracted with DCM (3×40 mL). The organic layers were combined, dried over MgSO$_4$, and evaporated *in vacuo*. Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-5%) gave a white solid (475 mg, 54%). $R_f = 0.81$ (2% MeOH/DCM); m.p. 73-75 °C; IR $\nu_{\text{max}}$/cm$^{-1}$ 3283 (N-H), 1716 (C=O), 1550 (N-H); $^1$H NMR (500 MHz, CD$_3$OD) $\delta$H 1.32 (3H, s, CH$_3$), 1.33 (1H, s, CH$_3$), 3.68 (2H, dd, $J = 5.6$ and 12.1 Hz, CH$_2$), 3.76-3.79 (1H, m, NHCH), 3.95 (2H, dd, $J = 3.8$ and 12.1 Hz, CH$_2$); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta$C 23.1 (CH$_3$), 24.8 (CH$_3$), 46.0 (NHCH), 62.8 (2×CH$_2$), 99.7 (CH$_2$OC(CH$_3$)$_2$), (d, $J = 286.5$ Hz, CF$_3$), 158.9 (d, $J = 37.3$ Hz, C=O). $^{19}$F NMR (470 MHz, CD$_3$OD) $\delta_F$ -77.1 (s).
2,2-Dimethyl-1,3-dioxan-5-amine (115)

![2,2-Dimethyl-1,3-dioxan-5-amine](image)

Compound 114 (500 mg, 2.2 mmol) was dissolved in THF (10 mL) and water (10 mL), LiOH monohydrate (184 mg, 4.4 mmol) was added and stirred at r.t. for 1.5 h. The solvent was evaporated *in vacuo*, the remaining residue was dissolved in water (20 mL) and extracted with DCM (5 × 15 mL). The organic layers were combined, dried over MgSO₄, and concentrated *in vacuo* to get a colourless liquid (157 mg, 54%) which was used for the next step without further purification. R<sub>f</sub> = 0.18 (2% MeOH/DCM); IR ν<sub>max</sub>/cm<sup>-1</sup> 3350 (NH<sub>2</sub> stretch), 2980, 2930; <sup>1</sup>H NMR (500 MHz, CDCl₃) δ<sub>H</sub> 1.42 (3H, s, CH₃), 1.54 (2H, br s, 2×NH₂), 2.84 (1H, br s, CH(CH₂)₂), 3.56 (2H, dd, <i>J</i> = 11.8 and 5.6 Hz, CH₂O), 4.01 (2H, dd, <i>J</i> = 11.8 and 3.5 Hz, CH₂O).

2-Bromo-2-(3,4-difluorophenyl)-N-(2,2-dimethyl-1,3-dioxan-5-yl)acetamide (116)

![2-Bromo-2-(3,4-difluorophenyl)-N-(2,2-dimethyl-1,3-dioxan-5-yl)acetamide](image)

Compound 109 (130 mg, 0.517 mmol), 115 (68 mg, 0.517) and DMAP (6.3 mg, 0.05 mmol) were dissolved in DCM (2.6 mL) and the reaction mixture was stirred at 0 °C for 20 min. DIC (0.09 mL, 0.56 mmol) was added and the solution was stirred at 0 °C for 3 h and at r.t. for 13 h. The reaction mixture was quenched with water (10 mL) and extracted with DCM (3 × 20 mL). The organic layers were combined, dried over MgSO₄, and concentrated *in vacuo*. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-90%) gave a white solid (149 mg, 79%). R<sub>f</sub> = 0.45 (50% EtOAc/Petrol); m.p. 145-147 °C; λ<sub>max</sub> (EtOH)/nm 398; IR ν<sub>max</sub>/cm<sup>-1</sup> 3279 (O-H stretch), 3045, 2921, 2868, 1525, 1488.; <sup>1</sup>H NMR (500 MHz, CDCl₃) δ<sub>H</sub> 1.44 (3H, s, CH₃), 1.50 (3H, s, CH₃), 3.72-3.77 (2H, m, CH₂O), 3.82-3.86 (1H, m, NHCH₃), 4.15 (2H, dd, <i>J</i> = 12.3, 2.0 Hz, CH₂O), 7.12-7.17 (1H, m, ArH), 7.20-7.23 (1H, m, ArH), 7.33-7.37 (1H, m, ArH), 7.51 (1H, br d, <i>J</i> = 7.7 Hz, NH); <sup>13</sup>C NMR (125 MHz, CDCl₃) δ<sub>C</sub> 18.3 (CH₃), 29.1 (CH₃), 44.8
(NHCH), 49.1 (d, J = 1.5 Hz, benzyl-CH), 63.2 (CH₂O), 63.2 (CH₂O), 98.9 (C(CH₃)₂), 117.8 (2C, dd, J = 17.9, 9.6 Hz, ArC² and ArC⁵), 124.8 (dd, J = 6.6, 3.6 Hz, ArC⁶), 134 (2C, dd, J = 5.8, 4.1 Hz, C¹), 166.0 (C=O), ArC³ and ArC⁴ could not be seen. ¹⁹F NMR (470 MHz; CDCl₃) δ F -135.3 (d, J = 22.1 Hz), -134.8 (d, J = 22.1 Hz); LRMS (ES⁺) m/z 362.2 [M(⁷⁹Br)-H]+, 364.2 [M(⁸¹Br)-H]⁺.

(Z)-2-((4-((6-chloro-7-methyl-1H-indol-3-yl)methylene)-2,5-dioxoimidazolidin-1-yl)-2-(3,4-difluorophenyl)-N-(2,2-dimethyl-1,3-dioxan-5-yl)acetamide (119)

Compound 118 (54 mg, 0.19 mmol) was dissolved in DMF (0.7 mL) and cooled to 0 °C. K₂CO₃ (26 mg, 0.28 mmol) was added and stirred for 20 min. Compound 116 (70 mg, 0.19 mmol) was added and the reaction mixture was stirred at 0 °C for 30 min at r.t. for 48 h. The reaction did not go into completion (as monitored by TLC), therefore, K₂CO₃ (10 mg, 0.07 mmol) was added and the reaction mixture was stirred at r.t. for 48 h. The reaction was poured into ice and extracted with EtOAc (3 × 15 ml). The organic layers were combined, dried over MgSO₄ and evaporated in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a yellow solid (18 mg, 17%). Rᵣ = 0.55 (8% MeOH/DCM); m.p. 250 °C (degradation); λₑmax (EtOH)/nm 371; IR νmax/cm⁻¹ 3048, 2945, 1742 (C=O), 1698 (C=O), 1638 (C=O); ¹H NMR (500 MHz, THF-d₈) δ H 1.16 (3H, s, CH₃), 1.25 (3H, s, CH₃), 2.41 (3H, s, CH₃), 3.50-3.60 (2H, m, CH₂O), 3.75-3.78 (1H, m, NHCH(CH₂OH)₂), 3.86-3.91 (2H, w, CH₂O), 5.75 (1H, s, benzyl-CH), 6.81 (1H, s, Indole-CH-hydantoin), 7.03 (1H, d, J = 8.5 Hz, ArH), 7.11-7.16 (1H, m, ArH), 7.20 (1H, d, J = 7.9), CONH), 7.26-7.28 (1H, m, ArH), 7.46-7.75 (2H, m, 2×ArH), 7.73 (1H, d, J = 2.5 Hz, ArH), 9.33 (1H, br s, Hydantoin-NH), 10.94 (1H, br s, Indole-NH); ¹³C NMR could not be obtained. ¹⁹F NMR (470 MHz; THF-d₈) δ F -140 (d, J = 21.8 Hz), -139.4 (d, J = 21.8 Hz); HRMS calcd for C₂₇H₂₆ClF₂N₄O₅ [M(⁷⁵Cl)+H]⁺ 559.1554, found 559.1540.
Compound 119 (18 mg, 0.032 mmol) was dissolved in THF (0.5 mL), 0.2 M HCl (0.17 mL) was added dropwise and stirred at 0 °C for 1 h and at r.t. for 2 h. The solvent was evaporated in vacuo and the remaining residue was neutralised with saturated aqueous NaHCO₃ and extracted with EtOAc (3 × 20 mL), dried over MgSO₄ and evaporated in vacuo. Purification by semi-preparative HPLC (C-18 silica, MeCN/formic acid aq., 65%) gave a yellow solid (6 mg, 36%). Rf = 0.36 (8% MeOH/DCM); m.p. 210 °C (degradation); λmax (EtOH)/nm 371; IR νmax/cm⁻¹ 3281 (O–H stretch), 2954, 1746 (C=O), 1700 (C=O), 1644 (C=O); ¹H NMR (500 MHz, THF-d₈) δ H 2.41 (3H, s, CH₃), 3.43-3.52 (4H, m, 2×CH₂OH), 3.63 (1H, br s, OH), 3.72 (1H, br s, OH), 3.76-3.82 (1H, m, NHCH(CH₂OH)₂), 5.72 (1H, s, Benzyl-CH), 6.81 (1H, s, Indole-CHCCO), 6.89 (1H, d, J = 7.8 Hz, CONH), 7.02 (1H, d, J = 8.5, ArH), 7.07-7.13 (1H, m, ArH), 7.22-7.25 (1H, m, ArH), 7.43-7.48 (2H, m, 2 × ArH), 7.73 (1H, d, J = 2.5 Hz, ArH), 9.33 (1H, br s, Hydantoin-NH), 10.91 (1H, br s, Indole-NH). ¹³C NMR (125 MHz, THF-d₈) δC 12.9 (CH₃), 54.0 (CH(CH₂OH)₂), 56.7 (BenzylCH), 60.8 (CH(CH₂OH)₂), 103.1 (Indole-CHCCO), 110.2, 116.7 (ArCH, multiplicity not clear), 116.8 (Indole-CH), 118.8 (ArC), 118.9 (d, J = 18.4 Hz, ArCH), 121.5 (Indole-CH), 123.2 (ArC), 118.7 (ArC), 121.5 (ArCH), 123.6 (ArC), 124.9 (dd, J = 3.6, 6.5 Hz, ArCH), 125.8 (ArC), 126.1 (ArC), 126.5 (ArCH, multiplicity not clear), 131.2 (ArC), 154.0 (CO), 163.3 (CO), 165.9 (CO). The expected dd of 2×CF were not seen due to the dilute NMR sample. ¹⁹F NMR (470 MHz; THF-d₈) δF -140 (d, J = 21.8 Hz), -139.4 (d, J = 21.8 Hz); HRMS calcd for C₂₄H₂₁ClF₂N₄O₅ [M(³⁵Cl)+H]⁺ 519.1241, found 559.1226.
16.3 MDMX Structural biology experimental

16.3.1 General procedures

16.3.1.1 Media supplementation

All cultures were supplemented with an antibiotic, according to the antibiotic selectivity of the pGEX-6P-1 vector and *E. coli* strain used during recombinant protein expression. During IPTG-based induction of protein expression, the lactose analogue IPTG was added to the culture to induce protein expression. Appropriate amounts of IPTG (200 mM) and ampicillin (50 mg/mL) were dissolved in sterile 18.2 MΩ/cm H₂O, before storing at -20 °C until required. Chloramphenicol (34 mg/mL) was dissolved in 100% ethanol and stored at -20 °C.

16.3.1.2 mHBS buffer

To prepare the mHBS buffer, 20 mL of aq. NaCl (5 M), 5 mL of aq. DTT (1 M) and 20 mL of aq. HEPES (1 mM, pH 7.4) were used in 1 L of solution and the pH was brought to 7.4 by addition of aq. NaOH (10 M). The buffer was filtered and degassed before use and, if required, stored at 4 °C for no longer than one week.

16.3.1.3 Glutathione elution buffer

To prepare the glutathione elution buffer, glutathione (0.123g) was dissolved in mHBS (20 mL) and the pH was brought to 7.4 by addition of aq. NaOH (10 M). The buffer was stored at 4 °C and used immediately after preparation.

16.3.1.4 Polyacrylamide gel electrophoresis

SDS-PAGE was used for protein identification and semi-quantitative analysis. Pre-cast 12-well acrylamide gels (RunBlue; 12% for GST-MDMX/GST-MDM2 and 16% for MDMX/MDM2) were used with SDS run buffer (RunBlue). Samples were mixed with SDS loading buffer.
(RunBlue) and denatured at 100 °C for 5 min before being loaded onto the gel. PageRuler pre-stained protein ladder (10-170 kDa, Thermo Scientific) was used. Electrophoresis was carried out at 180 V and gels were stained with InstantBlue™ protein stain.

**16.3.2 Expression from MDMX and MDM2 gene constructs**

**1.1.1.1 Transformation of competent Rosetta™ BL21(DE3) pLysS E. coli**

Recombinant MDMX pGEX-6P-1 expression vector plasmid were transformed into competent Rosetta™ (DE3)pLys S (Novagen), whilst recombinant MDM2 pGEX-6P-1 expression vector plasmid were transformed into competent BL21(DE3) pLys S E. coli (Novagen). Competent cells were mixed with 1 µL of plasmid and incubated on ice for 30 min. The cells were transferred to a water bath at 42 °C for 30 sec, before incubating on ice for 2 min. The cells were recovered through aseptic addition of 200 µL super optimal broth with catabolite repression (SOC) medium and incubated at 37 °C, 200 rpm for 1 h. The recovered cells were aseptically plated onto LB-agar plates supplemented with ampicillin and chloramphenicol and incubated overnight at 37 °C to allow growth of transformed bacterial colonies.

**1.1.1.2 Recombinant MDMX expression following IPTG induction**

Transformed Rosetta™ BL21(DE3) pLysS E. coli colonies were used to inoculate starter cultures containing 10 mL of Terrific Broth (TB), supplemented with ampicillin (20 µL) and chloramphenicol (10 µL). The starter cultures were incubated overnight at 37 °C, 200 rpm before aseptic transfer of a 1% (v/v) inoculum into an appropriate volume of expression media, supplemented with antibiotics. The culture was incubated at 37 °C, 160 rpm and the optical density (OD) of the culture was monitored by spectrometry during growth. Once an OD$_{600\,\text{nm}}$ of 0.6-1.0 was reached, 200 mM IPTG (1 mL) was added to the culture to induce protein expression. The culture was incubated at 20 °C, 160 rpm overnight.

**1.1.1.3 Recombinant MDM2 expression following IPTG induction**

BL21 (DE3) E. coli glycerol stocks for MDM2$_{17-125}^{K51A}$ and MDM2$_{17-125}^{E69K70A}$ were prepared previously by Judith Reeks and stored at -80 °C. A glycerol stock of BL21 (DE3) pLysS E. coli for MDM2$_{17-108}^{E69K70A}$ was prepared and stored similarly. Transformed BL21 E. coli cells were
inoculated in starter cultures containing 10 mL of LB, supplemented with ampicillin (20 µL) (BL21 (DE3)) or ampicillin (20 µL) and chloramphenicol (10 µL) (BL21 (DE3) pLysS). The starter cultures were incubated overnight at 37 °C, 200 rpm, before aseptic transfer of a 1% (v/v) inoculum into an appropriate volume of expression media, supplemented with chloramphenicol (1 mL) and/or ampicillin (1 mL). The culture was incubated at 37 °C, 160 rpm, whilst the optical density of the culture was monitored during growth. Once an OD$_{600\text{nm}}$ of 0.6-1.0 was reached, 200 mM IPTG (1 mL) was added to the culture to induce protein expression. The culture was incubated at 20 °C, 160 rpm overnight.

1.1.1.4 Cell harvesting

Following culture, the *E. coli* cells were harvested by centrifugation (5000 × g, 15 min, 4°C) to give a pellet which was resuspended in mHBS supplemented with a protease inhibitor tablet (Roche; 1 tablet/40 mL), flash-frozen in dry ice and stored at -20 °C until further use.

16.3.3 Protein purification

The resuspended cell pellets were thawed under running water and lysozyme (400 µL of 25 mg/mL stock), RNAase A (200 µL of 10 mg/mL stock), DNAase I (200 µL of 2 mg/mL stock) and MgCl$_2$ (100 µL of 2 M stock) were added before sonication (30% amplitude; 20 sec on/40 sec off intervals for 15 min) whilst on ice. The lysed cells were centrifuged (45,000 × g, 60 min, 4°C) and the supernatant (cell-free extract, CFE) was decanted from the pellet and retained. Diluted CFE samples were heated at 100 °C for 5 min and then analyzed by SDS-PAGE to establish whether the target protein had been overexpressed.

The supernatant was incubated overnight at 4 °C with glutathione Sepharose 4B resin (GE Healthcare, bed volume 2 mL). The mixture was loaded by gravity flow into a column and washed twice with mHBS (15 mL). The GST-MDMX (or GST-MDM2) was eluted with a fresh solution of glutathione in mHBS (20 mL). 3C protease (50:1 protein:3C protease by weight) was added to cleave MDMX or MDM2 from the GST tag and the mixture was incubated overnight at 4 °C. The desired protein was separated from the GST tag using gel filtration chromatography (Äkta Superdex 75 26/60, isocratic flow of mHBS, Äkta FPLC Chromatographic system, UV absorbance 280 nm).
The protein concentration was measured using a UV-vis spectrophotometer NanoDrop 2000 (Thermo Scientific).

16.3.4 Protein preparation for crystallography

The purified protein was incubated overnight at 4 °C with a 1.5× molar excess of inhibitor (20 mM stock in DMSO). Protein mixtures were concentrated using Amicon® Ultra-15 (Millipore) centrifugal filter devices (5000 × g, 4 °C) to a concentration between 1 and 20 mg/mL.

Crystallization trays were set up in 2-subwell 96-well plates by pipetting commercial screens (JCSG+ from Molecular Dimensions, Index (Hampton Research), and AmSO4 Suite from Qiagen) from deep well blocks prior to protein addition. Protein mixture and precipitant were mixed in the subwells by a Mosquito® robot (100 + 100 nL, 200 + 100 nL, precipitant:protein) using the sitting drop method. Plates were sealed and stored in the Minstrel (Rigaku) automated high-throughput monitoring system at 4 °C for up to five weeks. Crystals were transferred into a solution of 70% precipitant, 30% ethylene glycol, flash cooled in liquid nitrogen and shipped to Diamond Light Source (Oxford, UK) for data collection.

16.3.5 HTRF assay

The experimental detail described below is the final optimized procedure.

The inhibitor solutions (20 mM solution in DMSO) were dispensed in an Echo qualified 384-well low dead volume microplate source plate using a multi-channel pipette. Buffer A (50 mM Tris at pH 7.4, 100 mM NaCl, 100 μg/mL BSA, 1 mM DTT) and buffer B (50 mM Tris at pH 7.4, 100 mM NaCl, 100 μg/mL BSA) were prepared fresh. The inhibitors were dispensed into a 384-well black low-binding round bottom assay plate (Corning) using a Echo® Liquid Handler 550 (Labcyte), starting at 500 μM inhibitor (final assay concentration) and decreasing over a semi-log scale. Each well was backfilled with DMSO to a final volume of 250 nL and each condition was in duplicate.

IP3 peptide (300 µM in DMSO) was diluted in buffer A supplemented with 4.2 % DMSO to obtain a 500 nM solution and was added to all the wells on the assay plate (6 µL). GST-
MDMX\textsubscript{22-111} (107 µM stock) was diluted with buffer A, first to 1.07 µM and subsequently to 25 nM and was added to the plate (4 µL). Positive (peptide, MDMX and DMSO; no inhibitor) and negative (peptide, buffer A and DMSO; no protein or inhibitor) controls were included. The plate was incubated on a shaking platform for 1 h. The final concentrations were as follows: 10 nM GST-MDMX, 300 nM IP3 peptide, 5 % DMSO. A solution of Tb-anti-GST-antibody (3.3 µM stock) was prepared in buffer B (20 nM) and added to each well (10 µL) of the assay plate followed by 45 min incubation on the shaker. The plate was read using PHERAstar FS (BMG Labtech) microplate reader.
16.4 Synthesis of ATAD2 Inhibitors-Experimental Procedures

16.4.1 ATAD2 Biology Procedures

HTRF assay protocol

The ATAD2 bromodomain 981-1108 was expressed with an N-terminal GST fusion from the pGEX-6P-1 plasmid. The plasmids were transformed into BL21 (DE3) pLysS *Escherichia coli* (Novagen) and then the cells grown in luria bertani (LB) media at 37 °C until an OD$_{600}$ of 0.6 was reached. The temperature was reduced to 20 °C and expression induced using 0.2 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG). The cells were harvested after overnight incubation and resuspended in buffer A (20 mM HEPES pH 7.4, 100 mM NaCl, 5 mM Dithiothreitol (DTT)). The cells were lysed by sonication and the lysate cleared by centrifugation (50,000 x g, 4 °C, 1 hr). The protein was passed over 5ml of glutathione Sepharose 4B resin (GE Healthcare) at 4 °C. The resin was then washed with buffer A and the protein eluted with buffer A supplemented with 10 mM glutathione. The protein was then applied to a Superdex 200 26/60 gel filtration column (GE Healthcare) equilibrated in buffer A.

Compounds (dissolved to 100 mM in DMSO) were dispensed into black 384 well assay plates (Corning) over a final concentration range of 2000, 1350, 900, 600, 425, 300, 200, 100, 50, and 25 μM using an Echo 550 (Labcyte). Each well was backfilled to a final volume of 200 nl (for the MDM2 assay), resulting in final DMSO concentrations of 2 %. 5 μl of GST-ATAD2 was added to each well and incubated for 30mins resulting in a final concentration of 5 nM. 5μl of biotinylated-histone H4 peptide (SGRG-K(Ac)-GG-K(Ac)-GLG-K(Ac)-GGA-K(Ac)-RHRKVGG-K(Biotin)) was then added to each well at a final concentration of 500nM. Both GST-ATAD2 and peptide were diluted in buffer B (50 mM Tris pH 7.5, 100 mM NaCl, 1 mM DTT, 100 μg/ml bovine serum albumin (BSA)). The plate was then incubated at room temperature for 30mins and then 5 μl Lanthascreen® Tb-anti-GST Antibody (Life Technologies) at final concentration of 5 nM was added to each well and incubated for an additional 30mins. 5 μl of streptavidin-XLL65 (Cisbio Assay) at a final concentration of 62.5 μM was added to each assay well. Both dyes were diluted in buffer C (50 mM Tris pH 7.5, 100 nM NaCl, 100 μg/ml BSA). The plate was incubated at room temperature for a further 30mins.
and then read using a PheraStar FS (BMG Labtech). The data were analysed using Graphpad Prism.

16.4.2 Synthesis of ATAD2 Inhibitors: General Procedures

**General Procedure A: Substitution at N1**

The relevant compound (1 eq.) and Cs$_2$CO$_3$ (3 eq.) were suspended in DMF. The relevant alkyl halide (2.5 eq.) was added and stirred at 100 °C under microwave irradiation for 30 min. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with EtOAc (3×25 mL). The organic layers were combined, dried over MgSO$_4$ and the solvent removed *in vacuo*.

**General Procedure B: Pyridone formation**

The relevant pyridine (1 eq.) and alkyl halide (2 eq.) were dissolved in MeCN and the mixture was stirred at 170 °C under microwave irradiation for 1 h. The solvent was removed *in vacuo* and the residue was purified by MPLC.

**General Procedure C: Knoevenagel condensation**

The relevant compound (1 eq.) was dissolved in THF, the relevant aldehyde (1.1 eq.) and piperidine (1.5-3.1 eq.) were added. The reaction mixture was stirred at 100 °C until the starting material was completely consumed. The solvent was removed in vacuo and the residue was purified by MPLC.

**General Procedure D: Reduction of an alkene and subsequent methylation**

Step A: The relevant Knoevenagel product (1 eq.) was dissolved in THF and MeOH. 10% Pd/C was added and an atmosphere of hydrogen. The mixture was stirred at r.t. until the starting material was completely consumed. The mixture was filtered through a Celite plug using MeOH (3 × 20 mL). The solvent was removed in vacuo and the crude was taken forward without further purification.
Step B: The crude was dissolved in DMF, Cs$_2$CO$_3$ (2-2.5 eq.) and MeI (1.8-2.1 eq.) were added and the mixture was stirred at 60 °C for 1 h. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with EtOAc (3×25 mL). The organic layers were combined, dried over MgSO$_4$ and the solvent removed in vacuo.

**General Procedure E: Synthesis of spiro-pyrrolidine derivatives**

The relevant spirocyclopropane (1 eq.) and MgI$_2$ (0.1 eq.) were suspended in THF (1 mL). The relevant triazinane (1.5 eq.-3 eq.) was added and the reaction mixture was stirred at 90 °C until the SM was completely consumed. After cooling to r.t., solvent was removed in vacuo and the residue was purified by MPLC.

### 16.4.3 ATAD2 Inhibitors: Synthetic Procedures

**4-Bromo-2-hydroxy-3-methylbenzaldehyde (168)**

![Chemical Structure](image)

3-Bromo-2-methylphenol (500 mg, 2.67 mmol) was dissolved in MeCN (3.5 mL), MgCl$_2$ (381 mg, 4.00 mmol) and triethylamine (1.4 mL, 10.01 mmol) were added, respectively. To the solution, paraformaldehyde was added portion wise and the mixture was refluxed for 3 h. After cooling the reaction mixture to r.t., 5% HCl (6 mL) and Et$_2$O (10 mL) were added and stirred for 10 min. The aqueous layer was extracted with Et$_2$O (3×40 mL). The organic layers were combined, dried over MgSO$_4$ and the solvent removed in vacuo to get a light brown solid (535 mg, 93%) which did not require further purification. R$_f$=0.76 (25% EtOAc/Petrol); m.p. 40-42 °C; $\lambda_{max}$ (EtOH)/nm 330.2, 270.6; IR $\nu_{max}$/cm$^{-1}$ 3099 (OH), 3019, 2872, 1635 (C=O); $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$H 2.34 (3H, s, CH$_3$), 7.22 (1H, d, $J$ = 8.4 Hz, Ar-H), 7.25 (1H, d, $J$ = 8.4 Hz, Ar-H), 9.83 (1H, s, OH), 11.55 (1H, s, CHO); $^{13}$C NMR (125 MHz, CDCl$_3$) $\delta$C 14.8 (CH$_3$), 119.0 (C-Ar), 123.9 (CH-Ar), 127.5 (C-Ar), 131.4 (CH-Ar), 134.7 (C-Ar), 160.2 (C-Ar), 196.1 (CHO).
4-Bromo-2-hydroxy-3-methylbenzoic acid (169)

Compound 168 (480 mg, 2.23 mmol) was dissolved in MeCN (20 mL). A solution of sodium chlorite (344 mg, 3.80 mmol) in water (4 mL) and a solution of sulfamic acid (369 mg, 3.80 mmol) in water (4 mL) were added dropwise, respectively and the mixture was stirred at r.t. for 8 h. The solvent was evaporated in vacuo, water (30 mL) was added and the aqueous layer was extracted with EtOAc (3×30 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-20%) gave a beige solid (450 mg, 88%). Rₓ = 0.39 (20% MeOH/DCM); m.p. 206-209 °C; λmax (EtOH)/nm 307.0, 248.4; ¹H NMR (500 MHz, DMSO-d₆) δH 2.27 (3H, s, CH₃), 7.17 (1H, d, J = 8.5 Hz, Ar-H), 7.57 (1H, d, J = 8.5 Hz, Ar-H), COOH and OH could not be seen; ¹³C NMR (125 MHz, DMSO-d₆) δC 15.6 (CH₃), 112.1 (C-Ar), 123.0 (CH-Ar), 126.1 (C-Ar), 128.9 (CH-Ar), 131.6 (C-Ar), 160.5 (C-Ar), 172.6 (COOH); LRMS (ES⁻) m/z 229.0 [(M(⁷⁹Br)-H)⁻], 231.0 [(M(⁸¹Br)-H)⁻].

Methyl 4-bromo-2-methoxy-3-methylbenzoate (170)

Compound 169 (190 mg, 0.83 mmol) was dissolved in DMF (6 mL), MeI (0.13 mL, 2.15 mmol) was added and stirred at r.t. for 18 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3×30 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-7%) gave a light yellow oil (125 mg, 58%). Rₓ = 0.76 (5% MeOH/DCM); ¹H NMR (500 MHz, CDCl₃) δH 2.39 (3H, s, CH₃), 3.82 (3H, s, OCH₃), 3.90 (3H, s, COOCH₃), 7.37 (1H, d, J = 8.4 Hz, Ar-H), 7.52 (1H, dd, J = 8.4 Hz, Ar-H); ¹³C NMR (125 MHz, CDCl₃) δC 16.2 (CH₃), 52.3 (COOCH₃), 62.0 (OCH₃), 123.8 (C-Ar), 127.9 (CH-Ar), 129.4 (CH-Ar), 130.5 (C-Ar), 133.5 (C-Ar), 158.9 (C-Ar), 166.2 (C=O); LRMS (ES⁺) m/z 259.1 [(M(⁷⁹Br)+H)⁺], 261.1 [(M(⁸¹Br)+H)⁺].
4-bromo-2-methoxy-3-methylbenzoic acid (171)

Compound 170 (110 mg, 0.42 mmol) was dissolved in MeOH (4 mL) and 1 M NaOH in water (4 mL) was added and the mixture was stirred at 60 °C for 18 h. The solvent was evaporated in vacuo, water (30 mL) was added and the solution was acidified with 1 M HCl. The aqueous layer was extracted with EtOAc (3x30 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo to get a beige solid (97 mg, 95%). Rₛ = 0.26 (5% MeOH/DCM); m.p. 135-138 °C; ¹H NMR (500 MHz, DMSO-d₆) δH 2.32 (3H, s, CH₃), 3.75 (3H, s, OCH₃), 7.45 (1H, d, J = 8.4 Hz, Ar-H), 7.48 (1H, d, J = 8.4 Hz, Ar-H), 13.09 (1H, br s, COOH); ¹³C NMR (125 MHz, DMSO-d₆) δC 16.4 (CH₃), 62.2 (OCH₃), 125.9 (C-Ar), 128.1 (CH-Ar), 129.1 (C-Ar), 129.8 (C-Ar), 133.0 (C-Ar), 158.5 (C-Ar), 167.2 (COOH); HRMS calcd for C₉H₇BrO₃ [M⁺]+ 244.9808, found 244.9812.

4-Bromo-2-methoxy-N,3-dimethylbenzamide (172)

Compound 171 (60 mg, 0.24 mmol), DIC (40 μL, 0.28 mmol) and DMAP (2.9 mg, 0.024 mmol) were dissolved in THF (2.5 mL). A 2 M solution of methylamine in THF (0.4 mL, 0.80 mmol) was added and the reaction mixture was stirred at r.t. for 18 h. The carboxylic acid (93% by LC-MS) was still remaining, therefore, HATU (182 mg, 0.48 mmol) was added and the mixture was stirred at 60 °C for 4 h and at r.t. for 18 h. Water (30 mL) was added and the aqueous layer was extracted with EtOAc (3x20 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a white solid (50 mg, 71%). Rₛ = 0.71 (5% MeOH/DCM); m.p. 86-89 °C; λ_max (EtOH)/nm 242.4; IR ν_max/cm⁻¹ 3346 (NH), 2965, 2935, 2865, 1635 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 2.39 (3H, s, CH₃), 3.02 (3H, d, J = 4.8 Hz, NHC₃), 3.75 (3H, s, OCH₃), 7.43 (1H, d, J = 8.5 Hz, Ar-H), 7.66 (1H, br s, NH), 7.78 (1H, d, J = 8.5 Hz, Ar-H); ¹³C NMR (125 MHz, CDCl₃) δC
N-(4-hydroxy-2-methylphenyl)acetamide (175)

\[
\begin{align*}
\text{HN} & \text{\centerline{\text{\ H}}} \\
\text{\centerline{\ OH}} & \text{\centerline{\ OH}} \\
\end{align*}
\]

3-Methyl-4-nitrophenol (500 mg, 3.26 mmol) was dissolved in methanol (50 mL) and reduced using an H-Cube continuous flow reactor (Thalesnano Inc.) with a Pd/C CatCart (r.t., 1 mL/min). The solvent was removed in vacuo after 24 h and the residue was dissolved in AcOH (25 mL) and acetic anhydride (0.5 mL) and the mixture was stirred at r.t. for 30 min. The solvent was evaporated in vacuo and the residue was purified by MPLC on SiO\textsubscript{2} (DCM:MeOH, 0-15%) to give a brown solid (428 mg, 80%). R\textsubscript{f} = 0.21 (5% MeOH/DCM); m.p. 85-90 °C; \(\lambda_{\text{max}}\) (EtOH)/nm 229.8; IR \(\nu_{\text{max}}\) /cm\textsuperscript{-1} 3239-3181 (NH and OH), 3059, 2922, 2817, 1623 (C=O); \(^1\text{H}\) NMR (500 MHz, DMSO-d\textsubscript{6}) \(\delta_{\text{H}} 1.99\) (3H, s, COCH\textsubscript{3}), 2.08 (3H, s, CH\textsubscript{3}), 6.53 (1H, dd, \(J = 8.5\) and 2.6 Hz, Ar-H), 6.59 (1H, d, \(J = 2.6\) Hz, Ar-H), 7.02 (1H, d, \(J = 8.5\) Hz, Ar-H), 9.07 (1H, s, NH), 9.17 (1H, s, OH); \(^{13}\text{C}\) NMR (125 MHz, DMSO-d\textsubscript{6}) \(\delta_{\text{C}} 18.4\) (CH\textsubscript{3}), 23.4 (COCH\textsubscript{3}), 113.0 (CH-Ar), 117.0 (CH-Ar), 127.5 (CH-Ar), 128.4 (C-Ar), 134.4 (C-Ar), 155.3 (C-Ar), 168.6 (COCH\textsubscript{3}); HRMS calcd for C\textsubscript{9}H\textsubscript{12}NO\textsubscript{2} [M+H]\textsuperscript{+} 166.0863, found 166.0857.

N-(4-hydroxy-2-methoxyphenyl)acetamide (178)

\[
\begin{align*}
\text{MeO} & \text{\centerline{\text{\ H}}} \\
\text{\centerline{\ OH}} & \text{\centerline{\ OH}} \\
\end{align*}
\]

3-Methoxy-4-nitrophenol (200 mg, 1.28 mmol) was dissolved in methanol (18 mL) and reduced using an H-Cube\textsuperscript{®} continuous flow reactor (Thalesnano Inc.) with a Pd/C CatCart (r.t., 1 mL/min). The solvent was removed in vacuo after 24 h and the residue was dissolved in AcOH (9 mL) and acetic anhydride (0.18 mL) and the mixture was stirred at r.t. for 30 min. The solvent...
was evaporated in vacuo and the residue was purified by MPLC on SiO\textsubscript{2} (DCM:MeOH, 0-15%) which gave a brown solid (155 mg, 73%). R\textsubscript{f}=0.31 (5% MeOH/DCM); \(\lambda\text{max} (\text{EtOH})/\text{nm} 285.4, 250.4; \text{IR } \nu\text{max}/\text{cm}^{-1} 3265 (\text{OH}), 2936, 2844, 1597 (\text{C=O}); ^{1}\text{H NMR (500 MHz, DMSO-}d_{6}\text{) } \delta_{H} 1.99 (3H, s, COCH\text{3, } 3.74 (3H, s, OCH\text{3, } 6.28 (1H, dd, } J = 8.5 \text{ and } 2.5 \text{ Hz, Ar-}H\text{), } 6.42 (1H, d, } J = 2.5 \text{ Hz, Ar-}H\text{), } 7.46 (1H, d, } J = 8.5 \text{ Hz, Ar-}H\text{), } 8.87 (1H, s, NH\text{), } 9.27 (1H, s, OH); ^{13}\text{C NMR (125 MHz, DMSO-}d_{6}\text{) } \delta_{C} 23.9 \text{ (COCH\text{3, ) } 55.8 \text{ (OCH\text{3, ) } 99.7 \text{ (CH-}Ar\text{, ) } 106.5 \text{ (CH-}Ar\text{, ) } 119.2 \text{ (C-}Ar\text{, ) } 124.9 \text{ (CH-}Ar\text{, ) } 152.2 \text{ (C-}Ar\text{, ) } 155.4 \text{ (C-}Ar\text{, ) } 168.4 \text{ (COCH\text{3, ) HRMS calcld for } C_{9}H_{12}NO_{3} [M+H]^+ 182.0812, \text{ found } 182.0808.}

2-Hydrazinyl-5-methylpyridine (183)

\[
\begin{array}{c}
\text{N} \\
\text{N} \\
\text{N} \\
\text{NH}_2 \\
\end{array}
\]

2-Fluoro-5-methylpyridine (500 mg, 4.50 mmol) was dissolved in Ethanol (10 mL), hydrazine hydrate (2.2 mL, 45 mmol) was added and the reaction mixture was refluxed for 24 h. Starting material was still remaining, therefore, hydrazine hydrate (2.2 mL, 45 mmol) was added and the mixture was refluxed for 48 h. The solvent was removed in vacuo and the residue was purified by MPLC on SiO\textsubscript{2} (DCM:MeOH, 0-5%) which gave a beige solid (240 mg, 43%); \(^{1}\text{H NMR (500 MHz, DMSO-}d_{6}\text{) } \delta_{H} 2.11 (3H, s, CH\text{3, } 4.04 (2H, br s, NH\text{2, } 6.63 (1H, d, } J = 8.5 \text{ Hz, Ar-}H\text{), } 7.11 (1H, br s, NH\text{), } 7.29 (1H, dd, } J = 8.5 \text{ and } 2.3 \text{ Hz, Ar-}H\text{), } 7.81-7.83 (1H, m, Ar-}H\text{); } ^{13}\text{C NMR (125 MHz, DMSO-}d_{6}\text{) } \delta_{C} 17.5 \text{ (CH\text{3, ) } 106.7 \text{ (CH-}Ar\text{, ) } 121.3 \text{ (C-}Ar\text{, ) } 138.2 \text{ (CH-}Ar\text{, ) } 147.1 \text{ (CH-}Ar\text{, ) } 160.8 \text{ (C-}Ar\text{.)

3,6-Dimethyl-[1,2,4]triazolo[4,3-a]pyridine (182)

\[
\begin{array}{c}
\text{N} \\
\text{N} \\
\end{array}
\]

Compound 183 (235 mg, 1.59 mmol) was dissolved in acetic acid (5 mL) and acetic anhydride (5 mL). The solution was refluxed for 48 h. After cooling to r.t., the solvent was removed in vacuo and the residue was purified by MPLC on SiO\textsubscript{2} (DCM:MeOH, 0-7%). The purification was repeated to get a white solid (45 mg, 16%). R\textsubscript{f}=0.35 (5% MeOH/DCM); \(\lambda\text{max} (\text{EtOH})/\text{nm} 289.0, 212.0; \text{IR } \nu\text{max}/\text{cm}^{-1} 3033, 2925, 2868; ^{1}\text{H NMR (500 MHz, DMSO-}d_{6}\text{) } \delta_{H} 2.30 (3H, d, } J
= 0.9 Hz, CH₃-Pyridine), 2.64 (3H, s, CH₃-Triazole), 7.20 (1H, dd, J = 9.3 and 1.3 Hz, Ar-H), 7.62 (1H, d, J = 9.3 Hz, Ar-H), 8.15-8.18 (1H, m, Ar-H); ¹³C NMR (125 MHz, DMSO-d₆) δC 10.2 (CH₃-Triazole), 17.9 (CH₃-Pyridine), 114.9 (CH-Ar), 121.1 (CH-Ar), 122.9 (C-Ar), 130.7 (CH-Ar), 143.4 (C-Ar), 148.8 (C-Ar); HRMS calcd for C₈H₁₀N₃ [M+H]⁺ 148.0869, found 148.0869.

3-Methyl-6-(prop-1-en-2-yl)-[1,2,4]triazolo[4,3-a]pyridine (185)

![Chemical structure of 3-Methyl-6-(prop-1-en-2-yl)-[1,2,4]triazolo[4,3-a]pyridine]

6-Bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridine (100 mg, 0.47 mmol), isopropenylboronic acid pinacol ester (0.13 mL, 0.71 mmol), NaOH (19 mg, 0.47 mmol), N,N-dicyclohexylmethylamine (0.1 mL, 0.47 mmol) were suspended in THF (2.6 mL). The reaction mixture was sparged for 30 min with nitrogen, [1,1′-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (38 mg, 0.047 mmol) was added and the suspension was sparged with nitrogen for a further 15 min. The reaction mixture was heated at 95 °C for 2.5 h, cooled to r.t, and filtered through a pad of Celite using MeOH. The solvent was removed in vacuo and the residue was purified by MPLC on SiO₂ (DCM:MeOH, 0-15%) which gave a white solid (50 mg, 62%). Rₓ = 0.36 (5% MeOH/DCM); λmax (EtOH)/nm 282.6, 238.6; IR νmax/cm⁻¹ 2921, 2852, 2640; ¹H NMR (500 MHz, DMSO-d₆) δH 2.18 (3H, s, CH₃), 2.71 (3H, s, CH₃-Triazole), 5.25 (1H, s, 1×C=CH₂), 5.63 (1H, s, 1×C=CH₂), 7.64 (1H, dd, J = 9.7 and 1.6 Hz, 1×Ar-H), 7.68 (1H, dd, J = 9.7 and 1.0 Hz, 1×Ar-H), 8.23 (1H, ap s, Ar-H); ¹³C NMR (125 MHz, DMSO-d₆) δC 10.3 (CH₃-Triazole), 21.4 (CH₃), 115.0 (CH-Ar), 115.0 (C=CH₂), 120.4 (CH-Ar), 126.2 (CH-Ar), 126.3 (C-Ar), 138.9 (C=CH₂), 144.7 (C-Ar), 148.8 (C-Ar); LRMS (ES⁺) m/z 174.2 [(M+H)⁺].
3-Methyl-6-vinyl-[1,2,4]triazolo[4,3-a]pyridine (184)

![Chemical Structure](image)

6-Bromo-3-methyl-[1,2,4]triazolo[4,3-a]pyridine (100 mg, 0.47 mmol), vinylboronic acid pinacol ester (0.12 mL, 0.71 mmol), NaOH (19 mg, 0.47 mmol), N,N-dicyclohexylmethylamine (0.1 mL, 0.47 mmol) were suspended in THF (3 mL). The reaction mixture was sparged for 30 min with nitrogen, [1,1’-bis(diphenylphosphino)ferrocene]dichloropalladium(II), complex with dichloromethane (38 mg, 0.047 mmol) was added and the suspension was sparged with nitrogen for a further 15 min. The reaction mixture was heated at 95 °C for 2.5 h, cooled to r.t, and filtered through a pad of celite using MeOH. The solvent was removed in vacuo and the residue was purified by MPLC on SiO₂ (DCM:MeOH, 0-10%) followed by another purification by MPLC (H₂O:MeCN, reversed phase with 0.1% HCOOH modifier, 0-80%) to give a beige solid (38 mg, 51%). R½ = 0.27 (5% MeOH/DCM); ¹H NMR (500 MHz, DMSO-d₆) δ (H, s, CH₃-Triazole), 5.40 (1H, br d, J = 10.9 Hz, 1×CH=CH₂), 5.96 (1H, br d, J = 17.5 Hz, 1×CH=CH₂), 6.77 (1H, dd, 17.5 and 10.9 Hz, CH=CH₂), 7.66 (1H, dd, J = 9.5 and 1.4 Hz, Ar-H), 7.70 (1H, d, J = 9.5 Hz, Ar-H), 8.40 (1H, s, Ar-H); ¹³C NMR (125 MHz, DMSO-d₆) δ 10.2 (CH₃-Triazole), 115.5 (CH-Ar), 116.3 (CH=CH₂), 122.6 (CH-Ar), 123.9 (C-Ar), 124.9 (CH-Ar), 132.6 (CH=CH₂), 144.5 (C-Ar), 149.1 (C-Ar); LRMS (ES⁺) m/z 160.2 [(M+H)⁺].

2-(5-((Tert-butoxycarbonyl)amino)-2-methoxypyridin-4-yl)acetic acid (190)

![Chemical Structure](image)

Compound 189 (4 g, 16.78 mmol) was dissolved in THF (71 mL) and cooled to -78 °C. 1.4 M sec-BuLi in cyclohexane (36 mL, 50.34 mmol) was added dropwise and the reaction mixture was stirred at -78 °C for 15 min. 5-6 Pellets of dry ice were added and the reaction mixture was cooled to r.t. over a period of 45 min. The reaction was quenched with water (50 mL) and saturated NaHCO₃ (50 mL). The aqueous layer was extracted with EtOAc (2 × 100 mL). The aqueous layer was collected and acidified with 2 M HCl, stirred for 15 min and extracted with
EtOAc (2 × 100 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo to get an off-white solid (3.5 g, 74%). Rᵣ = 0.42 (20% MeOH/DCM); m.p. 116.120 °C; λₘₐₓ (EtOH)/nm 284.2, 234.6; IR νₘₐₓ/cm⁻¹ 3309, (NH), 2981, 2939, 2872, 2782, 1690 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.43 (9H, s, C(CH₃)₃), 3.58 (2H, s, CH₂COOH), 3.82 (3H, s, OCH₃), 6.74 (1H, s, 1×Pyridine-H), 7.97 (1H, s, 1×Pyridine-H), 8.62 (1H, br s, NH), 12.44 (1H, br s, COOH); ¹³C NMR (125 MHz, DMSO-d₆) δC 28.6 (C((CH₃)₃), 36.9 (CH₂CO), 53.7 (OCH₃), 79.4 (C(CH₃)₃), 111.9 (CH-Pyridine), 128.4 (C-Pyridine), 143.7 (CH-Pyridine), 144.4 (C-Pyridine), 154.5 (COO'Bu), 161.6 (Pyridine-C-OMe), 171.6 (COOH); HRMS calcd for C₁₃H₁₉N₂O₅ [M+H]+ 283.1288, found 283.1288.

**Tert-butyl 5-methoxy-2-oxo-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate (191)**

![Tert-butyl 5-methoxy-2-oxo-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate (191)](image)

Compound **190** (1.47 g, 5.20 mmol), tetrabutylammonium acetate (50 mg, 0.16 mmol) were dissolved in acetic anhydride (27 mL) and the mixture was stirred at 65 °C for 1 h. The solvent was removed in vacuo, water (30 mL) was added and the aqueous layer was extracted with DCM (3×40 mL). The organic layers were combined, washed with NaHCO₃ (3×30 mL), dried over MgSO₄ and evaporated in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a white solid (1.11 g, 81%). Rᵣ = 0.71 (5% MeOH/DCM); m.p. 161-163 °C; λₘₐₓ (EtOH)/nm 338.2, 230.0; IR νₘₐₓ/cm⁻¹ 3309, (NH), 2981, 2939, 2872, 2782, 1690 (C=O), 1628 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.57 (9H, s, C(CH₃)₃), 3.76 (2H, d, J = 1.0 Hz, CH₂CO), 3.83 (3H, s, OCH₃), 6.82 (1H, m, 1×Pyridine-H), 8.37 (1H, s, 1×Pyridine-H); ¹³C NMR (125 MHz, DMSO-d₆) δC 28.1 (C(CH₃)₃), 36.6 (CH₂CO), 53.8 (OCH₃), 84.2 (C(CH₃)₃), 106.9 (CH-Pyridine), 131.1 (CH-Pyridine), 133.4 (C-Pyridine), 138.4 (C-Pyridine), 148.9 (COO'Bu), 160.3 (Pyridine-C-OMe), 171.7 (C=O Pyrrolidinone); HRMS calcd for C₁₃H₁₇N₂O₄ [M+H]+ 265.1183, found 265.1187.
**Tert-butyl 5-methoxy-3,3-dimethyl-2-oxo-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate (192)**

![Tert-butyl 5-methoxy-3,3-dimethyl-2-oxo-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate](image)

Compound 191 (1.10 g, 4.16 mmol) and Cs₂CO₃ (3 g, 9.15 mmol) were suspended in MeCN (29 mL). MeI (0.65 mL, 10.4 mmol) was added and stirred at 60 °C for 3 h. The insoluble solids were removed by filtration using EtOAc and the filtrate was evaporated in vacuo. The residue was purified by MPLC on SiO₂ (Petrol:EtOAc, 0-20%) to get a white solid (945 mg, 78%). Rᵢ = 0.77 (5% MeOH/DCM); m.p. 175-178 °C; λₘₐₓ (EtOH)/nm 295.0, 233.0; IR νₘₐₓ/cm⁻¹ 2983, 2936, 1785 (C=O), 1630 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 1.41 (6H, s, 2×C(H₃)), 1.65 (9H, s, C(C₃H₃)), 3.92 (3H, s, OC₃H₃), 6.62 (1H, d, J = 0.6 Hz, 1×Pyridine-H), 8.54 (1H, d, J = 0.6 Hz, 1×Pyridine-H); ¹³C NMR (125 MHz, CDCl₃) δC 24.8 (2×C₃H₃), 28.1 (C(CH₃)₂), 44.7 (C(CH₃)₂), 53.7 (OCH₃), 84.8 (C(CH₃)₃), 104.8 (CH-Pyridine), 130.2 (C-Pyridine), 132.1 (CH-Pyrdine), 147.3 (C-Pyridine), 148.9 (COO'Bu), 161.1 (Pyridine-C-OMe), 178.4 (C=O pyrrolidine); HRMS calcd for C₁₅H₂₁N₂O₄ [M+H]^+ 293.1496, found 293.1497.

**3,3,6-Trimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (5)**

![3,3,6-Trimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione](image)

Compound 192 (630 mg, 2.15 mmol) was dissolved in MeCN (15 mL) and MeI (0.33 mL, 5.37 mmol) was added. The mixture was stirred at 170 °C under microwave irradiation for 1 h. The solvent was removed in vacuo and the residue was purified by MPLC on SiO₂ (DCM:MeOH, 0-10%) to get a white solid (148 mg, quant.). Rᵢ = 0.15 (5% MeOH/DCM); m.p. 162-163°C; λₘₐₓ (EtOH)/nm 336.2, 255.2, 218.6; IR νₘₐₓ/cm⁻¹ 3455 (NH), 3081, 3019, 2960, 2926, 2705, 2663, 1705 (C=O), 1589 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.24 (6H, s, 2×CH₃), 3.36 (3H, s, OCH₃), 6.48 (1H, s, 1×Pyridone-H), 7.14 (1H, s, 1×Pyridone-H), 10.25 (1H, s, NH); ¹³C NMR (125 MHz, DMSO-d₆) δC 24.0 (2×CH₃), 37.1 (C(CH₃)₂), 44.0 (NCH₃), 114.1 (CH-Pyridone), 116.9 (CH-Pyridone), 123.4 (C-Pyridone), 153.8 (C-Pyridone), 161.0 (C=O pyridone), 180.2 (C=O pyrrolidine); HRMS calcd for C₁₀H₁₃N₂O₂ [M+H]^+ 193.0972, found 193.0968.
1-Benzyl-3,3,6-trimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (193)

![Chemical Structure](attachment:structure.png)

Compound 5 (50 mg, 0.26 mmol) was dissolved in DMF (3 mL). NaH (60% in mineral oil, 31 mg, 0.78 mmol) was added portion wise and the mixture was stirred at r.t. for 15 min. Benzyl bromide (62 µL, 0.52 mmol) was added and the reaction was stirred at r.t. for 3 h. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over MgSO₄, the solvent removed in vacuo and the residue was purified by MPLC on SiO₂ (DCM:MeOH, 0-5%) to get a beige solid (45 mg, 61%). Rf = 0.35 (5% MeOH/DCM); m.p. 122-124°C; λmax (EtOH)/nm 257.8, 236.8; IR νmax/cm⁻¹ 3090, 3056, 2972, 2924, 2852, 1703 (C=O), 1593 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.32 (6H, s, 2×CH₃), 3.32 (3H, s, N-C₃H₃), 4.75 (2H, s, CH₂Ph), 6.57 (1H, s, 1×Pyridone-H), 7.28-7.37 (6H, m, 6×ArH); ¹³C NMR (125 MHz, DMSO-d₆) δC 24.2 (2×CH₃), 37.3 (N-CH₃), 43.2 (CH₂-phenyl), 43.9 (C(CH₃)₂), 114.2 (CH-Pyridone), 117.2 (CH-Pyridone), 124.3 (C-Ar), 127.6 (2×C-Ar), 127.9 (C-Ar), 129.2 (2×C-Ar), 136.3 (C-Ar), 152.2 (C-Ar), 161.2 (C=O pyridone), 178.3 (C=O pyrrolidine); HRMS calcd for C₁₇H₁₉N₂O₂ [M+H]⁺ 283.1441, found 283.1440.

3,3,6-Trimethyl-1-phenethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (194)

![Chemical Structure](attachment:structure1.png)

Compound 5 (50 mg, 0.26 mmol) was dissolved in DMF (3 mL). NaH (60% in mineral oil, 31 mg, 0.78 mmol) was added portion wise and the mixture was stirred at r.t. for 15 min. (2-bromoethyl)benzene (90 µL, 0.65 mmol) was added and the reaction was stirred at r.t. for 3 h. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over MgSO₄, the solvent removed in vacuo and the residue was purified by MPLC on SiO₂ (DCM:MeOH, 0-5%) to get a beige solid (23 mg, 30%). Rf = 0.36 (5% MeOH/DCM); λmax (EtOH)/nm 337.0, 258.2; IR νmax/cm⁻¹ 3059, 232
2967, 2926, 2866, 1706 (C=O), 1590 (C=O); $^1$H NMR (500 MHz, CDCl$_3$) δ$_H$ 1.28 (6H, s, 2×CH$_3$), 2.94 (2H, t, $J$ = 6.9 Hz, CH$_2$CH$_2$Ph), 3.39 (3H, s, N-CH$_3$), 3.82 (2H, t, $J$ = 6.9 Hz, CH$_2$CH$_2$Ph), 6.26 (1H, s, 1×Pyridone-H), 6.44 (1H, s, 1×Pyridone-H), 7.15-7.17 (2H, m, 2×ArH), 7.22-7.29 (3H, m, 3×ArH); $^{13}$C NMR (125 MHz, CDCl$_3$) δ$_C$ 24.0 (2×CH$_3$), 33.7 (CH$_2$CH$_2$Ph), 37.6 (N-CH$_3$), 42.0 (CH$_2$CH$_2$Ph), 43.8 (C(CH$_3$)$_2$), 114.6 (CH-Pyridone), 114.7 (CH-Pyridone), 125.6 (C-Ar), 126.9 (C-Ar), 128.7 (2×C-Ar), 129.0 (2×C-Ar), 138.2 (C-Ar), 152.2 (C-Ar), 161.7 (C=O pyridone), 178.3 (C=O pyrrolidinone); HRMS calcd for C$_{18}$H$_{21}$N$_2$O$_2$ [M+H]$^+$ 297.1598, 297.1597.

1-(2-((Tert-butyldimethylsilyl)oxy)ethyl)-3,3,6-trimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (201)

![Chemical Structure Image](image-url)

Prepared according to general procedure A using compound 5 (100 mg, 0.52 mmol), Cs$_2$CO$_3$ (508 mg, 1.56 mmol), compound 200 (311 mg, 1.30 mmol) and DMF (4.5 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-5%) gave an orange viscous liquid (120 mg, 55%). R$_f$ = 0.20 (5% MeOH/DCM); $\lambda_{max}$ (EtOH)/nm 336.2, 257.6, 219.8; IR $\nu_{max}$/cm$^{-1}$ 2929, 2855, 1703 (C=O), 1589 (C=O); $^1$H NMR (500 MHz, DMSO-$_d$$_6$) δ$_H$ 0.00 (6H, s, 2×CH$_3$ of TBDMS), 0.82 (9H, s, C(CH$_3$)$_3$ of TBDMS), 1.30 (6H, s, 2×CH$_3$), 3.42 (3H, s, N-CH$_3$), 3.71 (2H, t, $J$ = 5.3 Hz, CH$_2$CH$_2$OTBDMS), 3.83 (2H, t, $J$ = 5.3 Hz, CH$_2$CH$_2$OTBDMS), 6.56 (1H, s, 1×Pyridone-H), 7.48 (1H, s, 1×Pyridone-H); $^{13}$C NMR (125 MHz, DMSO-$_d$$_6$) δ$_C$ -5.1 (2×CH$_3$ of TBDMS), 18.2 (C(CH$_3$)$_3$ of TBDMS), 24.2 (2×CH$_3$), 26.0 (C(CH$_3$)$_3$ of TBDMS), 37.2 (N-CH$_3$), 42.6 (CH$_2$CH$_2$OTBDMS), 43.7 (C(CH$_3$)$_2$), 60.0 (CH$_2$CH$_2$OTBDMS) 113.7 (CH-Pyridone), 117.6 (CH-Pyridone), 125.1 (C-Pyridone), 152.2 (C-Pyridone), 161.1 (C=O pyridone), 178.1 (C=O pyrrolidinone); HRMS calcd for C$_{18}$H$_{31}$N$_2$O$_3$Si [M+H]$^+$ 351.2098, found 351.2100.
1-(2-Hydroxyethyl)-3,3,6-trimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (202)

Compound 201 (98 mg, 0.28 mmol) was dissolved in THF (2.5 mL). 1 M TBAF in THF (2.5 mL) was added and the reaction mixture was stirred at r.t. for 18 h. The solvent was removed in vacuo and the residue was purified by MPLC on SiO\(_2\) (DCM:MeOH, 0-20%) to get a white solid (56 mg, 90%). \(R_f = 0.30\) (15% MeOH/DCM); m.p. 140-142°C; \(\lambda_{\text{max}}\) (EtOH)/nm 238.0, 258.6; IR \(\nu_{\text{max}}\)/cm\(^{-1}\) 3271 (OH), 3043, 2973, 2932, 2870, 1708, (C=O), 1571 (C=O); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 1.26 (6H, s, 2×CH\(_3\)), 3.39 (3H, s, CH\(_3\)), 3.57-3.60 (4H, m, CH\(_2\)), 4.82 (1H, t, \(J = 5.4\) Hz, OH), 6.52 (1H, s, 1xPyridone-H), 7.44 (1H, s, 1xPyridone-H); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\) 24.2 (2×CH\(_3\)), 37.3 (N-CH\(_3\)), 42.9 (CH\(_2\)CH\(_2\)N), 43.7 (CH(CH\(_3\))), 58.0 (CH\(_2\)CH\(_2\)O), 113.7 (CH-Pyridone), 117.3 (CH-Pyridone), 125.2 (C-Pyridone), 152.4 (C-Pyridone), 161.1 (C=O pyridone), 178.2 (C=O pyrrolidinone); HRMS calcd for C\(_{12}\)H\(_{17}\)N\(_2\)O\(_3\) [M+H]\(^+\) 237.1234, found 237.1232.

3,3,6-Trimethyl-1-((phenylthio)methyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (197)

Prepared according to general procedure A using compound 5 (50 mg, 0.26 mmol), Cs\(_2\)CO\(_3\) (254 mg, 0.78 mmol), chloromethyl phenyl sulphide (0.09 mL, 0.65 mmol) and DMF (2 mL). Purification by MPLC on SiO\(_2\) (DCM:MeOH, 0-5%) gave a white solid (61 mg, 75%). \(R_f = 0.35\) (5% MeOH/DCM); m.p. 55-57 °C; \(\lambda_{\text{max}}\) (EtOH)/nm 335.2, 252.4; IR \(\nu_{\text{max}}\)/cm\(^{-1}\) 3422, 3055, 2968, 2927, 2866, 1712 (C=O), 1586 (C=O); \(^1\)H NMR (500 MHz, DMSO-\(d_6\)) \(\delta\) 1.09 (6H, s, 2xCH\(_3\)), 3.39 (3H, s, CH\(_3\)), 5.06 (2H, s, CH\(_2\)), 6.51 (1H, s, 1xPyridone-H), 7.27-7.30 (3H, m, 3xAr\(H\)), 7.40-7.42 (2H, m, 2xAr\(H\)), 7.44 (1H, s, 1xPyridone-H); \(^{13}\)C NMR (125 MHz, DMSO-
$d_6$ δ C 24.0 (2×CH₃), 37.4 (N-CH₃), 43.8 (C(CH₃)₂), 44.3 (NCH₂S), 113.8 (CH-Pyridone), 118.8 (CH-Pyridone), 122.4 (C-Ar), 128.5 (C-Ar), 129.5 (2×C-Ar), 132.2 (C-Ar), 133.4 (2×C-Ar), 151.8 (C-Ar), 161.2 (C=O pyridone), 177.7 (C=O pyrrolidinone); HRMS calcd for C₁₇H₁₅N₂O₄S [M+H]+ 315.1162, found 315.1164.

3,3,6-Trimethyl-1-((phenylsulfonyl)methyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (198)

![Chemical Structure]

Compound 197 (35 mg, 0.11 mmol) was dissolved in MeOH (1 mL) and a solution of oxone (101 mg, 0.33 mL) in water (1 mL) was added. The reaction mixture was stirred at r.t. for 18 h. The solvent was removed in vacuo, water (20 mL) was added and the aqueous layer was extracted with EtOAc (3 × 20 mL). The organic layers were combined, dried over MgSO₄, and the solvent removed in vacuo to get a white solid (20 mg, 52%). Rₐ = 0.35 (5% MeOH/DCM); m.p. 185-188 °C; λₘₐₓ (EtOH)/nm 334.4, 256.0; IR νₘₐₓ/cm⁻¹ 3053, 2970, 2920, 2865, 1718, (C=O), 1581 (C=O), 1309 (SO), 1134 (SO); $^1$H NMR (500 MHz, DMSO-$d_6$) δ H 1.12 (6H, s, 2×CH₃), 3.33 (3H, s, CH₃), 5.27 (2H, s, CH₂), 6.53 (1H, s, 1×Pyridone-H), 7.37 (1H, s, 1×Pyridone-H), 7.60-7.63 (2H, m, 2×ArH), 7.74-7.78 (1H, m, 1×ArH), 7.82-7.84 (2H, m, 2×ArH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δ C 24.0 (2×CH₃), 37.5 (N-CH₃), 43.6 (C(CH₃)₂), 61.0 (NCH₂SO₂), 113.8 (CH-Pyridone), 119.2 (CH-Pyridone), 122.5 (C-Ar), 129.3 (2×C-Ar), 130.0 (2×C-Ar), 135.1 (C-Ar), 137.5 (C-Ar), 151.3 (C-Ar), 161.1 (C=O pyridone), 177.5 (C=O pyrrolidinone); HRMS calcd for C₁₇H₁₉N₂O₄S [M-H]⁻ 347.1060, found 347.1056.
3,3,6-Trimethyl-1-(2-morphinoethyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (196)

Prepared according to general procedure A using compound 5 (43 mg, 0.22 mmol), Cs₂CO₃ (287 mg, 0.88 mmol), 4-(2-chloroethyl)morpholine hydrochloride (102 mg, 0.55 mmol) and DMF (2.5 mL). The product is insoluble in EtOAc, therefore aqueous layer was collected and the solvent removed in vacuo. The residue was purified by MPLC (H₂O:MeCN, reversed phase with 0.1% HCOOH modifier, 0-50%) to get a white solid (55 mg, 82%). Rₜ = 0.15 (5% MeOH/DCM); m.p. 172-174°C; λ max (EtOH)/nm 336.8, 258.2, 220.6; IR ν max/cm⁻¹ 3034, 2939, 2852, 2811, 1712 (C=O), 1590 (C=O); ¹H NMR (500 MHz, CD₃OD) δ H 1.27 (6H, s, 2×CH₃), 2.41 (4H, br s, 4×Morpholine-CH₂), 2.52 (2H, t, J = 6.4 Hz, 2×CON-CH₂CH₂), 3.46 (3H, s, CH₃), 3.51 (4H, br t, J = 4.5 Hz, 4×Morpholine-CH₂), 3.70 (2H, t, J = 6.4 Hz, 2×CON-CH₂), 6.47 (1H, s, 1×Pyridone-H), 7.34 (1H, s, 1×Pyridone-H); ¹³C NMR (125 MHz, CD₃OD) δ C 22.8 (2×CH₃), 36.8 (CON-CH₂), 36.9 (N-CH₃), 44.0 (C(CH₃)₂), 53.2 (2×CH₂-Morpholine), 53.9 (CON-CH₂CH₂), 66.6 (2×CH₂-Morpholine), 113.0 (CH-Pyridone), 116.8 (CH-Pyridone), 126.1 (C-Pyridone), 153.5 (C-Pyridone), 162.5 (C=O pyridone), 179.4 (C=O pyrrolidinone); HRMS calcd for C₁₆H₂₄N₃O₃ [M+H]⁺ 306.1812, found 306.1812.

3,3,6-Trimethyl-1-(2-pyrrolidin-1-yl)ethyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (195)

Prepared according to general procedure A using compound 5 (43 mg, 0.22 mmol), Cs₂CO₃ (287 mg, 0.88 mmol), 1-(2-chloroethyl)pyrrolidine hydrochloride (94 mg, 0.55 mmol) and DMF (2.5 mL). The product is insoluble in EtOAc, therefore aqueous layer was collected and
the solvent removed in vacuo. The residue was purified by MPLC (H₂O:MeCN, reversed phase with 0.1% HCOOH modifier, 0-50%) to get a white solid (25 mg, 40%). Rₜ = 0.15 (5% MeOH/DCM); λₘₚₖ (EtOH)/nm 336.0, 257.8, 220.0; IR νₘₚₖ/cm⁻¹ 3029, 2962, 2923, 2787, 1712 (C=O), 1587 (C=O); ¹H NMR (500 MHz, CD₃OD) δH 1.26 (6H, s, 2×CH₃), 1.69-1.72 (4H, m, 4×Pyrrolidine-CH₂), 2.51-2.54 (4H, m, 4×Pyrrolidine-CH₂), 3.70 (2H, t, J = 6.9 Hz, 2×CON-C₂H₂), 6.47 (1H, s, 1×Pyridone-CH), 7.32 (1H, s, 1×Pyridone-CH), 7.48 (1H, t, J = 7.6 Hz, ArCH), 7.54 (1H, d, J = 7.8 Hz, ArH), 7.67 (1H, td, J = 7.8 and 1.1 Hz, ArH), 7.88 (1H, dd, J = 7.7 and 1.1 Hz, ArH), 10.33 (1H, s, NH); ¹³C NMR (125 MHz, CD₃OD) δC 22.7 (2×CH₃), 22.9 (2×CH₂-Pyrrolidine), 36.9 (N-CH₃), 38.9 (CON-CH₂), 44.1 (C(CH₃)₂), 51.6 (CON-CH₂CH₂), 53.7 (2×CH₂-Pyrrolidine), 113.1 (CH-Pyridone), 116.6 (CH-Pyridone), 126.2 (C-Pyridone), 153.5 (C-Pyridone), 162.5 (C=O pyridone), 179.2 (C=O pyrrolidine); HRMS calcd for C₁₆H₂₄N₃O₂ [M+H]⁺ 290.1863, found 290.1865.

2-((3,3-Dimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)methyl)benzonitrile (207)

Prepared according to general procedure B using compound 192 (120 mg, 0.411 mmol), 2-(bromomethyl)benzonitrile (162 mg, 0.824 mmol) and MeCN (3.8 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-7%) gave a white solid (75 mg, 63%). Rₜ = 0.32 (5% MeOH/DCM); m.p. 315 °C (degraded); λₘₚₖ (EtOH)/nm 339.2, 224.0; IR νₘₚₖ/cm⁻¹ 3069, 3019, 2972, 2726, 2224 (CN), 1708 (C=O), 1588 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.27 (6H, s, 2×CH₃), 5.24 (2H, s, CH₂), 6.58 (1H, s, 1×Pyridone-CH), 7.12 (1H, d, J = 7.8 Hz, ArH), 7.27 (1H, s, 1×Pyridone-CH), 7.48 (1H, t, J = 7.6 Hz, ArH), 7.67 (1H, td, J = 7.8 and 1.1 Hz, ArH), 7.88 (1H, dd, J = 7.7 and 1.1 Hz, ArH), 10.33 (1H, s, NH); ¹³C NMR (125 MHz, DMSO-d₆) δC 24.0 (2×CH₃), 44.1 (C(CH₃)₂), 50.4 (NCH₂Ar), 110.9 (C-Ar), 114.8 (CH-Pyridone), 116.2 (CH-Pyridone), 117.7 (CN), 124.1 (C-Ar), 127.8 (C-Ar), 128.5 (C-Ar), 133.5 (C-Ar), 134.1 (C-Ar), 141.5 (C-Ar), 154.7 (C-Ar), 160.7 (C=O pyridone), 180.1 (C=O pyrrolidinone); HRMS calcd for C₁₇H₁₆N₃O₂ [M+H]⁺ 294.1237, found 224.1240.
2-((1,3,3-Trimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)methyl)benzonitrile (208)

Prepared according to general procedure A using compound 207 (50 mg, 0.17 mmol), Cs$_2$CO$_3$ (166 mg, 0.51 mmol), MeI (30 μL, 0.43 mmol) and DMF (1.5 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-5%) gave a white solid (50 mg, 96%). R$_f$ = 0.42 (5% MeOH/DCM); m.p. 195-197 °C; λ$_{max}$ (EtOH)/nm 258.4, 225.6; IR ν$_{max}$/cm$^{-1}$ 3054, 2973, 2924, 2221 (CN), 1703 (C=O), 1599 (C=O); $^1$H NMR (500 MHz, CD$_3$OD) δH 1.28 (6H, s, 2×CH$_3$), 3.03 (3H, s, N-CH$_3$), 5.28 (2H, s, CH$_2$), 6.51 (1H, s, 1×Pyridone-H), 7.17 (1H, d, J = 7.9 Hz, Ar-H), 7.35-7.38 (2H, m, Ar-H + Pyridone-H), 7.52 (1H, td, J = 7.7, 1.2 Hz, Ar-H), 7.67 (1H, dd, J = 7.7, 1.0 Hz, Ar-H); $^{13}$C NMR (125 MHz, CD$_3$OD) δC 22.6 (2×CH$_3$), 44.2 (C(CH$_3$)$_2$), 51.2 (NCH$_2$Ar), 111.2 (C-Ar), 113.7 (CH-Pyridone), 115.7 (CH-Pyridone), 116.9 (CN), 127.5 (C-Ar), 127.7 (C-Ar), 128.1 (C-Ar), 132.9 (C-Ar), 133.1 (C-Ar), 139.9 (C-Ar), 154.0 (C-Ar), 162.2 (C=O pyridone), 179.0 (C=O pyrrolidine); HRMS calcd for C$_{18}$H$_{18}$N$_3$O$_2$ [M+H]$^+$ 308.1394, found 308.1396.

3-((3,3-Dimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)methyl)benzonitrile (209)

Prepared according to general procedure B using compound 192 (100 mg, 0.342 mmol), 3-(bromomethyl)benzonitrile (134 mg, 0.684 mmol) and MeCN (2.5 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-7%) gave a white solid (65 mg, 65%). R$_f$ = 0.32 (5% MeOH/DCM); m.p. 310 °C (degraded); λ$_{max}$ (EtOH)/nm 338.4, 255.8, 223.0; IR ν$_{max}$/cm$^{-1}$ 3059, 2964, 2925, 2723, 2227 (CN), 1709 (C=O), 1584 (C=O); $^1$H NMR (500 MHz, DMSO-$d_6$) δH 1.25 (6H, s, 2×CH$_3$), 5.09 (2H, s, CH$_2$), 6.56 (1H, s, 1×Pyridone-H), 7.29 (1H, s, 1×Pyridone-H), 7.55-7.58 (1H, m, ArH), 7.62-7.65 (1H, m, ArH), 7.76-7.79 (2H, m, ArH), 10.31 (1H, s, NH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δC 24.0 (2×CH$_3$), 44.1 (C(CH$_3$)$_2$), 50.9 (NCH$_2$Ar), 111.8 (C-Ar) 114.8 (CH-Pyridone), 115.8 (CH-Pyridone), 119.1 (CN), 124.1 (C-Ar), 130.3 (C-Ar), 131.8 (C-Ar), 238
131.9 (C-Ar), 133.2 (C-Ar), 139.8 (C-Ar), 154.4 (C-Ar), 160.7 (C=O pyridone), 180.2 (C=O pyrrolidinone); HRMS calcd for C$_{17}$H$_{16}$N$_3$O$_2$ [M+H]$^+$ 294.1237, found 294.1242.

3-((1,3,3-trimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)methyl)benzonitrile (210)

Prepared according to general procedure A using compound 209 (40 mg, 0.136 mmol), Cs$_2$CO$_3$ (133 mg, 0.408 mmol), MeI (21 μL, 0.34 mmol) and DMF (1.5 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-5%) gave a white solid (38 mg, 91%). R$_f$ = 0.40 (5% MeOH/DCM); m.p. 170-172 °C; λ$_{max}$ (EtOH)/nm 338.4, 259.4, 225.4; IR ν$_{max}$/cm$^{-1}$ 3033, 2926, 2864, 2229 (CN), 1713 (C=O), 1579 (C=O); $^1$H NMR (500 MHz, CD$_3$OD) δ$_H$ 1.26 (6H, s, 2×CH$_3$), 3.03 (3H, s, -N-C$_3$H$_3$), 5.11 (2H, s, CH$_2$), 6.51 (1H, s, 1×Pyridone-H), 7.38 (1H, s, Ar-H), 7.43 (1H, app t, J = 7.8 Hz, Ar-H), 7.55-7.58 (2H, m, 2×Ar-H), 7.63-7.65 (1H, m, Ar-H); $^{13}$C NMR (125 MHz, CD$_3$OD) δ$_C$ 22.6 (2×CH$_3$), 25.5 (N-CH$_3$), 44.2 (C(CH$_3$)$_2$), 51.8 (NCH$_2$Ar), 112.4 (C-Ar), 113.7 (CH-Pyridone), 115.2 (CH-Pyridone), 118.0 (CN), 127.7 (C-Ar), 129.5 (C-Ar), 131.2 (C-Ar), 131.3 (C-Ar), 132.3 (C-Ar), 138.5 (C-Ar), 153.8 (C-Ar), 162.1 (C=O pyridone), 179.0 (C=O pyrrolidinone); HRMS calcd for C$_{18}$H$_{18}$N$_3$O$_2$ [M+H]$^+$ 308.1394, found 308.1393.

3,3-Dimethyl-6-(4-(methylsulfonyl)benzyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (211)

Prepared according to general procedure B using compound 192 (87 mg, 0.298 mmol), 1-(bromomethyl)-4-(methylsulfonyl)benzene (148 mg, 0.596 mmol) and MeCN (2.5 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-7%) gave a white solid (55 mg, 53%). R$_f$ = 0.27 (5% MeOH/DCM); m.p. 320 °C (degraded); λ$_{max}$ (EtOH)/nm 339.4, 255.6, 223.2; IR ν$_{max}$/cm$^{-1}$ 3083, 3008, 2973, 2926, 2731, 1717 (C=O), 1549, 1306 (SO), 1146 (SO); $^1$H NMR (500 MHz, DMSO-$d_6$) δ$_H$ 1.26 (6H, s, 2×CH$_3$), 3.20 (1H, s, SO$_2$CH$_3$), 5.16 (2H, s, CH$_2$), 6.58 (1H, s, 1×Pyridone-H), 7.28 (1H, s, 1×Pyridone-H), 7.52-7.54 (2H, m, 2×Ar-H), 7.89-7.91 (2H,
m, 2×Ar-H), 10.31 (1H, s, NH); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta_{c} 24.0\) (2×CH\(_3\)), 44.0 (SO\(_2\)CH\(_3\)), 44.1 (C(CH\(_3\))\(_2\)), 51.2 (N(CH\(_2\)Ar)), 114.8 (CH-Pyridone), 115.9 (CH-Pyridone), 124.0 (C-Ar), 127.8 (2×C-Ar), 128.9 (2×C-Ar), 140.3 (C-Ar), 144.1 (C-Ar), 154.4 (C-Ar), 160.7 (C=O pyridone), 180.2 (C=O pyrrolidinone); HRMS calcd for C\(_{17}\)H\(_{19}\)N\(_2\)O\(_4\)S [M+H]\(^+\) 347.1060, found 347.1062.

1,3,3-Trimethyl-6-(4-(methylsulfonyl)benzyl)-1,6-dihydro-2\(H\)-pyrrolo[2,3-c]pyridine-2,5(3\(H\))-dione (212)

Prepared according to general procedure A using compound 211 (36 mg, 0.104 mmol), Cs\(_2\)CO\(_3\) (102 mg, 0.312 mmol), MeI (16 \(\mu\)L, 0.260 mmol) and DMF (1 mL). Purification by MPLC on SiO\(_2\) (DCM:MeOH, 0-5%) gave a white solid (32 mg, 86%). \(R_f=0.35\) (5% MeOH/DCM); m.p. 95-97 °C; \(\lambda_{max}\) (EtOH)/nm 338.8, 259.2, 224.6; IR \(\nu_{max}/\text{cm}^{-1}\) 3050, 2969, 2927, 1709 (C=O), 1587 (C=O), 1145 (SO); \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta_{H} 1.27\) (6H, s, 2×CH\(_3\)), 2.99 (1H, s, SO\(_2\)CH\(_3\)), 3.03 (1H, s, N-C(CH\(_3\))\(_3\)) 5.19 (2H, s, C\(_2\)H\(_5\)), 6.52 (1H, s, 1×Pyridone-H), 7.37 (1H, s, 1×Pyridone-H), 7.46-7.48 (2H, m, 2×Ar-H), 7.82-7.84 (2H, m, 2×Ar-H); \(^{13}\)C NMR (125 MHz, CD\(_3\)OD) \(\delta_{C} 22.6\) (2×CH\(_3\)), 25.5 (N-CH\(_3\)), 42.9 (SO\(_2\)CH\(_3\)), 44.2 (C(CH\(_3\))\(_2\)), 52.0 (NCH\(_2\)Ar), 113.7 (CH-Pyridone), 115.3 (CH-Pyridone), 127.5 (2×C-Ar), 127.7 (C-Ar), 128.3 (2×C-Ar), 140.1 (C-Ar), 143.1 (C-Ar), 153.9 (C-Ar), 162.1 (C=O pyridone), 179.0 (C=O pyrrolidinone); HRMS calcd for C\(_{18}\)H\(_{21}\)N\(_2\)O\(_4\)S [M+H]\(^+\) 361.1217, found 361.1215.

Methyl 2-((3,3-dimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6\(H\)-pyrrolo[2,3-c]pyridin-6-yl)methyl)benzoate (222)

Prepared according to general procedure B using compound 192 (150 mg, 0.513 mmol), methyl 2-(bromomethyl)benzoate (235 mg, 1.02 mmol) and MeCN (4 mL). Purification by MPLC on SiO\(_2\) (DCM:MeOH, 0-7%) gave a white solid (36 mg, 22%). \(R_f=0.32\) (5% MeOH/DCM); m.p.
284-286 °C; \( \lambda_{\text{max}} \) (EtOH)/nm 337.4, 256.4, 223.4; IR \( \nu_{\text{max}}/\text{cm}^{-1} \) 3085, 3015, 2964, 2783, 2739, 1711 (C=O), 1546 (C=O); \(^1\)H NMR (500 MHz, DMSO-\( \text{d}_6 \)) \( \delta_H \) 1.28 (6H, s, 2×C\( \text{H}_3 \)), 3.88 (3H, s, COOC\( \text{H}_3 \)), 5.38 (2H, s, CH\( _2 \)), 6.58 (1H, s, 1×Pyridone-\( H \)), 6.90 (1H, d, \( J = 7.5 \) Hz, Ar-\( H \)), 7.18 (1H, s, 1×Pyridone-\( H \)), 7.42 (1H, dt, \( J = 7.6 \) Hz, \( J = 0.8 \) Hz, Ar\( H \)), 7.55 (1H, td, \( J = 7.6 \) and \( J = 1.4 \) Hz, Ar\( H \)), 7.93 (1H, dd, \( J = 7.8 \) and 1.2 Hz, Ar\( H \)), 10.28 (1H, s, NH); \(^{13}\)C NMR (125 MHz, DMSO-\( \text{d}_6 \)) \( \delta_C \) 24.1 (2×C\( \text{H}_3 \)), 44.1 (C(CH\( _3 \))\( _2 \)), 50.3 (N(CH\( _2 \)Ar)), 52.7 (COO\( \text{C}_3 \))), 114.8 (CH-Pyrrolone), 116.1 (CH-Pyridone), 124.0 (C-Ar), 127.4 (C-Ar), 127.7 (C-Ar), 129.0 (C-Ar), 130.8 (C-Ar), 133.2 (C-Ar), 139.2 (C-Ar), 154.4 (C-Ar), 160.8 (C=O pyridone), 167.4 (COO\( \text{C}_3 \))), 180.2 (C=O pyrrolidinone); HRMS calcd for C\( _{18} \)H\( _{19} \)N\( _2 \)O\( _4 \)[M+H]\(^+ \) 327.1339, found 327.1341.

**Methyl 2-((1,3,3-trimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)methyl)benzoate (223)**

![Diagram](image)

Prepared according to general procedure A using compound 222 (25 mg, 0.07 mmol), Cs\( _2 \)CO\( _3 \) (75 mg, 0.23 mmol), MeI (12 μL, 0.19 mmol) and DMF (3 mL). Purification by MPLC on SiO\( _2 \) (DCM:MeOH, 0-5%) gave a white solid (19 mg, 74%). \( R_f \) = 0.40 (5% MeOH/DCM); \( \lambda_{\text{max}} \) (EtOH)/nm 337.0, 226.0; IR \( \nu_{\text{max}}/\text{cm}^{-1} \) 3062, 2968, 2924, 2863, 1716 (C=O), 1591 (C=O); \(^1\)H NMR (500 MHz, CD\( _3 \)OD) \( \delta_H \) 1.29 (6H, s, 2×C\( \text{H}_3 \)), 3.00 (1H, s, N-C\( \text{H}_3 \)), 3.84 (3H, s, COOC\( \text{H}_3 \)), 5.49 (2H, s, CH\( _2 \)), 6.54 (1H, s, 1×Pyridone-\( H \)), 6.88 (1H, d, \( J = 7.8 \) Hz, Ar-\( H \)), 7.28-7.31 (2H, m, 1×Ar-\( H \)+1×Pyridone-\( H \)), 7.42 (1H, td, \( J = 7.6 \) Hz, Ar\( H \)), 7.92 (1H, dd, \( J = 7.8 \) and 1.2 Hz, Ar-\( H \)); \(^{13}\)C NMR (125 MHz, CD\( _3 \)OD) \( \delta_C \) 22.7 (2×C\( \text{H}_3 \))), 25.4 (N-C\( \text{H}_3 \))), 44.2 (C(CH\( _3 \))\( _2 \)), 51.2 (NCH\( _2 \)Ar), 51.3 (COO\( \text{C}_3 \))), 113.6 (CH-Pyrrolone), 115.7 (CH-Pyridone), 126.8 (C-Ar), 127.2 (C-Ar), 127.6 (C-Ar), 128.7 (C-Ar), 130.6 (C-Ar), 132.5 (C-Ar), 138.0 (C-Ar), 153.7 (C-Ar), 162.4 (C=O pyridone), 167.4 (COO\( \text{C}_3 \))), 179.1 (C=O pyrrolidinone); HRMS calcd for C\( _{19} \)H\( _{20} \)N\( _2 \)O\( _4 \)[M+H]\(^+ \) 341.1496, found 341.1497.
6-(4-Chlorobenzyl)-3,3-dimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (213)

Prepared according to general procedure B using compound 192 (75 mg, 0.25 mmol), 4-chlorobenzyl bromide (105 mg, 0.51 mmol) and MeCN (2 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-7%) gave a white solid (62 mg, 80%). Rₚ = 0.30 (5% MeOH/DCM); m.p. 255-257 °C; λmax (EtOH)/nm 338.8, 256.6, 221.4; IR νmax/cm⁻¹ 3071, 3022, 2967, 2930, 2864, 2734, 1711 (C=O), 1559 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.24 (6H, s, 2×CH₃), 5.04 (2H, s, CH₂), 6.55 (1H, s, 1×Pyridone-H), 7.22 (1H, d, J = 0.4 Hz, 1×Pyridone-H), 7.32-7.34 (2H, m, 2×Ar-H), 7.40-7.42 (2H, m, 2×Ar-H), 10.27 (1H, s, NH); ¹³C NMR (125 MHz, DMSO-d₆) δC 24.0 (2×CH₃), 44.0 (C(CH₃)₂), 50.7 (NCH₂Ar), 114.8 (CH-Pyridone), 115.7 (CH-Pyridone), 123.9 (C-Ar), 129.0 (2×C-Ar), 130.2 (2×C-Ar), 132.6 (C-Ar), 137.3 (C-Ar), 154.2 (C-Ar), 160.6 (C=O pyridone), 180.2 (C=O pyrrolidinone); HRMS calcd for C₁₆H₁₆ClN₂O₂ [M⁺]+ 303.0895, found 303.0901.

6-(4-Chlorobenzyl)-1,3,3-trimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (6)

Prepared according to general procedure A using compound 213 (34 mg, 0.11 mmol), Cs₂CO₃ (110 mg, 0.33 mmol), MeI (17 μL, 0.28 mmol) and DMF (1.2 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a white solid (32 mg, 90%). Rₚ = 0.37 (5% MeOH/DCM); m.p. 181-183 °C; λmax (EtOH)/nm 338.4, 259.4, 221.2; IR νmax/cm⁻¹ 3061, 3018, 2973, 2927, 2864, 1713 (C=O), 1597 (C=O); ¹H NMR (500 MHz, CD₃OD) δH 1.26 (6H, s, 2×CH₃), 3.01 (1H, s, N-CH₃), 5.06 (2H, s, CH₂), 6.50 (1H, s, 1×Pyridone-H), 7.24 (4H, s, 4×Ar-H) 7.31 (1H, s, 1×Pyridone-H); ¹³C NMR (125 MHz, CD₃OD) δC 22.6 (2×CH₃), 25.4 (N-CH₃), 44.1 (C(CH₃)₂), 51.6 (NCH₂Ar), 113.6 (CH-Pyridone), 115.1 (CH-Pyridone), 127.6 (C-Ar), 128.4 (2×C-Ar), 129.2 (2×C-Ar), 133.4 (C-Ar), 135.6 (C-Ar), 153.5 (C-Ar), 162.1 (C=O pyridone), 179.0 (C=O pyrrolidinone); HRMS calcd for C₁₇H₁₈ClN₂O₂ [M⁺]+ 317.1051, found 317.1056.
2-((1,3,3-Trimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)methyl)benzoic acid (226)

![Chemical Structure Image]

Compound 208 (32 mg, 0.10 mmol) was dissolved in ethanol (4 mL), 1 M NaOH in water (2 mL) was added and the mixture was stirred at 80 °C for 16 h. The reaction did not go into completion, therefore, 1 M NaOH (1 mL) was added and the reaction mixture was stirred at 100 °C for 7 h. Ethanol was evaporated in vacuo and the residue was acidified to pH 1 using 1 M HCl. The aqueous layer was extracted with EtOAc (4×20 mL), the organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo (11 mg, 32%). Rf = 0.29 (5% MeOH/DCM); m.p. 268 °C (degraded); λmax (EtOH)/nm 339.0, 258.8; IR νmax/cm⁻¹ 3071-2180 (broad spectrum), 2919, 2851, 1713 (C=O), 1545 (C=O); ¹H NMR (500 MHz, CD₃OD) δH 1.29 (6H, s, 2×C₃H₃), 2.99 (1H, s, N-C₃H₃), 5.52 (2H, s, CH₂), 6.54 (1H, s, 1×Pyridone-H), 6.88 (1H, d, J = 7.8 Hz, Ar-H), 7.26-7.29 (1H, m, 1×Ar-H), 7.31 (1H, s, 1×Pyridone-H), 7.37 (1H, td, J = 7.8 and 1.3 Hz, Ar-H), 7.93 (1H, dd, J = 7.8 and 1.0 Hz, Ar-H); ¹³C NMR (125 MHz, CD₃OD) δC 22.7 (2×C₃H₃), 25.4 (N-CH₃), 44.2 (CH(C₃H₃)₂), 51.1 (NCH₂Ar), 113.6 (CH-Pyridone), 115.8 (CH-Pyridone), 126.9 (C-Ar), 127.1 (C-Ar), 127.6 (C-Ar), 130.8 (C-Ar), 132.0 (C-Ar), 135.8 (C-Ar), 137.7 (C-Ar), 153.6 (C-Ar), 162.4 (C=O pyridone), 169.4 (COOCH₃), 179.1 (C=O pyrrolidinone); HRMS calcd for C₁₈H₁₉N₂O₄ [M+H]^+ 327.1339, found 327.1343.

Methyl 4-((3,3-dimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)methyl)-3-methoxybenzoate (224)

![Chemical Structure Image]

Prepared according to general procedure B using compound 192 (115 mg, 0.39 mmol), methyl 4-(bromomethyl)-3-methoxybenzoate (168 mg, 0.65 mmol) and MeCN (2.5 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-7%) gave a white solid (92 mg, 66%). Rf = 0.31 (5% MeOH/DCM); m.p. 308-310 °C; λmax (EtOH)/nm 247.0, 211.2; IR νmax/cm⁻¹ 3076, 3004, 2964, 2735, 1715 (C=O), 1556 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.27 (6H, s, 2×CH₃), 3.85
(3H, s, COOCH$_3$), 3.92 (3H, s, OCH$_3$), 5.04 (2H, s, CH$_2$), 6.56 (1H, s, 1×Pyridone-H), 6.98 (1H, d, $J = 8.0$ Hz, Ar-H), 7.15 (1H, s, 1×Pyridone-H), 7.52-7.54 (2H, m, 2×Ar-H), 10.23 (1H, s, NH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δC 24.0 (2×CH$_3$), 44.1 (C(CH$_3$)$_2$), 47.4 (NCH$_2$Ar), 52.7 (COOCH$_3$), 56.2 (OCH$_3$), 111.1 (C-Ar), 114.7 (CH-Pyridone), 116.3 (CH-Pyridone), 122.0 (C-Ar), 123.8 (C-Ar), 128.3 (C-Ar), 130.4 (C-Ar), 131.3 (C-Ar), 154.3 (C-Ar), 157.1 (C-Ar), 160.7 (C=O pyridone), 166.4 (COOCH$_3$), 180.1 (C=O pyrrolidinone); HRMS calcd for C$_{19}$H$_{21}$N$_2$O$_5$ [M+H]$^+$ 357.1445, found 357.1448.

Methyl 3-methoxy-4-((1,3,3-trimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]pyridin-6-yl)methyl)benzoate (225)

Prepared according to general procedure A using compound 224 (66 mg, 0.18 mmol), Cs$_2$CO$_3$ (180 mg, 0.55 mmol), MeI (28 μL, 0.46 mmol) and DMF (1.5 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-5%) gave a white solid (55 mg, 80%). $R_f = 0.36$ (5% MeOH/DCM); m.p. 195-198°C; $\lambda_{max}$ (EtOH)/nm 247.4, 212.0; IR $\nu_{max}$/cm$^{-1}$ 2963, 2933, 1715 (C=O), 1606 (C=O); $^1$H NMR (500 MHz, DMSO-$d_6$) δH 1.29 (6H, s, 2×CH$_3$), 3.01 (N-CH$_3$) 3.85 (3H, s, COOCH$_3$), 3.94 (3H, s, OCH$_3$), 5.05 (2H, s, CH$_2$), 6.62 (1H, s, 1×Pyridone-H), 6.91-6.92 (1H, m, Ar-H), 7.44 (1H, s, 1×Pyridone-H), 7.52-7.54 (2H, m, 2×Ar-H); $^{13}$C NMR (125 MHz, DMSO-$d_6$) δC 24.1 (2×CH$_3$), 26.7 (N-CH$_3$), 43.9 (C(CH$_3$)$_2$), 47.8 (NCH$_2$Ar), 52.7 (COOCH$_3$), 56.2 (OCH$_3$), 111.0 (C-Ar), 114.5 (CH-Pyridone), 116.3 (CH-Pyridone), 122.0 (C-Ar), 126.1 (C-Ar), 127.8 (C-Ar), 130.3 (C-Ar), 131.1 (C-Ar), 152.8 (C-Ar), 157.0 (C-Ar), 161.0 (C=O pyridone), 166.4 (COOCH$_3$), 177.9 (C=O pyrrolidinone); HRMS calcd for C$_{20}$H$_{23}$N$_2$O$_5$ [M+H]$^+$ 371.1601, found 371.1600.
3-Methoxy-4-((1,3,3-trimethyl-2,5-dioxo-1,2,3,5-tetrahydro-6H-pyrrolo[2,3-c]245yridine-6-yl)methyl)benzoic acid (227)

Compound 225 (26 mg, 0.07 mmol) was dissolved in MeOH (1.5 mL), 1 M NaOH (1.5 mL) was added and the reaction mixture was stirred at 70 °C for 2 h. The solvent was removed in vacuo and water (2 mL) was added to the residue and the mixture was acidified to pH 1 with 1 M HCl. The aqueous layer was extracted with EtOAc (4×20 mL), the organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo (24 mg, 96%). Rᵢ = 0.56 (15% MeOH/DCM); m.p. 246-248 °C; λₘₐₓ (EtOH)/nm 236.8, 209.6; IR νₘₐₓ/cm⁻¹ 3049-2200 (broad stretch), 1707 (C=O), 1688 (C=O), 1581 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.29 (6H, s, 2×C₃H₃), 3.01 (N-C₃H₃), 3.93 (3H, s, OCH₃), 5.04 (2H, s, CH₂), 6.62 (1H, s, 1×Pyridone-H), 6.90 (1H, d, J = 7.8 Hz, Ar-H), 7.43 (1H, s, 1×Pyridone-H), 7.49-7.52 (2H, m, 2×Ar-H), 13.03 (1H, s, COO); ¹³C NMR (125 MHz, DMSO-d₆) δC 24.1 (2×C₃H₃), 26.7 (N-C₃H₃), 43.9 (C(CH₃)₂), 47.8 (NCH₂Ar), 56.1 (OCH₃), 111.2 (C-Ar), 114.5 (CH-Pyridone), 116.3 (CH-Pyridone), 122.1 (C-Ar), 126.1 (C-Ar), 127.7 (C-Ar), 130.6 (C-Ar), 131.6 (C-Ar), 152.7 (C-Ar), 156.9 (C-Ar), 161.0 (C=O pyridone), 167.5 (COOH), 177.9 (C=O pyrroldinone); HRMS calcd for C₁₉H₂₁N₂O₅ [M+H]⁺ 357.1445, found 357.1449.

6-(2,6-dichlorobenzyl)-3,3-dimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (214)

Prepared according to general procedure B using compound 192 (76 mg, 0.26 mmol), 2-(bromomethyl)-1,3-dichlorobenzene (187 mg, 0.78 mmol) and MeCN (2.5 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-7%) gave a white solid (72 mg, 82%). Rᵢ = 0.32 (5% MeOH/DCM); m.p. 310 °C (degraded); λₘₐₓ (EtOH)/nm 338.6, 256.6; IR νₘₐₓ/cm⁻¹ 3078, 3012, 2962, 2924, 2735, 1712 (C=O), 1550 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.24 (6H, s,
2×C\(\text{H}_3\)), 5.25 (2H, s, CH\(_2\)), 6.50 (1H, d, \(J = 0.5\) Hz, 1×Pyridone-H), 6.57 (1H, d, \(J = 0.5\) Hz, 1×Pyridone-H), 7.49 (1H, dd, \(J = 7.5\) and \(8.5\) Hz, 1×Ar-H), 7.61 (2H, ap d, \(J = 8.1\) Hz, 2×Ar-H), 7.49 (1H, dd, \(J = 7.5\) and \(8.5\) Hz, 1×Ar-H), 7.61 (2H, ap d, \(J = 8.1\) Hz, 2×Ar-H), 9.87 (1H, s, NH); \(^{13}\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\)C 24.0 (2×C\(\text{H}_3\)), 44.1 (C(CH\(_3\))\(_2\)), 46.3 (NCH\(_2\)Ar), 112.8 (CH-Pyridone), 114.6 (CH-Pyridone), 124.0 (C-Ar), 129.6 (2×C-Ar), 131.9 (2×C-Ar), 136.6 (2×C-Ar), 153.7 (C-Ar), 160.5 (C=O pyridone), 179.8 (C=O pyrrolidinone); HRMS calcd for C\(_{16}\)H\(_{15}\)Cl\(_2\)N\(_2\)O\(_2\) [M+H\(^+\)] 337.0505, found 337.0509.

6-(2,6-dichlorobenzyl)-1,3,3-trimethyl-1,6-dihydro-2\(\text{H}\)-pyrrolo[2,3-\(c\)]pyridine-2,5(3\(\text{H}\))-dione (215)

Prepared according to general procedure A using compound 214 (50 mg, 0.148 mmol), Cs\(_2\)CO\(_3\) (145 mg, 0.44 mmol), MeI (23 \(\mu\)L, 0.37 mmol) and DMF (1.5 mL). Purification by MPLC on SiO\(_2\) (DCM:MeOH, 0-5%) gave a white solid (48 mg, 92%). \(R_t = 0.37\) (5% MeOH/DCM); m.p. 153-155°C; \(\lambda_{\text{max}}\) (EtOH)/nm 338.0, 258.8; IR \(\nu_{\text{max}}\)/cm\(^{-1}\) 3027, 2970, 2926, 2867, 1711 (C=O), 1578 (C=O); \(^1\)H NMR (500 MHz, CD\(_3\)OD) \(\delta\)H 1.25 (6H, s, 2×CH\(_3\)), 5.36 (2H, s, C\(\text{H}_2\)), 6.50 (1H, s, 1×Pyridone-H), 6.64 (1H, s, 1×Pyridone-H), 7.29-7.32 (1H, m, Ar-H), 7.40-7.42 (2H, m, 2×Ar-H); \(^{13}\)C NMR (125 MHz, CD\(_3\)OD) \(\delta\)C 22.6 (2×C\(\text{H}_3\)), 44.0 (C(CH\(_3\))\(_2\)), 47.1 (NCH\(_2\)Ar), 112.6 (CH-Pyridone), 113.5 (CH-Pyridone), 127.4 (C-Ar), 128.8 (2×C-Ar), 130.9 (C-Ar), 130.9 (C-Ar), 136.7 (2×C-Ar), 153.1 (C-Ar), 162.1 (C=O pyridone), 178.9 (C=O pyrrolidinone); HRMS calcd for C\(_{17}\)H\(_{17}\)Cl\(_2\)N\(_2\)O\(_2\) [M+H\(^+\)] 351.0662, found 351.0655.

6-(4-Bromobenzyl)-3,3-dimethyl-1,6-dihydro-2\(\text{H}\)-pyrrolo[2,3-\(c\)]pyridine-2,5(3\(\text{H}\))-dione (216)

Prepared according to general procedure B using compound 192 (80 mg, 0.27 mmol), 1-bromo-4-(bromomethyl)benzene (135 mg, 0.54 mmol) and MeCN (2 mL). Purification by MPLC on
SiO$_2$ (DCM:MeOH, 0-7%) gave a white solid (73 mg, 78%). $R_f$=0.32 (5% MeOH/DCM); m.p. 269-271 °C; $\lambda_{\text{max}}$ (EtOH)/nm 339.4, 259.4, 221.6; IR $\nu_{\text{max}}$/cm$^{-1}$ 3025, 2965, 2928, 2860, 2734, 1705 (C=O), 1545 (C=O); $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta_H$ 1.24 (6H, s, 2×CH$_3$), 5.02 (2H, s, CH$_2$), 6.55 (1H, s, 1×Pyridone-$H$), 7.22 (1H, s, 1×Pyridone-$H$), 7.26-7.28 (2H, m, 2×Ar-$H$), 7.53-7.55 (2H, m, 2×Ar-$H$), 10.27 (1H, s, NH); $^{13}$C NMR (125 MHz, DMSO-$d_6$) $\delta_C$ 24.0 (2×CH$_3$), 44.0 (C(CH$_3$)$_2$), 50.7 (NCH$_2$Ar), 114.8 (CH-Pyridine), 115.7 (CH-Pyridone), 121.1 (C-Ar), 123.9 (C-Ar), 130.5 (2×C-Ar), 131.9 (2×C-Ar), 137.7 (C-Ar), 154.2 (C-Ar), 160.6 (C=O pyridone), 180.2 (C=O pyrrolidinone); HRMS calcd for C$_{16}$H$_{16}$BrN$_2$O$_2$ [M($^{79}$Br)+H]$^+$ 347.0390, found 347.0390.

6-(4-bromobenzyl)-1,3,3-trimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (217)

![Chemical structure](image)

Prepared according to general procedure A using compound 216 (50 mg, 0.14 mmol), Cs$_2$CO$_3$ (137 mg, 0.42 mmol), MeI (22 μL, 0.35 mmol) and DMF (1.4 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-5%) gave a white solid (48 mg, 95%). $R_f$=0.40 (5% MeOH/DCM); m.p. 198-200 °C; $\lambda_{\text{max}}$ (EtOH)/nm 338.2, 259.4, 222.4; IR $\nu_{\text{max}}$/cm$^{-1}$ 3063, 3019, 2977, 2933, 2870, 1715 (C=O), 1598 (C=O); $^1$H NMR (500 MHz, CD$_3$OD) $\delta_H$ 1.26 (6H, s, 2×CH$_3$), 3.01 (1H, s, N-CH$_3$), 5.04 (2H, s, CH$_2$), 6.50 (1H, s, 1×Pyridone-$H$), 7.16-7.18 (2H, m, 2×Ar-$H$), 7.31 (1H, s, 1×Pyridone-$H$), 7.38-7.40 (2H, m, 2×Ar-$H$); $^{13}$C NMR (125 MHz, CD$_3$OD) $\delta_C$ 22.6 (2×CH$_3$), 25.4 (N-CH$_3$), 44.1 (C(CH$_3$)$_2$), 51.7 (NCH$_2$Ar), 113.6 (CH-Pyridine), 115.1 (CH-Pyridone), 121.3 (C-Ar), 127.6 (C-Ar), 129.5 (2×C-Ar), 131.4 (2×C-Ar), 136.0 (C-Ar), 153.5 (C-Ar), 162.1 (C=O pyridone), 179.0 (C=O pyrrolidinone); HRMS calcd for C$_{17}$H$_{17}$BrN$_2$O$_2$ [M($^{79}$Br)+H]$^+$ 361.0546, found 361.0544.
3,3-Dimethyl-6-(4-(trifluoromethyl)benzyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (218)

![Chemical structure](image)

Prepared according to general procedure B using compound 192 (80 mg, 0.27 mmol), 1-(bromomethyl)-4-(trifluoromethyl)benzene (129 mg, 0.54 mmol) and MeCN (2 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-7%) gave a white solid (67 mg, 71%). Rᵣ = 0.30 (5% MeOH/DCM); m.p. 275-277 °C; λ_max (EtOH)/nm 338.0, 257.0, 217.4; IR ν_max/cm⁻¹ 3073, 3008, 2973, 2932, 2867, 2735, 1716 (C=O), 1562 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δ_H 1.25 (6H, s, 2×CH₃), 5.13 (2H, s, CH₂), 6.57 (1H, s, 1×Pyridone-H), 7.26 (1H, s, 1×Pyridone-H), 7.49 (2H, d, J = 8.1 Hz, 2×Ar-H), 7.72 (2H, d, J = 8.1 Hz, 2×Ar-H); ¹³C NMR (125 MHz, DMSO-d₆) δ_C 24.0 (2×CH₃), 44.1 (CH(CH₃)₂), 51.1 (NCH₂Ar), 114.8 (CH-Pyridone), 115.9 (CH-Pyridone), 124.0 (C-Ar), 124.7 (CF₃, q, J = 127.9 Hz), 125.9 (2×CF₃CH-CH, q, J = 3.6 Hz), 128.4 (CF₃C-Ar, q, J = 31.9 Hz), 128.8 (2×CF₃CHCH-CH, Ar), 143.0 (2×C-Ar), 154.4 (C-Ar), 160.7 (C=O pyridone), 180.2 (C=O pyrrolidinone); ¹⁹F NMR (470 MHz, DMSO-d₆) δ_-60.92 (3F, s, CF₃); HRMS calcd for C₁₇H₁₆F₃N₂O₂ [M+H]+ 337.1158, found 337.1155.

1,3,3-Trimethyl-6-(4-(trifluoromethyl)benzyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (219)

![Chemical structure](image)

Prepared according to general procedure A using compound 218 (42 mg, 0.13 mmol), Cs₂CO₃ (122 mg, 0.38 mmol), MeI (20 μL, 0.31 mmol) and DMF (1.2 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a white solid (40 mg, 92%). Rᵣ = 0.40 (5% MeOH/DCM); m.p. 172-174 °C; λ_max (EtOH)/nm 338.8, 258.8, 218.8; IR ν_max/cm⁻¹ 3035, 2970, 2928, 2865, 1706 (C=O), 1580 (C=O); ¹H NMR (500 MHz, CD₂OD) δ_H 1.27 (6H, s, 2×CH₃), 3.02 (1H, s, N-C₃H₇), 5.16 (2H, s, CH₂), 6.52 (1H, s, 1×Pyridone-H), 7.33 (1H, s, 1×Pyridone-H), 7.41 (2H, d, J = 8.1 Hz, 2×Ar-H), 7.54 (2H, d, J = 8.1 Hz, 2×Ar-H); ¹³C NMR (125 MHz, CD₂OD) δ_C 22.6 (2×CH₃), 25.4 (N-CH₃), 44.1 (CH(CH₃)₂), 51.9 (NCH₂Ar), 113.7 (CH-Pyridone), 115.2 (CH-Pyridone), 125.2 (2×CF₃CH-CH, q, J = 3.6 Hz), 127.6 (C-Ar), 127.9 (2×CF₃CHCH-CH, Ar), 129.6 (CF₃C-Ar, q, J = 32.2 Hz), 141.2 (C-Ar), 153.7 (C-Ar), 162.2 (C=O pyridone), 179.0
(C=O pyrrolidinone), CF₃ quartet was not observed; ¹⁹F NMR (470 MHz, CD₃OD) δ -64.10 (3F, s, CF₃); HRMS calcd for C₁₈H₁₈F₃N₂O₂ [M+H]⁺ 551.1310, found 551.1297.

3,3-Dimethyl-6-(4-methylbenzyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (220)

Prepared according to general procedure B using compound 192 (80 mg, 0.27 mmol), 1-(bromomethyl)-4-methylbenzene (75 mg, 0.54 mmol) and MeCN (2.7 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-7%) gave a white solid (66 mg, 85%). Rₓ = 0.30 (5% MeOH/DCM); m.p. 252-254 °C; λₓₓ (EtOH)/nm 339.4, 256.8, 217.6; IR νₓₓ/cm⁻¹ 3021, 2968, 2922, 2863, 2736, 1719 (C=O), 1586 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.24 (6H, s, 2×CH₃), 2.27 (3H, s, CH₃-Ar), 5.00 (2H, s, CH₂), 6.53 (1H, s, 1×Pyridone-H), 7.12-7.15 (2H, m, 2×Ar-H), 7.20-7.22 (2H, m, 2×Ar-H), 10.18 (1H, s, NH); ¹³C NMR (125 MHz, DMSO-d₆) δC 21.2 (CH₃-Ar), 24.0 (2×CH₃), 44.0 (C(CH₃)₂), 50.8 (NCH₂Ar), 114.7 (CH-Pyridone), 115.6 (CH-Pyridone), 123.7 (C-Ar), 128.4 (2×C-Ar), 129.6 (2×C-Ar), 135.3 (C-Ar), 137.2 (C-Ar), 153.9 (C-Ar), 160.6 (C=O pyridone), 180.2 (C=O pyrrolidinone); HRMS calcd for C₁₇H₁₉N₂O₂ [M+H]⁺ 283.1441, found 283.1440.

1,3,3-Trimethyl-6-(4-methylbenzyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (221)

Prepared according to general procedure A using compound 220 (50 mg, 0.18 mmol), Cs₂CO₃ (173 mg, 0.53 mmol), MeI (22 μL, 0.35 mmol) and DMF (1.7 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a white solid (48 mg, 92%). Rₓ = 0.42 (5% MeOH/DCM); m.p. 180-182 °C; λₓₓ (EtOH)/nm 337.8, 256.0, 218.2; IR νₓₓ/cm⁻¹ 3056, 2973, 2928, 2865, 1713 (C=O), 1601 (C=O); ¹H NMR (500 MHz, CD₃OD) δH 1.25 (6H, s, 2×CH₃), 2.20 (3H, s, CH₃-Ar), 3.00 (1H, s, N-CH₃), 5.04 (2H, s, CH₂), 6.49 (1H, d, J = 0.3 Hz, 1×Pyridone-H), 7.04-7.06 (2H, m, 2×Ar-H), 7.12-7.14 (2H, m, 2×Ar-H), 7.24 (1H, d, J = 0.3 Hz, 1×Pyridone-H); ¹³C
NMR (125 MHz, CD$_3$OD) δ: 21.1 (CH$_3$-Ar), 24.1 (2×CH$_3$), 26.8 (N-CH$_3$), 45.5 (C(CH$_3$)$_2$), 53.3 (NCH$_2$Ar), 115.0 (CH-Pyridone), 116.6 (CH-Pyridone), 128.9 (C-Ar), 129.0 (2×C-Ar), 130.4 (2×C-Ar), 135.2 (C-Ar), 138.9 (C-Ar), 154.7 (C-Ar), 163.6 (C=O pyridone), 180.5 (C=O pyrrolidinone); HRMS calcd for C$_{18}$H$_{21}$N$_2$O$_2$ [M+H]$^+$ 297.1598, found 297.1596.

6-(3,4-Dichlorobenzyl)-3,3-dimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (228)

Prepared according to general procedure B using compound 192 (80 mg, 0.27 mmol), 4-(bromomethyl)-1,2-dichlorobenzene (132 mg, 0.54 mmol) and MeCN (2 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-7%) gave a white solid (85 mg, 93%). R$_f$ = 0.39 (5% MeOH/DCM); m.p. 248-250°C; λ$_{max}$ (EtOH)/nm 339.0, 256.8, 220.0; IR ν$_{max}$/cm$^{-1}$ 2961, 2870, 2789, 2729; 1H NMR (500 MHz, DMSO-d$_6$) δ: 1.25 (6H, s, 2×CH$_3$), 5.04 (2H, s, C(CH$_3$)$_2$), 6.55 (1H, s, 1×Pyridone-H), 7.27 (1H, s, 1×Pyridone-H), 7.30 (1H, dd, J = 8.2 and 2.0 Hz, 1×Ar-H), 7.60-7.63 (2H, m, 2×Ar-H), 10.27 (1H, s, NH); 13C NMR (125 MHz, DMSO-d$_6$) δ: 24.0 (2×CH$_3$), 44.0 (C(CH$_3$)$_2$), 50.4 (NCH$_2$Ar), 114.8 (CH-Pyridone), 115.7 (CH-Pyridone), 124.1 (C-Ar), 128.8 (C-Ar), 130.5 (C-Ar), 131.3 (C-Ar), 131.4 (C-Ar), 139.4 (2×C-Ar), 154.4 (C-Ar), 160.6 (C=O pyridone), 180.2 (C=O pyrrolidinone); HRMS calcd for C$_{18}$H$_{15}$Cl$_2$N$_2$O$_2$ [M($^{35}$Cl)$_2$]+H]$^+$ 337.0505, found 337.0505.

6-(3,4-Dichlorobenzyl)-1,3,3-trimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (229)

Prepared according to general procedure A using compound 228 (50 mg, 0.15 mmol), Cs$_2$CO$_3$ (145 mg, 0.44 mmol), MeI (23 μL, 0.37 mmol) and DMF (1.5 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-5%) gave a white solid (49 mg, 94%). R$_f$ = 0.41 (5% MeOH/DCM); m.p. 151-153 °C; λ$_{max}$ (EtOH)/nm 340.2, 259.6, 220.6; IR ν$_{max}$/cm$^{-1}$ 3063, 3022, 2974, 2929, 2866, 1712 (C=O), 1595 (C=O); 1H NMR (500 MHz, CD$_3$OD) δ: 1.26 (6H, s, 2×CH$_3$), 3.02 (1H, s, 2×CH$_3$), 3.02 (1H, s,
N-CH₃), 5.04 (2H, s, CH₂), 6.50 (1H, s, 1×Pyridone-H), 7.18 (1H, dd, J = 8.3 and 2.0 Hz, Ar-H), 7.33 (1H, s, 1×Pyridone-H), 7.39 (1H, d, J = 8.3 Hz, Ar-H), 7.44 (1H, d, J = 2.0 Hz, Ar-H); 

¹³C NMR (125 MHz, CD₃OD) δC 24.0 (2×CH₃), 26.9 (N-C-CH₃), 45.5 (C(CH₃)₂), 52.7 (NCH₂Ar), 115.1 (CH-Pyridone), 116.5 (CH-Pyridone), 128.9 (C-Ar), 129.1 (C-Ar), 131.1 (C-Ar), 131.8 (C-Ar), 132.8 (C-Ar), 133.5 (C-Ar), 138.9 (C-Ar), 155.1 (C-Ar), 163.5 (C=O pyridone), 180.4 (C=O pyrrolidinone); HRMS calcd for C₁₁H₁₇Cl₂N₂O₂ [M+H]+ 351.0662, found 351.0661.

3-Benzylidene-5-methoxy-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one (231)

Prepared according to general procedure C using compound 191 (250 mg, 0.95 mmol), benzaldehyde (0.10 mL, 1.04 mmol), piperidine (0.29 mL, 2.93 mmol) and THF (2 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-10%) gave an orange solid (210 mg, 88%, E:Z = 1:4 by ¹H NMR). Rf = 0.36 (5% MeOH/DCM); m.p. 221-224 °C; λmax (EtOH)/nm 323.2, 260.6; IR νmax/cm⁻¹ 3161, 2978, 2938, 2345, 1694 (C=O); 

¹H NMR (500 MHz, DMSO-d₆) δH 3.76 (3H, s, OCH₃, Z-isomer), 3.83 (3H, s, OCH₃, E-isomer), 6.84 (1H, s, 1×Pyridine-H, Z-isomer), 7.26 (1H, s, 1×Pyridine-H, E-isomer), 7.50-7.59 (3H, m 3×Ar-H, E and Z-isomers), 7.64 (1H, s, 1×Pyridine-H, E-isomer), 7.71-7.74 (3H, m, 2×Ar-H + 1×Pyridine-H, Z-isomer), 7.90 (1H, s, CHAr, Z-isomer), 8.09 (1H, s, CHAr, E-isomer), 8.43-8.45 (2H, m, 2×Ar-H, E-isomer), 10.67 (1H, s, NH, E and Z-isomers); 

¹³C NMR (125 MHz, DMSO-d₆) δC 53.7 (OCH₃, Z-isomer), 53.7 (OCH₃, E-isomer), 101.8 (CH-Pyridine, E-isomer), 104.0 (CH-Pyridine, Z-isomer), 125.5 (CH-Pyridine, E-isomer), 125.8 (C-Ar), 126.4 (CH-Pyridine, Z-isomer), 126.9 (C-Ar), 128.8 (C-Ar), 129.4 (C-Ar), 130.0 (C-Ar), 131.1 (C-Ar), 132.0 (C-Ar), 132.4 (C-Ar), 132.8 (C-Ar), 133.1 (C-Ar), 133.8 (C-Ar), 134.2 (C-Ar), 137.7 (C-Ar), 142.1 (CH-Ar, Z-isomer), 142.3 (CHAr, E-isomer), 159.4 (C-Ar), 159.7 (C-Ar), 167.0 (C=O, E-isomer), 168.2 (C=O, Z-isomer); HRMS calcd for C₁₅H₁₃N₂O₂ [M+H]+ 551.1310, found 551.1297.
3-Benzyl-5-methoxy-1,3-dimethyl-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one (235)

Prepared according to general procedure D using compound 231 (122 mg, 0.483 mmol), 10% Pd/C (40 mg), THF (18 mL) and MeOH (9 mL) in step A and Cs₂CO₃ (315 mg, 0.97 mmol), MeI (55 μL, 0.88 mmol), DMF (7.5 mL) in Step B. Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a colourless sticky solid (75 mg, 55%). Rᵣ=0.70 (5% MeOH/DCM); m.p. 73-76 °C; λₘₐₓ (EtOH)/nm 305.0, 251.0; IR νₘₐₓ/cm⁻¹ 2927, 2655, 1708 (C=O); ¹H NMR (500 MHz, CD₃Cl) δ H 1.45 (3H, s, C₃H₃), 2.97 (1H, d, J = 13.2 Hz, C₂H₂Ar), 3.00 (3H, s, N-C₃H₃), 3.14 (1H, d, J = 13.2 Hz, CH₂Ar), 3.89 (3H, s, OCH₃), 6.55 (1H, d, J = 0.6 Hz, 1×Pyridine-H), 6.88-6.90 (2H, m, 2×Ar-H), 7.09-6.10 (3H, m, 3×Ar-H), 7.41 (1H, d, J = 0.6 Hz, 1×Pyridine-H); ¹³C NMR (125 MHz, CD₃Cl) δC 22.6 (2×C₃H₃), 26.2 (N-C₃H₃), 44.0 (CH₂Ar), 50.3 (C(CH₃)₂CH₂Ar), 53.7 (OCH₃), 106.8 (CH-Pyridine), 123.5 (CH-Pyridine), 126.8 (C-Ar), 127.8 (2×C-Ar), 129.8 (2×C-Ar), 134.8 (C-Ar), 135.4 (C-Ar), 146.5 (C-Ar), 160.2 (C=O pyridone), 178.4 (C=O pyrrolidinone); HRMS calcd for C₁₇H₁₈N₂O₂ [M+H]⁺ 253.0972, found 253.0971.

3-Benzyl-6-(4-chlorobenzyl)-1,3-dimethyl-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (237)

Prepared according to general procedure B using compound 235 (60 mg, 0.21 mmol), 1-(bromomethyl)-4-chlorobenzene (87 mg, 0.42 mmol) and MeCN (2 mL). Reaction time: 30 min. Purification by MPLC on SiO₂ (DCM:MeOH, 0-6%) gave a white solid (53 mg, 64%). Rᵣ= 0.45 (5% MeOH/DCM); m.p. 175-178 °C; λₘₐₓ (EtOH)/nm 340.4, 260.0; IR νₘₐₓ/cm⁻¹ 3049, 2971, 2926, 1718 (C=O), 1594 (C=O); ¹H NMR (500 MHz, CD₃Cl) δ H 1.48 (3H, s, CH₃), 2.81 (3H, s, N-C₃H₃), 2.90 (1H, d, J = 13.1 Hz, CH₂Ar), 3.22 (1H, d, J = 13.1 Hz, CH₂Ar), 5.01 (1H,
d, \( J = 14.9 \) Hz, 1×4-Chlorophenyl-\( CH_2 \), 5.06 (1H, d, \( J = 14.9 \) Hz, 1×4-Chlorophenyl-\( CH_2 \)), 6.33 (1H, s, 1×Pyridone-\( H \)), 6.58 (1H, s, 1×Pyridone-\( H \)), 6.95-6.96 (2H, m, 2×4-Chlorophenyl-\( H \)), 7.09-7.12 (5H, m, 5×Ph-\( H \)), 7.29-7.31 (2H, m, 2×4-Chlorophenyl-\( H \)); \(^{13}\)C NMR (125 MHz, CD\(_3\)Cl) \( \delta C \) 22.6 (2×\( CH_3 \)), 26.2 (N-\( CH_3 \)), 44.7 (CH\(_2\)Ar), 50.3 (C(CH\(_3\))CH\(_2\)Ar), 51.6 (4-Chlorophenyl-\( CH_2 \)N), 112.4 (CH-Pyridone), 116.3 (CH-Pyridone), 127.1 (C-Ar), 127.6 (C-Ar), 128.0 (2×C-Ar), 129.1 (2×C-Ar), 129.2 (2×C-Ar), 129.9 (2×C-Ar), 134.0 (C-Ar), 135.0 (C-Ar), 135.4 (C-Ar), 150.5 (C-Ar), 161.3 (C=O pyridone), 177.0 (C=O pyrrolidinone); HRMS calcd for C\(_{23}\)H\(_{22}\)ClN\(_2\)O\(_2\) [M+H]\(^+\) 393.1364, found 393.1359.

5-Methoxy-3-(pyridin-2-ylmethylene)-1,3-dihydro-2\( H \)-pyrrolo[2,3-c]pyridin-2-one (232)

Prepared according to general procedure C using compound 191 (250 mg, 0.95 mmol), picolinaldehyde (99 \( \mu \)L, 1.04 mmol), piperidine (291 \( \mu \)L, 2.95 mmol) and THF (2 mL). Purification by MPLC on SiO\(_2\) (DCM:MeOH, 0-10%) gave an orange solid (120 mg, 50%). \( R_f = 0.39 \) (5% MeOH/DCM); m.p. 217-230 °C; \( \lambda_{max} \) (EtOH)/nm 333.2, 263.6, 210.2; IR \( \nu_{max}/cm^-1 \) 3163, 3105, 2992, 2943, 2851, 1717 (C=O); \(^1\)H NMR (500 MHz, DMSO-\( d_6 \)) \( \delta H \) 3.83 (3H, s, OCH\(_3\)), 7.52-7.55 (1H, m, Pyridine-\( H \)), 7.71 (1H, s, 1×Methoxypyridine-\( H \)), 7.80 (1H, s, CHAr), 7.97-8.02 (2H, m, 2×Pyridine-\( H \)), 8.47 (1H, s, 1×Methoxypyridine-\( H \)), 8.93-8.95 (2H, m, 2×Ar-\( H \)), 10.68 (1H, s, NH); \(^{13}\)C NMR (125 MHz, DMSO-\( d_6 \)) \( \delta C \) 53.7 (OCH\(_3\)), 109.6 (CH-Methoxypyridine), 125.5 (CH-Methoxypyridine), 125.8 (C-Ar), 128.3 (C-Ar), 130.1 (C-Ar), 133.1 (C=CH), 134.2 (C-Ar), 138.0 (C-Ar), 139.4 (C=CH), 150.3 (C-Ar), 152.9 (C-Ar), 160.0 (C-Ar), 168.8 (C=O); HRMS calcd for C\(_{14}\)H\(_{11}\)N\(_3\)O\(_2\) [M+H]\(^+\) 254.0924, found 254.0927.
5-Methoxy-1,3-dimethyl-3-(pyridin-2-ylmethyl)-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one (236)

Prepared according to general procedure D using compound 232 (95 mg, 0.37 mmol), 10% Pd/C (30 mg), THF (12 mL) and MeOH (6 mL) in step A and Cs₂CO₃ (257 mg, 0.79 mmol), Mel (47 μL, 0.75 mmol), DMF (4 mL) in Step B. Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a beige solid (60 mg, 56%). Rᶠ = 0.48 (5% MeOH/DCM); m.p. 105-108 °C; λₓₓₓ(ETOH)/nm 306.2, 252.2, 205.0; IR νₓₓₓ/cm⁻¹ 3005, 3009, 2976, 2937, 1700 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 1.46 (3H, s, CH₃), 3.14 (3H, s, N-CH₃), 3.22 (1H, d, J = 13.8 Hz, CH₂Ar), 3.41 (1H, d, J = 13.8 Hz, CH₂Ar), 3.85 (3H, s, OC₃H₃), 6.54 (1H, s, Methoxypyridine-H), 6.98-7.03 (2H, m, 2×Ar-H), 7.45-7.49 (2H, m, 2×Ar-H); ¹³C NMR (125 MHz, CDCl₃) δC 23.4 (2×CH₃), 26.5 (N-CH₃), 45.0 (CH₂Ar), 48.9 (CH₃CH₂Ar), 53.5 (OCH₃), 106.9 (CH-Methoxypyridine), 121.7 (C-Ar), 123.5 (CH-Methoxypyridine), 123.8 (C-Ar), 134.9 (C-Ar), 136.1 (C-Ar), 144.6 (C-Ar), 148.7 (C-Ar), 156.3 (C-Ar), 160.3 (C-Ar), 178.8 (C=O); HRMS calcd for C₁₆H₁₈N₃O₂ [M+H]⁺ 284.1394, found 284.1393.

6-(4-Chlorobenzyl)-1,3-dimethyl-3-(pyridin-2-ylmethyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (238)

Prepared according to general procedure B using compound 236 (46 mg, 0.16 mmol), 1-(bromomethyl)-4-chlorobenzene (67 mg, 0.32 mmol) and MeCN (1 mL). Reaction time: 30 min. Purification by MPLC on SiO₂ (DCM:MeOH, 0-6%) gave a brown solid (32 mg, 50%). Rᶠ = 0.30 (5% MeOH/DCM); m.p. 182-185 °C; λₓₓₓ(ETOH)/nm 340.6, 260.8, 222.0; IR νₓₓₓ/cm⁻¹ 3187, 3044, 2966, 2927, 1711 (C=O), 1586 (C=O); ¹H NMR (500 MHz, CD₃OD) δH 1.48 (3H, s, CH₃), 3.02 (3H, s, N-CH₃), 3.26 (1H, d, J = 14.1 Hz, CH₂-Pyridine), 3.41 (1H, d,
\[ J = 14.1 \text{ Hz}, \ CH_2-\text{Pyridine}, \] 5.04 (1H, d, \( J = 14.8 \text{ Hz}, \) 1×4-Chlorophenyl-\( CH_2 \)), 5.12 (1H, d, \( J = 14.8 \text{ Hz}, \) 1×4-Chlorophenyl-\( CH_2 \)), 6.51 (1H, s, 1×Pyridone-H), 7.09-7.14 (2H, m, 2×Pyridine-H), 7.16 (1H, s, 1×Pyridine-H), 7.18-7.19 (2H, m, 2×4-Chlorophenyl-H), 7.30-7.32 (2H, m, 2×4-Chlorophenyl-H), 7.57 (1H, td, \( J = 11.5 \text{ and } 1.9 \text{ Hz}, \) 1×Pyridine-H), 8.22-8.23 (1H, m, 1×Pyridine-H);

\[ ^{13}\text{C} \text{ NMR (125 MHz, CD}_3\text{OD)} \delta 23.5 (2×C_\text{H}_3), 26.8 (N-\text{C}_\text{H}_3), 45.8 (\text{CH}_2-\text{Pyridine}), 50.0 (\text{C(CH}_3)_2\text{CH}_2\text{Ar}), 52.8 (4-\text{Chlorophenyl-CH}_2\text{N}), 116.0 (\text{CH-Pyridone}), 116.1 (\text{CH-Pyridone}), 123.2 (\text{C-Ar}), 125.3 (\text{C-Ar}), 129.7 (\text{C-Ar}), 129.8 (2×\text{C-Ar}), 130.2 (2×\text{C-Ar}), 134.7 (\text{C-Ar}), 137.0 (\text{C-Ar}), 137.8 (\text{C-Ar}), 149.6 (\text{C-Ar}), 152.6 (\text{C-Ar}), 157.5 (\text{C-Ar}), 163.2 (\text{C}=\text{O pyridone}), 179.5 (\text{C}=\text{O pyrrolidinone}); \text{HRMS calcd for } C_{22}H_{21}ClN_3O_2 [\text{M}(35\text{Cl})+H]^+ 394.1311, \text{ found } 394.1311.

### 3-Methyloxetane-3-carbaldehyde (240)

![3-Methyloxetane-3-carbaldehyde](image)

(3-methyloxetan-3-yl)methanol (0.49 mL, 4.89 mmol) was dissolved in DCM (15 mL) and cooled to 0 °C. Dess-Martin periodinane (3.11 g, 7.34 mmol) in DCM (30 mL) was added and the solution was stirred at 0 °C for 30 min and at r.t. for 3 h. The reaction mixture was poured into a solution of saturated aqueous Na_2S_2O_3 (30 mL) and saturated aqueous NaHCO_3 (30 mL) and stirred for 30 min. The aqueous layer was extracted with DCM (3×40 mL), the organic layers were combined, dried over MgSO_4 and the solvent removed \textit{in vacuo} to get a colourless oil (380 mg, 78%).

\[ ^1\text{H} \text{ NMR (500 MHz, CD}_3\text{Cl)} \delta H 1.47 (3H, s, CH_3), 4.49 (2H, d, J = 6.3 \text{ Hz}, \ CH_2), 4.86 (2H, d, J = 6.3 \text{ Hz}, \ CH_2), 9.94 (1H, s, CHO); ^{13}\text{C} \text{ NMR (125 MHz, CD}_3\text{Cl)} \delta C 17.5 (CH_3), 49.3 (Cq), 76.7 (2×CH_2), 200.6 (C=O).

### 5-Methoxy-3-((3-methyloxetan-3-yl)methylene)-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one (241)

![5-Methoxy-3-((3-methyloxetan-3-yl)methylene)-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one](image)

Prepared according to general procedure C using compound 191 (200 mg, 0.76 mmol), compound 240 (121 mg, 1.21 mmol), piperidine (150 μL, 1.51 mmol) and THF (0.7 mL).
Purification by MPLC on SiO\textsubscript{2} (DCM:MeOH, 0-10%) gave an light-orange solid (120 mg, 50%). R\textsubscript{f} = 0.27 (5% MeOH/DCM); m.p. 154-156 °C; \(\lambda_{\text{max}}\) (EtOH)/nm 255.8; IR \(\nu_{\text{max}}/\text{cm}^{-1}\) 3157, 3069, 3023, 2955, 2855, 1715 (C=O); Ratio of diastereoisomers by \(^1\)H NMR = 3:5; \(^1\)H NMR (500 MHz, DMSO-\text{d}\textsubscript{6}) \(\delta\)H 1.59 (3H, s, CH\textsubscript{3}-Oxetane, isomer 1), 1.60 (3H, s, CH\textsubscript{3}-Oxetane, isomer 2), 3.80 (3H, s, OCH\textsubscript{3}, isomer 1), 3.80 (3H, s, OCH\textsubscript{3}, isomer 2), 4.48 (1H, d, \(J = 6.1\) Hz, 2×Oxetane-H, isomer 1), 4.67-4.69 (4H, m, 2×Oxetane-H, isomer 1 and 2), 4.76 (1H, d, \(J = 5.8\) Hz, 1×Oxetane-H, isomer 2), 6.38 (1H, s, 1×Methoxypyridine-H, isomer 2), 7.06 (1H, s, 1×Methoxypyridine-H, isomer 1), 7.34 (1H, s, C\textsubscript{H}=CCO, isomer 2), 7.60 (1H, s, C\textsubscript{H}=CCO, isomer 2), 7.63 (1H, s, 1×Methoxypyridine-H, isomer 1), 7.70 (1H, s, 1×Methoxypyridine-H, isomer 2), 10.53 (1H, s, NH, isomer 1), 10.62 (1H, s, NH, isomer 2); \(^13\)C-NMR was not be obtained; HRMS calcd for C\textsubscript{13}H\textsubscript{14}N\textsubscript{3}O\textsubscript{2}\ [M+H]\textsuperscript{+} 247.1077, found 247.1080.

5-Methoxy-1,3-dimethyl-3-((3-methylxetan-3-yl)methyl)-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one (243)

Prepared according to general procedure D using compound 241 (58 mg, 0.24 mmol), 10% Pd/C (20 mg), THF (10 mL) and MeOH (5 mL) in step A and Cs\textsubscript{2}CO\textsubscript{3} (176 mg, 0.54 mmol), MeI (30 \(\mu\)L, 0.48 mmol), DMF (4.7 mL) in Step B. Purification by MPLC on SiO\textsubscript{2} (DCM:MeOH, 0-5%) gave an off-white solid (30 mg, 42%). R\textsubscript{f} = 0.40 (5% MeOH/DCM); m.p. 98-101 °C; \(\lambda_{\text{max}}\) (EtOH)/nm 305.6, 249.8, 205.2; IR \(\nu_{\text{max}}/\text{cm}^{-1}\) 3060, 2931, 2866, 1715 (C=O); \(^1\)H NMR (500 MHz, CD\textsubscript{3}Cl) \(\delta\)H 0.96 (3H, s, CH\textsubscript{3}-Oxetane), 1.34 (3H, s, CH\textsubscript{3}), 2.32 (1H, d, \(J = 14.5\) Hz, CH\textsubscript{2}-Oxetane), 2.39 (1H, d, \(J = 14.5\) Hz, CH\textsubscript{2}-Oxetane), 3.20 (3H, s, N-CH\textsubscript{3}), 3.78 (1H, d, \(J = 5.8\) Hz, 1×Oxetane-H\textsubscript{2}), 3.90 (3H, s, OCH\textsubscript{3}), 4.05 (1H, d, \(J = 5.8\) Hz, 1×Oxetane-H\textsubscript{2}), 4.37 (1H, d, \(J = 5.8\) Hz, 1×Oxetane-H\textsubscript{2}), 4.46 (1H, d, \(J = 5.8\) Hz, 1×Oxetane-H\textsubscript{2}), 6.64 (1H, s, 1×Methoxypyridine-H), 7.65 (1H, s, 1×Methoxypyridine-H); \(^13\)C-NMR (125 MHz, CD\textsubscript{3}Cl) \(\delta\)C 22.9 (CH\textsubscript{3}-Oxetane), 26.6 (N-CH\textsubscript{3}), 27.0 (CH\textsubscript{3}), 39.2 (C-Oxetane), 45.5 (Pyrrolidinone-CH\textsubscript{2}-Oxetane), 47.3 (C(CH\textsubscript{3})\textsubscript{2}Oxetane), 53.7 (OCH\textsubscript{3}), 83.5 (CH\textsubscript{2}-Oxetane), 83.8 (CH\textsubscript{2}-Oxetane), 106.8 (CH-Methoxypyridine), 124.1 (CH-Methoxypyridine), 134.6 (C-Ar), 146.6 (C-Ar), 160.4 (C-Ar), 178.7 (C=O); HRMS calcd for C\textsubscript{15}H\textsubscript{21}N\textsubscript{3}O\textsubscript{2}\ [M+H]\textsuperscript{+} 277.1547, found 277.1544.
6-(4-Chlorobenzyl)-1,3-dimethyl-3-((3-methyloxetan-3-yl)methyl)-1,6-dihydro-2H-pyrrolo[2,3-c]pyridine-2,5(3H)-dione (244)

Prepared according to general procedure B using compound 243 (18 mg, 0.065 mmol), 1-(bromomethyl)-4-chlorobenzene (27 mg, 0.13 mmol) and MeCN (1 mL). Reaction time: 45 min. Purification by MPLC on SiO₂ (DCM:MeOH, 0-6%) gave an off-white solid (21 mg, 83%). Rᵣ = 0.32 (5% MeOH/DCM); m.p. 167-170 °C; IR νₘₐₓ/cm⁻¹ 3049, 2964, 2923, 2863, 1709 (C=O) 1598 (C=O); ¹H NMR (500 MHz, CD₃Cl) δ 1.05 (3H, s, C₃H₃-Oxetane), 1.35 (3H, s, C₃H₃), 2.22 (1H, d, J = 14.5 Hz, CH₂-Oxetane), 2.40 (1H, d, J = 14.5 Hz, CH₂-Oxetane), 3.04 (3H, s, N-C₃H₃), 3.93 (1H, d, J = 5.8 Hz, 1×Oxetane-H₂), 4.05 (1H, d, J = 5.8 Hz, 1×Oxetane-H₂), 4.44 (1H, d, J = 5.8 Hz, 1×Oxetane-H₂), 4.48 (1H, d, J = 5.8 Hz, 1×Oxetane-H₂), 4.98 (1H, d, J = 14.6 Hz, 1×4-Chlorophenyl-CH₂), 5.21 (1H, d, J = 14.6 Hz, 1×4-Chlorophenyl-CH₂), 6.54 (1H, s, 1×Pyridone-H), 6.64 (1H, s, 1×Pyridone-H), 7.23-7.24 (2H, m, 2×4-Chlorophenyl-H₂), 7.31-7.33 (2H, m, 2×4-Chlorophenyl-H₂); ¹³C NMR (125 MHz, CD₃Cl) δc 23.4 (CH₃-Oxetane), 26.7 (N-CH₃), 27.1 (CH₃), 39.4 (C-Oxetane), 45.7 (Pyrrolidinone-CH₂-Oxetane), 46.9 (C(CH₃)CH₂-Oxetane), 51.7 (NCH₂-Ar), 83.6 (CH₂-Oxetane), 83.6 (CH₂-Oxetane), 113.0 (CH-Methoxypyridine), 116.3 (CH-Methoxypyridine), 127.0 (C-Ar), 129.3 (2×C-Ar), 129.5 (2×C-Ar), 134.3 (C-Ar), 135.0 (C-Ar), 150.6 (C-Ar), 161.1 (C-Ar), 177.3 (C=O); HRMS calcd for C₂₁H₂₄ClN₂O₃ [M+(3Cl)+H]⁺ 387.1470, found 387.1465.

Tert-butyl 5'-methoxy-2'-oxospiro[cyclopropane-1,3'-pyrrolo[2,3-c]pyridine]-1'(2'H)-carboxylate (260)

Compound 191 (600 mg, 2.27 mmol) and Cs₂CO₃ (1.62 g, 4.99 mmol) were suspended in MeCN (15 mL). 1,2-dibromoethane (0.22 mL, 2.50 mmol) was added and the mixture was stirred at 40 °C for 3.5 h. The starting material was not completely consumed, therefore, 1,2-
dibromoethane (50 μL, 0.57 mmol) was added and stirred at 40 °C for 1.5 h. The reaction mixture was filtered using EtOAc (3×30 mL) and the filtrate was evaporated in vacuo. Water (30 mL) was added to the residue and the aqueous layer was extracted with EtOAc (3×25 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-10%) gave a white solid (360 mg, 55%). Rₕ = 0.75 (5% MeOH/DCM); m.p. 146-149 °C; λ_max (EtOH)/nm 293.8; IR ν_max/cm⁻¹ 3085, 2977, 1767 (C=O), 1720 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 1.59 (2H, q, J = 4.2 Hz, CH₂-spirocycle), 1.65 (9H, s, C(CH₃)₃), 1.92 (2H, q, J = 4.2 Hz, CH₂-spirocycle), 3.92 (3H, s, OC₃H₃), 6.23 (1H, d, J = 0.6 Hz, 1×Pyridine-H), 8.55 (1H, d, J = 0.6 Hz, 1×Pyridine-H); ¹³C NMR (125 MHz, CDCl₃) δC 22.5 (C₂H₂-spirocycle), 27.8 (C(CH₃)₃), 28.1 (C(CH₃)₃), 53.7 (OCH₃), 84.7 (C(CH₃)₃), 100.3 (CH-Pyridine), 131.5 (C-Pyridine), 131.6 (CH-Pyridine), 143.1 (C-Pyridine), 148.7 (COO'Bu), 160.8 (Pyridine-C-OMe), 174.3 (C=O pyrrolidinone); HRMS calcd for C₁₅H₁₉N₂O₄ [M+H]⁺ 291.1339, found 291.1337.

6'-4-Chlorobenzyl)-1',6'-dihydrospiro[cyclopropane-1,3'-pyrrolo[2,3-c]pyridine]-2',5'-dione (264)

Prepared according to general procedure B using compound 260 (100 mg, 0.34 mmol), 1-(bromomethyl)-4-chlorobenzene (141 mg, 0.69 mmol) and MeCN (3.4 mL). Reaction time: 45 min. Purification by MPLC on SiO₂ (DCM:MeOH, 0-10%) gave a white solid (70 mg, 68%). Rₕ = 0.24 (5% MeOH/DCM); m.p. 298 °C; λ_max (EtOH)/nm 339.4, 230.6; IR ν_max/cm⁻¹ 3073, 3026, 2997, 2936, 2876, 2735, 1714 (C=O), 1554 (C=O); ¹H NMR (500 MHz, DMSO-d₆) δH 1.53-1.61 (4H, m, 2×CH₂), 5.06 (2H, s, CH₂-Ar), 6.19 (1H, s, 1×Pyridone-H), 7.25 (1H, s, 1×Pyridone-H), 7.28-7.30 (2H, m, 2×Ar-H), 7.39-7.41 (2H, m, 2×Ar-H), 10.42 (1H, s, NH); ¹³C NMR (125 MHz, DMSO-d₆) δC 20.5 (2×CH₂), 27.1 (C(CH₂)₂), 50.7 (NCH₂Ar), 109.8 (CH-Pyridone), 115.7 (CH-Pyridone), 124.8 (C-Ar), 128.9 (2×C-Ar), 130.0 (2×C-Ar), 132.4 (C-Ar), 137.4 (C-Ar), 150.1 (C-Ar), 160.3 (C=O pyridone), 176.0 (C=O pyrrolidinone); HRMS calcd for C₁₆H₁₄ClN₂O₂ [M(³⁵Cl)+H]⁺ 301.0738, found 301.0738.
6′-(4-Chlorobenzyl)-1′-methyl-1′,6′-dihydrospiro[cyclopropane-1,3′-pyrrolo[2,3-c]pyridine]-2′,5′-dione (265)

Prepared according to general procedure A using compound 264 (60 mg, 0.199 mmol), Cs₂CO₃ (130 mg, 0.398 mmol), Mel (25 μL, 0.398 mmol) and DMF (4 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a white solid (50 mg, 80%). Rₐ = 0.32 (5% MeOH/DCM); m.p. 155-157 °C; λₘₐₓ (EtOH)/nm 339.4, 231.2; ¹H NMR (500 MHz, CD₃Cl) δ H 1.51 (2H, q, J = 4.1 Hz, CH₂-spirocyclopropane), 1.83 (2H, q, J = 4.1 Hz, CH₂-spirocyclopropane), 3.12 (1H, s, N-C₃H₃), 5.11 (2H, s, CH₂-Ar), 6.13 (1H, s, 1×Pyridone-H), 6.67 (1H, s, 1×Pyridone-H), 7.23-7.25 (2H, m, 2×Ar-H), 7.30-7.32 (2H, m, 2×Ar-H); ¹³C NMR (125 MHz, CD₃Cl) δ C 21.0 (2×CH₂), 26.9 (N-C₃H₃), 51.6 (N-C₃H₂Ar), 109.9 (CH-Pyridone), 128.0 (C-Ar), 129.2 (2×C-Ar), 129.4 (2×C-Ar), 134.1 (C-Ar), 135.3 (C-Ar), 148.8 (C-Ar), 161.3 (C=O pyridone), 174.3 (C=O pyrrolidinone); HRMS calcd for C₁₇H₁₆ClN₂O₂ [M(³⁵Cl)+H]⁺ 315.0895, found 315.0893.

6′-(4-Chlorobenzyl)-1,1′-dimethyl-1′,6′-dihydrospiro[pyrrolidine-3,3′-pyrrolo[2,3-c]pyridine]-2′,5′-dione (271)

Prepared according to general procedure E using Compound 265 (150 mg, 0.476 mmol), MgI₂ (13 mg, 0.047 mmol), 1,3,5-trimethyl-1,3,5-triazinane (0.17 mL, 1.19 mmol) and THF (1.6 mL). Purification by MPLC on SiO₂ (DCM:MeOH, 0-12%) gave a beige solid (92 mg, 53%). Rₐ = 0.27 (5% MeOH/DCM); m.p. 78-80 °C; λₘₐₓ (EtOH)/nm 339.4, 259.8; IR νₘₐₓ/cm⁻¹ 3047, 2936, 2844, 2783, 1707 (C=O), 1588 (C=O); ¹H NMR (500 MHz, CD₃Cl) δ H 2.00 (1H, dt, J = 12.9, 7.5 Hz, CH-spiropyrrolidine), 2.35-2.40 (1H, m, CH-spiropyrrolidine), 2.40 (3H, s, N-CH₃), 2.65 (1H, dd, J = 16.3 and 8.4 Hz, CH-spiropyrrolidine), 2.78 (2H, s, CH₂-Spiropyrrolidine), 2.99-3.03 (1H, m, CH-spiropyrrolidine), 3.05 (3H, s, N-CH₃), 5.09 (2H, s, CH₂-Ar), 6.59 (1H, s, 1×Pyridone-H), 6.81 (1H, s, 1×Pyridone-H), 7.25-7.27 (2H, m, 2×Ar-H), 7.30-7.33 (2H, m, 2×Ar-H); ¹³C NMR (125 MHz, CD₃Cl) δ C 26.6 (N-CH₃), 37.9 (CH₂-
Spiropyrrolidine), 41.4 (N-CH₃), 51.5 (NCH₂Ar), 53.0 (C-Spiropyrrolidine), 56.0 (CH₂-Spiropyrrolidine), 66.1 (CH₂-Spiropyrrolidine), 112.1 (CH-Pyridone), 115.9 (CH-Pyridone), 127.1 (C-Ar), 129.1 (2×C-Ar), 129.4 (2×C-Ar), 134.0 (C-Ar), 135.1 (C-Ar), 152.7 (C-Ar), 161.7 (C=O pyridone), 177.0 (C=O pyrrolidinone); LRMS (ES⁺) m/z 358.3 [M(35Cl)+H]⁺, 360.3 [M(37Cl)+H]⁺.

*Tert*-butyl 3,3-diallyl-5-methoxy-2-oxo-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate (255)

![Tert-butyl 3,3-diallyl-5-methoxy-2-oxo-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate](image)

Compound 191 (493 mg, 1.865 mmol) and Cs₂CO₃ (1.40 g, 4.289 mmol) were suspended in MeCN (12.5 mL). Allyl bromide (0.4 mL, 4.662 mmol) was added and the mixture was stirred at 40 °C for 2.5 h. The reaction mixture was filtered using EtOAc (3×30 mL) and the filtrate was evaporated *in vacuo*. Water (40 mL) was added to the residue and the aqueous layer was extracted with EtOAc (3×25 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed *in vacuo*. Purification by MPLC on SiO₂ (Petrol:EtOAc, 0-20%) gave a white solid (350 mg, 55%). Rₜ = 0.56 (20% EtOAc/Petrol); m.p. 84-87 °C; λₚₚₚ (EtOH)/nm 295.2, 230.6; IR νₚₚₚ/cm⁻¹ 3084, 2980, 2947, 2908, 1759 (C=O), 1719 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 1.63 (9H, s, C(CH₃)₃), 2.49-2.53 (2H, m, CH₂CH=CH₂), 2.60-2.64 (2H, m, CH₂CH=CH₂), 3.93 (3H, s, OCH₃), 4.98-5.05 (4H, m, 2×CH₂CH=CH₂), 5.40-5.49 (2H, m, 2×CH₂CH=CH₂), 6.61 (1H, d, J = 0.7 Hz, 1×Pyridine-H), 8.51 (1H, d, J = 0.7 Hz, 1×Pyridine-H); ¹³C NMR (125 MHz, CDCl₃) δC 28.1 (C(CH₃)₃), 41.6 (2×CH₂CH=CH₂), 53.4 (C(CH₂CH=CH₂)₂), 53.7 (OCH₃), 84.7 (C(CH₃)₃), 105.7 (CH-Pyridine), 120.2 (2×CH=CH₂), 130.9 (2×CH=CH₂), 131.4 (C-Pyridine), 132.0 (CH-Pyridine), 143.2 (C-Pyridine), 148.5 (COO'Bu), 160.8 (Pyridine-C-OMe), 176.3 (C=O pyrrolidinone); HRMS calcd for C₁₉H₂₅N₂O₄ [M+H]⁺ 345.1809, found 345.1804.
Tert-butyl 5'-methoxy-2'-oxospiro[cyclopentane-1,3'-pyrrolo[2,3-c]pyridin]-3-ene-1'(2'H)-carboxylate (256)

Compound 255 (329 mg, 0.955 mmol) was dissolved in DCM (19.5 mL). Grubbs catalyst 2nd generation (41 mg, 5 mol%) was added and the reaction mixture was stirred at 80 °C under microwave irradiation for 20 min. The solvent was removed in vacuo and the residue was purified by MPLC on SiO$_2$ (Petrol:EtOAc, 0-20%) to get a white solid (272 mg, 90%). R$_f$=0.48 (20% EtOAc/Petrol); m.p. 163-166 °C; $^1$H NMR (500 MHz, CD$_3$Cl) δ 1.65 (9H, s, C(CH$_3$)$_3$), 2.60-2.63 (2H, m, CH$_2$CH=CHC$_2$H$_2$), 3.08-3.11 (2H, m, CH$_2$CH=CHC$_2$H$_2$), 3.91 (3H, s, OC$_2$H$_3$), 5.79 (2H, s, CH$_2$C=CHCH$_2$), 6.68 (1H, d, $J$ = 0.7 Hz, 1×Pyridine-H), 8.51 (1H, d, $J$ = 0.7 Hz, 1×Pyridine-H); $^{13}$C NMR (125 MHz, CDCl$_3$) δC 28.1 (C((CCH$_3$)$_3$)), 46.2 (2×CH$_2$CH=CHC$_2$H$_2$), 52.7 (CH$_2$C=CH), 53.7 (OCH$_3$), 84.8 (C(CH$_3$)$_3$), 103.8 (CH-Pyridine), 128.5 (2×CH=CH), 130.1 (C-Pyridine), 131.9 (CH-Pyridine), 148.4 (C-Pyridine), 148.7 (COO'Bu), 161.4 (Pyridine-C-OMe), 178.7 (C=O pyrrolidinone); HRMS calcd for C$_{17}$H$_{21}$N$_2$O$_4$ [M+H]$^+$ 317.1496, found 317.1493.

6’-(4-Chlorobenzyl)-1’,6’-dihydrospiro[cyclopentane-1,3’-pyrrolo[2,3-c]pyridin]-3-ene-2’,5’-dione (257)

Prepared according to general procedure B using compound 256 (257 mg, 0.81 mmol), 1-(bromomethyl)-4-chlorobenzene (334 mg, 1.63 mmol) and MeCN (5.4 mL). Reaction time: 45 min. Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-10%) gave a white solid (237 mg, 89%). R$_f$ = 0.29 (5% MeOH/DCM); m.p. 274-277 °C; $\lambda_{max}$ (EtOH)/nm 343.0, 258.4, 221.4; IR $\nu_{max}$/cm$^{-1}$ 3021, 2933, 2844, 2792, 2734, 1718 (C=O), 1588 (C=O); $^1$H NMR (500 MHz, DMSO-$d_6$) δH 2.55-2.59 (2H, m, CH$_2$CH=CHCH$_2$), 2.79-2.82 (2H, m, CH$_2$CH=CHCH$_2$), 5.04 (2H, s, CH$_2$-Ar), 5.78 (2H, s, CH$_2$CH=CHCH$_2$), 6.29 (1H, s, 1×Pyridone-H), 7.21 (1H, s, 1×Pyridone-H), 7.31-7.33 (2H, m, 2×Ar-H), 7.39-7.41 (2H, m, 2×Ar-H), 10.31 (1H, s, NH);
$^{13}$C NMR (125 MHz, DMSO-$d_6$) δC 45.2 (2×CH$_2$CH=CHCH$_2$), 50.7 (NCH$_2$Ar), 51.9 (CCH$_2$CH=CH), 113.1 (CH-Pyridone), 115.7 (CH-Pyridone), 124.1 (C-Ar), 129.0 (2×C-Ar), 129.1 (2×CH=CH), 130.2 (2×C-Ar), 132.6 (C-Ar), 137.2 (C-Ar), 155.5 (C=O pyridone), 180.1 (C=O pyrrolidinone); HRMS calcd for C$_{18}$H$_{16}$ClN$_2$O$_2$ [M($^{35}$Cl)+H]$^+$ 327.0895, found 327.0895.

6'-[(4-Chlorobenzyl)-1'-methyl]-1',6'-dihydropyrrolo[cyclopentane-1,3'-pyrrolo[2,3-c]pyridin]-3-ene-2',5'-dione (258)

Prepared according to general procedure A using compound 257 (225 mg, 0.69 mmol), Cs$_2$CO$_3$ (673 mg, 2.07 mmol), MeI (110 μL, 1.72 mmol) and DMF (7 mL). Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-10%) gave an off-white solid (205 mg, 87%). $R_f = 0.48$ (5% MeOH/DCM); m.p. 56-58 °C; $\lambda_{\text{max}}$ (EtOH)/nm 339.6, 261.0, 221.6; IR $\nu_{\text{max}}$/cm$^{-1}$ 3058, 2918, 2844, 1704 (C=O), 1582 (C=O); $^1$H NMR (500 MHz, CD$_3$Cl) δH 2.56-2.62 (2H, m, CH$_2$CH=CHC$_2$H$_2$), 2.98-3.03 (2H, m, CH$_2$CH=CHCH$_2$), 3.06 (3H, s, N-C$_3$H$_3$), 5.08 (2H, s, CH$_2$-Ar), 5.78 (2H, s, CH$_2$CH=CHCH$_2$), 6.60 (1H, s, 1×Pyridone-H), 6.62 (1H, s, 1×Pyridone-H), 7.25-7.27 (2H, m, 2×Ar-H), 7.31-7.33 (2H, m, 2×Ar-H); $^{13}$C NMR (125 MHz, CD$_3$Cl) δC 25.6 (N-C$_3$H$_3$), 44.0 (2×CH$_2$CH=CHCH$_2$), 50.5 (NCH$_2$Ar), 50.9 (CCH$_2$CH=CH), 111.3 (CH-Pyridone), 113.1 (CH-Pyridone), 126.0 (C-Ar), 127.6 (2×CH=CH), 128.1 (2×C-Ar), 128.4 (2×C-Ar), 133.0 (C-Ar), 134.0 (C-Ar), 152.7 (C-Ar), 160.8 (C=O pyridone), 177.2 (C=O pyrrolidinone); HRMS calcd for C$_{19}$H$_{18}$ClN$_2$O$_2$ [M($^{35}$Cl)+H]$^+$ 341.1051, found 341.1050.
(1s,3R,4S)-6’-(4-Chlorobenzyl)-3,4-dihydroxy-1’-methyl-1’,6’-dihydrospiro[cyclopentane-1,3’-pyrrolo[2,3-c]pyridine]-2’,5’-dione (252)

Compound 258 (25 mg, 0.073 mmol) was dissolved in THF:water (3:1, 2.4 mL) and cooled to 0 °C. N-methylmorpholine (12 mg, 0.099 mmol) and 2.5 wt% OsO₄ in ‘BuOH (30 μL, 4 mol%) were added, respectively and the mixture was stirred at r.t. for 18 h. The starting material was not completely consumed, therefore, 2.5 wt% OsO₄ in ‘BuOH (70 μL, 9.3 mol%) was added and the reaction was stirred at r.t. for 24 h. The mixture was quenched with saturated Na₂SO₃ (30 mL) and stirred for 30 min. The aqueous layer was extracted with EtOAc (3×20 mL), the organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-10%) gave a white solid (16 mg, 59%) and other isomer 259 (5 mg, 18%). Rₘ = 0.18 (5% MeOH/DCM); m.p. 68-71 °C; λₘₐₓ (EtOH)/nm 339.4, 260.8, 221.6; IR νₘₐₓ/cm⁻¹ 3355 (OH), 3049, 2925, 2853, 1698 (C=O), 1582 (C=O); ¹H NMR (500 MHz, CD₃Cl) δH 2.17-2.20 (4H, m, 2×C₂H₂CHOH), 3.09 (3H, s, N-C₃H₃), 3.81 (2H, d, J = 9.8 Hz, 2×OH), 4.28-4.33 (2H, m, 2×CHOH), 5.08 (2H, s, CH₂-Ar), 6.52 (1H, s, 1×Pyridone-H), 6.65 (1H, s, 1×Pyridone-H), 7.25-7.27 (2H, m, 2×Ar-H), 7.31-7.33 (2H, m, 2×Ar-H); ¹³C NMR (125 MHz, CD₃Cl) δC 26.5 (N-CH₃), 42.2 (2×CH₂CHOH), 51.1 (NCH₂Ar), 51.2 (CCH₂CHOH), 74.4 (2×CHOH), 112.9 (CH-Pyridone), 114.8 (CH-Pyridone), 126.4 (C-Ar), 128.7 (2×C-Ar), 129.0 (2×C-Ar), 133.8 (C-Ar), 134.2 (C-Ar), 151.0 (C-Ar), 160.9 (C=O pyridone), 179.9 (C=O pyrrolidinone); HRMS calcd for C₁₉H₂₀ClN₂O₄[M(³⁵Cl)+H]⁺ 375.1106, found 375.1103.
(1r,3R,4S)-6’-(4-Chlorobenzyl)-3,4-dihydroxy-1’-methyl-1’,6’-dihydrospiro[cyclopentane-1,3’-pyrrolo[2,3-c]pyridine]-2’,5’-dione (259)

Obtained as a white solid from the reaction above (5 mg, 18%). \( R_t = 0.11 \) (5% MeOH/DCM); \( \lambda_{\text{max}} \) (EtOH)/nm 339.4, 260.8, 221.8; IR \( \nu_{\text{max}} \)/cm\(^{-1}\) 3374 (OH), 2924, 2853, 1700 (C=O), 1567 (C=O); \(^1\)H NMR (500 MHz, CD\(_3\)Cl) \( \delta_H \) 2.00 (2H, dd, \( J = 13.7 \) and 5.2 Hz, CH\(_2\)CHOH), 2.34 (2H, dd, \( J = 13.7 \) and 6.0 Hz, CH\(_2\)CHOH), 3.04 (3H, s, N-CH\(_3\)), 3.31 (2H, br s, 2×OH), 4.43 (2H, br s, 2×CHOH), 5.09 (2H, s, CH\(_2\)-Ar), 6.58 (1H, s, 1×Pyridone-\( H \)), 7.03 (1H, s, 1×Pyridone-\( H \)), 7.24-7.26 (2H, m, 2×Ar-\( H \)), 7.31-7.33 (2H, m, 2×Ar-\( H \)); \(^13\)C NMR (125 MHz, CD\(_3\)Cl) \( \delta_C \) 26.6 (N-CH\(_3\)), 42.6 (2×CH\(_2\)CHOH), 50.5 (NCH\(_2\)Ar), 51.6 (CH\(_2\)-CHOH), 74.2 (2×OH), 112.1 (CH-Pyridone), 116.8 (CH-Pyridone), 127.9 (C-Ar), 129.2 (2×C-Ar), 129.5 (2×C-Ar), 134.1 (C-Ar), 134.8 (C-Ar), 153.5 (C-Ar), 162.2 (C=O pyridone), 179.1 (C=O pyrrolidinone); HRMS calcd for C\(_{19}\)H\(_{20}\)ClN\(_2\)O\(_4\) [M\(^{35}\)Cl+H]\(^+\) 375.1106, found 375.1102.

3-Hydroxy-1-methyl-3-((trimethylsilyl)methyl)indolin-2-one (280)

\( N \)-methylisatin (200 mg, 1.24 mmol) was dissolved in THF (6.2 mL) and cooled to -78 °C. 1 M (trimethylsilyl)methylmagnesium chloride in Et\(_2\)O (2.5 mL, 2.5 mmol) was added dropwise and the solution was stirred at r.t. for 1.5 h. The solvent was removed \textit{in-vacuo} and the residue was purified by MPLC on SiO\(_2\) (Petrol:EtOAc, 0-20%) to get a light yellow solid (220 mg, 71%). \( R_t = 0.31 \) (5% MeOH/DCM); m.p. 143-145 °C; \( \lambda_{\text{max}} \) (EtOH)/nm 256.8, 209.4; IR \( \nu_{\text{max}} \)/cm\(^{-1}\) 3352 (OH), 3057, 2950, 2899, 2871, 1690 (C=O), 1609 (C=O); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \( \delta_H \) 0.31 (9H, s, 3×CH\(_3\)), 1.53 (1H, d, \( J = 13.5 \) Hz, 1×CH\(_2\)), 1.56 (1H, d, \( J = 13.5 \) Hz, 1×CH\(_2\)), 2.80 (1H, s, O\( H \)), 3.18 (3H, s, NCH\(_3\)), 6.83 (1H, d, \( J = 7.7 \) Hz, 1×Ar-\( H \)), 7.09 (1H, td, \( J = 7.5 \) and 0.9 Hz, 1×Ar-\( H \)), 7.32 (1H, td, \( J = 7.7 \) and 1.3 Hz, 1×Ar-\( H \)), 7.36 (1H, dd, \( J = 7.5 \) and 0.9 Hz, 1×Ar-\( H \)), 8.54 (1H, d, \( J = 0.6 \) Hz, 1×Pyridine-\( H \)); \(^13\)C NMR (125 MHz, CDCl\(_3\)) \( \delta_C \) 0.0 (3×CH\(_3\)), 27.4
(N-CH₃), 29.9 (CH₂), 76.9 (COH), 109.7 (CH-Ar), 124.4 (CH-Ar), 125.4 (CH-Ar), 131.0 (CH-Ar), 132.3 (C-Ar), 144.2 (C-Ar), 179.5 (C=O pyrrolidinone); HRMS calcd for C₁₃H₂₀N₂O₆Si [M+H]⁺ 250.1258, found 250.1257.

2-(1,3-Dimethyl-2-oxoindolin-3-yl)acetamide (277)

To a solution of compound 282 (60 mg, 0.37 mmol) in DMF (4 mL), Cs₂CO₃ (182 mg, 0.55 mmol) and 2-bromoacetamide (77 mg, 0.55 mmol) were added, respectively. The reaction mixture was stirred at 50 °C for 2 h. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with EtOAc (3×20 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-7%) gave a white solid (10 mg, 12%). Rf = 0.28 (5% MeOH/DCM); m.p. 177-178 °C; λmax (EtOH)/nm 252.8, 205.6; IR νmax/cm⁻¹ 3371 (NH), 3189, 3052, 2920, 1674 (C=O), 1609 (C=O); ¹H NMR (500 MHz, CDCl₃) δH 1.42 (3H, s, C₆H₃), 2.66 (1H, d, J = 14.9 Hz, 1×C₆H₂), 2.78 (1H, d, J = 14.9 Hz, 1×CH₂), 3.21 (3H, s, NCH₃), 5.30 (1H, br s, 1×NH₂), 6.40 (1H, br s, 1×NH₂), 6.83 (1H, d, J = 7.7 Hz, 1×Ar-H), 7.05 (1H, td, J = 7.7 and 0.9 Hz, 1×Ar-H), 7.23-7.28 (2H, m, 2×Ar-H); ¹³C NMR (125 MHz, CDCl₃) δC 23.5 (CH₃), 26.4 (N-C₆H₃), 43.4 (CH₂), 46.0 (C(CH₃)), 108.4 (CH-Ar), 122.7 (CH-Ar), 122.9 (CH-Ar), 128.3 (CH-Ar), 133.3 (C-Ar), 142.8 (C-Ar), 171.3 (CONH₂), 180.6 (C=O pyrrolidinone); HRMS calcd for C₁₂H₁₅N₂O₂ [M+H]⁺ 219.1128, found 219.1125.

4-Formyl-N,N-dimethyl-1H-imidazole-1-sulfonamide (285)

1H-imidazole-4-carbaldehyde (200 mg, 2.08 mmol) was suspended in DCM (7 mL), trimethylamine (0.32 mL, 2.29 mmol) and N,N-dimethylsulfamoyl chloride (0.25 mL, 2.29 mmol) were added, respectively. The reaction mixture was stirred at r.t. for 24 h. N,N-
dimethylsulfamoyl chloride (0.12 mL, 1.14 mmol) and the reaction mixture was stirred at 40 °C for 24 h. The reaction was quenched with water (30 mL) and the aqueous layer was extracted with EtOAc (3×30 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo. Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave colourless crystals (360 mg, 85%). Rₚ = 0.36 (5% MeOH/DCM); m.p. 99-101 °C; λ_max (EtOH)/nm 245.8; IR ν_max/cm⁻¹ 3134, 3092, 3032, 2951, 2838, 1692 (C=O), 1387 (SO), 1174 (SO); ¹H NMR (500 MHz, CDCl₃) δ H 2.91 (6H, s, 2×CH₃), 7.88 (1H, d, J = 1.3 Hz, 1×Ar-H), 7.95 (1H, d, J = 1.3 Hz, 1×Ar-H), 9.93 (1H, s, CHO); ¹³C NMR (125 MHz, CDCl₃) δ C 38.2 (2×CH₃), 121.8 (CH-Ar), 137.5 (CH-Ar), 142.3 (C-Ar), 185.8 (C=O pyrrolidinone); HRMS calcd for C₆H₁₀N₃O₃S [M+H]⁺ 204.0437, found 204.0433.

**Tert-butyl 3-((1-(N,N-dimethylsulfamyl)-1H-imidazol-4-yl)methylene)-5-methoxy-2-oxo-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate (286)**

![Structure of Compound 286](image)

Compound 191 (275 mg, 1.04 mmol) and compound 285 (275 mg, 1.35 mmol) were dissolved in THF (0.5 mL), piperidine (0.2 mL, 2.08 mmol) was added and the reaction mixture was stirred at 50 °C for 1 h. The reaction mixture was filtered using Et₂O (5×20 mL) and water (2×10 mL) to get a yellow solid (140 mg, 30%). Rₚ = 0.63 (5% MeOH/DCM); m.p. 197-200 °C; λ_max (EtOH)/nm 339.4, 213.2; IR ν_max/cm⁻¹ 3108, 3019, 2971, 2927, 1773 (C=O), 1636 (C=O), 1118 (SO); ¹H NMR (500 MHz, DMSO-d₆) δ H 1.60 (9H, s, C(CH₃)₃), 2.91 (6H, s, N(CH₃)₂), 3.88 (3H, s, OCH₃), 7.81 (1H, s, Pyrrole-H), 8.51 (1H, s, 1×Pyridine-H), 8.63 (1H, s, Pyrrole-H), 8.65 (1H, s, C=CH), 8.87 (1H, s, 1×Pyridine-H); ¹³C NMR (125 MHz, DMSO-d₆) δ C 28.2 (C(CH₃)₃), 38.3 (N(CH₃)₂), 53.9 (OCH₃), 84.4 (C(CH₃)₃), 107.6 (C-Ar), 121.5 (C-Ar), 128.8 (C-Ar), 130.5 (C-Ar), 131.3 (C-Ar), 132.2 (C-Ar), 133.0 (C-Ar), 138.2 (C=CH), 139.6 (C=CH), 148.7 (COO'-Bu), 160.9 (C-OMe), 166.0 (C=O pyrrolidinone); HRMS calcd for C₁₉H₂₄N₅O₆S [M+H]⁺ 450.1442, found 450.1430.

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**Tert-butyl 3-((1-(N,N-dimethylsulfamoyl)-1H-imidazol-4-yl)methyl)-5-methoxy-3-methyl-2-oxo-2,3-dihydro-1H-pyrrolo[2,3-c]pyridine-1-carboxylate (288)**

Prepared according to general procedure D using compound 286 (75 mg, 0.16 mmol), 10% Pd/C (20 mg), THF (12 mL) and MeOH (6 mL) in step A and Cs₂CO₃ (81 mg, 0.25 mmol), MeI (20 μL, 0.25 mmol), DMF (4.5 mL) in Step B. Purification by MPLC on SiO₂ (DCM:MeOH, 0-5%) gave a white solid (53 mg, 69%). R_f = 0.51 (5% MeOH/DCM); λ_max (EtOH)/nm 229.4; IR ν_max/cm⁻¹ 3112, 2972, 2935, 1792 (C=O), 1385 (SO), 1144 (SO); ¹H NMR (500 MHz, CD₃Cl) δ H 1.52 (3H, s, CH₃), 1.63 (9H, s, C(CH₃)₃), 2.67 (6H, s, N(CH₃)₂), 2.99 (1H, d, J = 14.3 Hz, CH₂), 3.21 (1H, d, J = 14.3 Hz, CH₂), 3.88 (3H, s, OCH₃), 6.54 (1H, d, J = 0.5 Hz, 1×Pyridine-H), 6.78 (1H, s, 1×Pyrrole-H), 7.62 (1H, d, J = 1.2 Hz, 1×Pyrrole-H), 8.43 (1H, d, J = 0.5 Hz, 1×Pyridine-H); ¹³C NMR (125 MHz, CD₃Cl) δ C 23.6 (CH₃), 28.1 (C(CH₃)₃), 36.9 (CH₂), 38.0 (N(CH₃)₂), 49.3 (C(CH₃)), 53.6 (OCH₃), 84.7 (C(CH₃)₃), 105.6 (C-Ar), 115.3 (C-Ar), 130.9 (C-Ar), 131.8 (C-Ar), 136.1 (C-Ar), 138.5 (C-Ar), 145.0 (C-Ar), 148.8 (COO'Bu), 160.8 (C-OMe), 177.2 (C=O pyrroldinone); HRMS calcd for C₂₀H₂₆N₅O₆S [M+H]⁺ 466.1755, found 466.1743.

**1-((2-(Trimethylsilyl)ethoxy)methyl)-1H-pyrrole-3-carbaldehyde (292)**

1H-pyrrole-3-carbaldehyde (500 mg, 5.25 mmol) was dissolved in THF (21 mL) and cooled to 0 °C. NaH (60% in mineral oil, 252 mg, 6.30 mmol) was added portion wise and the mixture was stirred at 0 °C for 30 min. 2-(chloromethoxy)ethyltrimethylsilane (1.02 mL, 5.78 mmol) was added and the reaction mixture was stirred at r.t. for 2.5 h. The reaction was quenched with saturated NH₄Cl (30 mL) and the aqueous layer was extracted with EtOAc (3×50 mL). The organic layers were combined, dried over MgSO₄ and the solvent removed in vacuo.
Purification by MPLC on SiO$_2$ (Petrol:EtOAc, 0-20%) gave colourless oil (1 g, 85%). $^1$H NMR (500 MHz, CD$_3$Cl) $\delta$H 0.00 (9H, s, Si(CH$_3$)$_3$), 0.91-0.95 (2H, m, CH$_2$), 3.56-3.59 (2H, m, CH$_2$), 5.74 (2H, s, NCH$_2$O), 6.33 (1H, dd, $J = 3.9$ and 2.6 Hz, Ar-H), 7.01 (1H, dd, $J = 3.9$ and 1.7 Hz, Ar-H), 7.17-7.18 (1H, m, 1×Ar-H), 7.64 (1H, d, $J = 1.0$ Hz, CHO); $^{13}$C NMR (125 MHz, CD$_3$Cl) $\delta$C 0.0 (Si(CH$_3$)$_3$), 19.3 (CH$_2$), 67.6 (CH$_2$), 78.1 (NCH$_2$O), 112.0 (C-Ar), 126.7 (C-Ar), 132.5 (C-Ar), 133.3 (C-Ar), 181.1 (C=O).

5-Methoxy-3-(((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrol-2-yl)methylene)-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one (293)

Prepared according to general procedure C using compound 191 (335 mg, 1.26 mmol), compound 292 (343 mg, 1.52 mmol), piperidine (250 μL, 2.53 mmol) and THF (250 μL), except temperature was 60 °C. Purification by MPLC on SiO$_2$ (DCM:MeOH, 0-5%) gave an orange solid (372 mg, 79%), ratio of diastereomers = 4:3; $R_f$ = 0.32 (5% MeOH/DCM); m.p. 142-145 °C; $\lambda_{max}$ (EtOH)/nm 399.8, 260.4, 209.0; IR $\nu_{max}$/cm$^{-1}$ 3137, 3085, 2946, 2888, 1688 (C=O); $^1$H NMR (500 MHz, DMSO-d$_6$) $\delta$H 0.12 (9H, s, Si(CH$_3$)$_3$, isomer 1), 0.10 (9H, s, Si(CH$_3$)$_3$, isomer 2), 0.78-0.83 (2H, m, CH$_2$, isomer 1 and 2), 3.43-3.47 (2H, m, CH$_2$, isomer 1 and 2), 3.80 (3H, s, OCCH$_3$, isomer 1), 3.81 (3H, s, OCCH$_3$, isomer 2), 5.50 (2H, s, NC=H$_2$O, isomer 1), 5.67 (2H, s, NCH$_2$O, isomer 2), 6.35 (1H, dd, $J = 4.1$ and 2.7 Hz, Ar-H, isomer 2), 6.42 (1H, dd, $J = 3.7$ and 2.7 Hz, Ar-H, isomer 1), 7.12 (1H, s, Ar-H, isomer 2), 7.15 (1H, d, $J = 3.3$ Hz, Ar-H, isomer 1), 7.29 (1H, s, Ar-H, isomer 1), 7.45 (1H, dd, $J = 2.5$ and 1.4 Hz, Ar-H, isomer 1), 7.46 (1H, dd, $J = 2.5$ and 1.7 Hz, Ar-H, isomer 2), 7.60 (1H, s, Ar-H, isomer 2), 7.68 (1H, s, Ar-H, isomer 1), 7.79 (1H, s, Ar-H, isomer 1), 7.84 (1H, s, Ar-H, isomer 2), 8.42 (1H, dd, $J = 4.0$ and 1.5 Hz, Ar-H, isomer 2), 10.43 (1H, s, NH, isomer 2), 10.49 (1H, s, NH, isomer 2); $^{13}$C NMR (125 MHz, DMSO-d$_6$) $\delta$C -0.6 (Si(CH$_3$)$_3$, isomer 1), -0.5 (Si(CH$_3$)$_3$, isomer 2), 17.9 (CH$_3$, isomer 1 and 2), 54.0 (OCH$_3$, isomer 1 and 2), 65.9 (CH$_2$, isomer 1), 66.0 (CH$_2$, isomer 2), 76.2 (NCH$_2$O, isomer 1), 76.7 (NCH$_2$O, isomer 2), 100.5, 103.5, 111.1, 111.2, 117.3, 119.2, 120.5, 124.0, 125.3, 126.1, 127.5, 128.0, 128.9, 129.2, 130.8, 132.0, 132.1, 133.5, 133.8, 138.6, 268
159.7 (C-OMe, isomer 1), 160.0 (C-OMe, isomer 2), 167.8 (C=O, isomer 1), 169.6 (C=O, isomer 2); HRMS calcd for C_{19}H_{26}N_{3}O_{3}Si [M+H]^+ 372.1738, found 372.1732.

5-Methoxy-1,3-dimethyl-3-((1-((2-(trimethylsilyl)ethoxy)methyl)-1H-pyrrol-2-yl)methyl)-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one (295)

Prepared according to general procedure D using compound 293 (100 mg, 0.27 mmol), 10% Pd/C (20 mg), THF (15 mL) and MeOH (7.5 mL) in step A and Cs_{2}CO_{3} (202 mg, 0.62 mmol), MeI (40 μL, 0.62 mmol), DMF (5 mL) in Step B; reaction done at r.t. Purification by MPLC on SiO_{2} (DCM:MeOH, 0-5%) gave a beige sticky liquid (72 mg, 67%). R_{f} = 0.57 (5% MeOH/DCM); \lambda_{max} (EtOH)/nm 305.4, 250.0; IR ν_{max}/cm\(^{-1}\) 2949, 2892, 1708 (C=O); \textsuperscript{1}H NMR (500 MHz, CD_{3}Cl) δ_{H} 0.047 (9H, s, SiC(CH_{3})_{3}), 0.77-0.87 (2H, m, CH_{2}), 1.43 (3H, s, CH_{3}), 3.11 (1H, d, J = 15.2 Hz, Pyrrolidinone-CH_{2}-Pyrrole), 3.11 (3H, s, NCH_{3}), 3.19 (1H, d, J = 15.2 Hz, CH_{2}-Pyrrole), 3.27-3.35 (2H, m, CH_{2}), 3.88 (3H, s, OCH_{3}), 4.90 (1H, d, J = 11.2 Hz, NCH_{2}O), 5.10 (1H, d, J = 11.2 Hz, NCH_{2}O), 5.59 (1H, dd, J = 3.4 and 1.6 Hz, Pyrrole-H), 5.89 (1H, app t, Pyrrole-H), 6.44 (1H, d, J = 0.6 Hz, 1×Pyridine-H), 6.52 (1H, dd, J = 2.7 and 1.7 Hz, Pyrrole-H), 7.54 (1H, d, J = 0.6 Hz, 1×Pyridine-H); \textsuperscript{13}C NMR (125 MHz, CD_{3}Cl) δ_{C} 0.0 (SiC(CH_{3})_{3}), 19.1 (CH_{2}), 24.4 (CH_{3}), 27.9 (N(CH_{3})_{2}), 34.5 (Pyrrolidinone-CH_{2}-Pyrrole), 50.8 (C(CH_{3})_{3}), 55.0 (OCH_{3}), 66.7 (CH_{2}), 77.4 (NCH_{2}O), 108.0 (C-Ar), 108.7 (C-Ar), 111.0 (C-Ar), 123.2 (C-Ar), 125.2 (C-Ar), 128.3 (C-Ar), 161.9 (C-OMe), 180.1 (C=O pyrrolidinone), 2 aromatic carbons not observed due to dilute NMR sample; HRMS calcd for C_{21}H_{32}N_{3}O_{3}Si [M+H]^+ 402.2207, found 402.2203.
3-((1H-Pyrrol-2-yl)methyl)-5-methoxy-1,3-dimethyl-1,3-dihydro-2H-pyrrolo[2,3-c]pyridin-2-one (298)

Compound 295 (55 mg, 0.13 mmol) was dissolved in THF (0.25 mL), 1 M TBAF in THF (0.7 mL, 0.7 mmol) was added and the reaction mixture was stirred at 50 °C for 48 h. THF (20 mL) and TBAF scavenger (1 g) were added to the reaction and the mixture was stirred at r.t. for 48 h. The reaction mixture was filtered and the filtrate was evaporated in vacuo to get an off-white solid (19 mg, 51%). R\textsubscript{f} = 0.42 (5% MeOH/DCM); m.p. 146-149 °C; \lambda\textsubscript{max} (EtOH)/nm 249.4; IR \nu\textsubscript{max}/cm\textsuperscript{-1} 3348 (NH), 2976, 2928, 2863, 1706 (C=O); \textsuperscript{1}H NMR (500 MHz, CD\textsubscript{3}Cl) \delta H 1.30 (3H, s, CH\textsubscript{3}), 2.91 (1H, d, J = 14.5 Hz, CH\textsubscript{2}), 3.03 (1H, d, J = 14.5 Hz, CH\textsubscript{2}), 3.06 (3H, s, N-CH\textsubscript{3}), 3.79 (3H, s, OC\textsubscript{H}\textsubscript{3}), 5.40-5.42 (1H, m, Pyrrole-H), 5.77 (1H, dd, J = 5.6 and 2.6 Hz, Pyrrole-H), 6.44-6.46 (1H, m, Pyrrole-H), 6.59 (1H, d, J = 0.7 Hz, 1×Pyridone-H), 7.69 (1H, d, J = 0.7 Hz, 1×Pyridine-H), 10.1 (1H, s, NH); \textsuperscript{13}C NMR (125 MHz, CD\textsubscript{3}Cl) \delta C 23.0 (CH\textsubscript{3}), 26.6 (N-CH\textsubscript{3}), 35.3 (CH\textsubscript{2}), 49.2 (C(CH\textsubscript{3})), 53.6 (OCH\textsubscript{3}), 106.2 (C-Ar), 106.8 (C-Ar), 107.8 (C-Ar), 116.7 (C-Ar), 124.3 (C-Ar), 126.3 (C-Ar), 135.4 (C-Ar), 147.1 (C-Ar), 159.9 (C-Ar), 178.5 (C=O pyrrolidinone); HRMS calcd for C\textsubscript{15}H\textsubscript{18}N\textsubscript{3}O\textsubscript{2} [M+H]+ 272.1394, found 272.1394.

1-Allyl-6’-(4-chlorobenzyl)-1’-methyl-1’,6’-dihydrospiro[pyrrolidine-3,3’-pyrrolo[2,3-c]pyridine]-2’,5’-dione (273)

Prepared according to general procedure E using Compound 265 (30 mg, 0.095 mmol), MgI\textsubscript{2} (5 mg), compound 276 (118 mg, 0.57 mmol) and THF (0.3 mL). The residue was purified by MPLC on SiO\textsubscript{2} (DCM:MeOH, 0-7%) gave a sticky beige solid (16 mg). The compound was not pure enough by \textsuperscript{1}H-NMR. However, to avoid the loss of any material, the compound was taken forward without further purification and characterisation.
6’-(4-Chlorobenzyl)-1'-methyl-1’,6’-dihydrospiro[pyrrolidine-3,3’-pyrrolo[2,3- 
c]pyridine]-2’,5’-dione (253)

To a mixture of tetrakistriphenylphosphine palladium (2 mg, 4.4 mol%) and N,N-
dimethylbarbituric acid (15 mg, 0.097 mmol), a solution of compound 273 (15 mg, 0.039 
mmol) in DCM (1 mL) purged with N\textsubscript{2} was added and the mixture was stirred at 35 °C for 16 
h. The solvent was removed \textit{in vacuo} and the residue was treated with saturated NaHCO\textsubscript{3} (10 
\text{mL}) and Et\textsubscript{2}O (10 \text{mL}) and the mixture was stirred for 30 min. The aqueous layer was collected 
and the residue was purified by eluting through a pad of amine silica (DCM:MeOH, 10%) to 
get beige sticky solid (7 mg, 22% over 2 steps). R\text{f}=0.48 (15% MeOH/DCM); \lambda_{\text{max}} (EtOH)/nm 
339.4, 221.2; IR \nu_{\text{max}}/\text{cm}^{-1} 3415 (NH), 3045, 2921, 2851, 1705 (C=O), 1585 (C=O); \textsuperscript{1}H NMR 
(500 MHz, CD\textsubscript{3}Cl) \delta \text{H} 2.00-2.06 (1H, m, Pyrrolidine-\text{H}), 2.29-2.35 (1H, m, Pyrrolidine-\text{H}), 
2.92-2.96 (1H, m, Pyrrolidine-\text{H}), 3.06 (1H, s, N-\text{CH\textsubscript{3}}), 3.14-3.19 (1H, m, Pyrrolidine-\text{H}), 3.36- 
3.39 (2H, m, Pyrrolidine-\text{H}), 5.09 (2H, s, CH\textsubscript{2}-Ar), 6.55 (1H, s, 1xPyridone-\text{H}), 6.62 (1H, s, 
1xPyridone-\text{H}), 7.25-7.27 (2H, m, 2xAr-\text{H}), 7.31-7.33 (2H, m, 2xAr-\text{H}); \textsuperscript{13}C NMR (125 MHz, 
CD\textsubscript{3}Cl) \delta \text{C} 26.5 (N-\text{CH\textsubscript{3}}), 39.6 (C-Pyrrolidine), 49.0 (C-Pyrrolidine), 51.5 (NCH\textsubscript{2}Ar), 54.2 (C), 
60.7 (C-Pyrrolidine), 112.4 (CH-Pyridone), 114.8 (CH-Pyridone), 127.4 (C-Ar), 129.1 (2xC-Ar), 
129.4 (2xC-Ar), 134.1 (C-Ar), 135.0 (C-Ar), 150.9 (C-Ar), 161.4 (C=O pyridone), 178.0 
(C=O pyrrolidinone); HRMS calcd for C\textsubscript{18}H\textsubscript{19}ClN\textsubscript{3}O\textsubscript{2} [M(\textsuperscript{35}Cl)+H]\textsuperscript{+} 344.1160, found 344.1160.
Appendices

Crystal data and structure refinement for RO2443 3.

Identification code rjg130001
Chemical formula (moiety) C_{24}H_{20}ClF_{2}N_{3}O_{4}
Chemical formula (total) C_{24}H_{20}ClF_{2}N_{3}O_{4}
Formula weight 487.88
Temperature 150(2) K
Radiation, wavelength MoKα, 0.71073 Å
Crystal system, space group triclinic, P\overline{1}
Unit cell parameters a = 8.8755(5) Å \quad \alpha = 78.372(6)^{\circ}
b = 10.4934(7) Å \quad \beta = 81.700(5)^{\circ}
c = 13.5493(9) Å \quad \gamma = 69.763(6)^{\circ}
Cell volume 1155.88(13) Å^{3}
Z 2
Calculated density 1.402 g/cm^{3}
Absorption coefficient \mu 0.218 mm\(^{-1}\)
F(000) 504
Crystal colour and size 0.40 \times 0.02 \times 0.02 mm^{3}
Reflections for cell refinement 2773 (θ range 3.8 to 28.5\(^{\circ}\))
Data collection method Xcalibur, Atlas, Gemini ultra
thick-slice ω scans
θ range for data collection 3.0 to 26.0\(^{\circ}\)
Index ranges h –10 to 10, k –12 to 12, l –16 to 16
Completeness to θ = 25.0\(^{\circ}\) 99.8 \%
Reflections collected 10284
Independent reflections 4441 (R_{int} = 0.0335)
Reflections with F^{2}>2σ 3486
Absorption correction semi-empirical from equivalents
Min. and max. transmission 0.9180 and 0.9957
Structure solution direct methods
Refinement method Full-matrix least-squares on F^{2}
Weighting parameters a, b 0.0624, 0.5323
Data / restraints / parameters 4441 / 0 / 311
Final R indices [F^{2}>2σ] R1 = 0.0478, wR2 = 0.1155
R indices (all data) R1 = 0.0669, wR2 = 0.1293
Goodness-of-fit on F^{2} 1.030
Extinction coefficient 0.0031(16)
Largest and mean shift/su 0.005 and 0.000
Largest diff. peak and hole 0.77 and –0.26 e Å\(^{-3}\)
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