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Synthesis of Novel Agents for the treatment of Infectious and Neurodegenerative diseases

Benjamin Joe Eduful

University of South Florida, beduful@mail.usf.edu

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Synthesis of Novel Agents for the treatment of Infectious and Neurodegenerative diseases

by

Benjamin Joe Eduful

A dissertation submitted in partial fulfilment of the requirements for the degree of Doctor of Philosophy
Department of Chemistry
College of Arts and Sciences
University of South Florida

Major Professor: James W. Leahy, Ph.D.
Edward Turos, Ph.D.
Jianfeng Cai, Ph.D.
Nicholas J. Lawrence, Ph.D.
Yu Chen, Ph.D.

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Dedication

To the loving memories of Emelia and Peter
Acknowledgements

Attaining this height has been nothing short of a great miracle - given the many challenges along the way. Indeed without the help of my God, in whom I live, move and have being, I am certain I could not have made it to this far. So I am profoundly grateful to God for bringing me to this place.

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List of Abbreviations

AA  Axenic amastigote
Aβ  Beta amyloid peptides
ACH  Acetylcholine
ACHE  Acetylcholinesterase
ACHEI  Acetylcholinesterase inhibitor
AcOH  Acetic acid
AD  Alzheimer’s disease
ADF  Actin-depolymerizing factor
APP  Beta amyloid precursor protein
BACE1  β-secretase
BBB  Blood brain barrier
BCG  Bacille Calmette Guerin
Bn  Benzyl
Boc  tert-butoxycarbonyl
CL  Cutaneous leishmaniasis
CNS  Central nervous system
DBU  1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM  Dichloromethane (methylene chloride)
DIPEA  Diisopropylethylamine
DMF  Dimethylformamide
DMAP  Dimethylaminopyridine
DMSO  Dimethyl sulfoxide
DPPA  Diphenylphosphoryl azide
DPPF  1,1'-Bis(diphenylphosphino)ferrocene
ESI  Electrospray ionization
Et3N  Triethylamine
EtOAc  Ethyl acetate
EtOH  Ethanol
GA  Geldanamycin
h  Hour(s)
H2O2  Hydrogen peroxide
HCS  High content screen
HPLC  High pressure liquid chromatography
Hsp 90  Heat shock protein 90
HTS  High throughput screen
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>IM</td>
<td>Infected macrophages</td>
</tr>
<tr>
<td>IPA</td>
<td>Isopropyl alcohol</td>
</tr>
<tr>
<td>LCMS</td>
<td>Liquid chromatography mass spectrometry</td>
</tr>
<tr>
<td>LC/QToF-MS</td>
<td>Liquid chromatography quadrupole time-of-flight mass spectrometry</td>
</tr>
<tr>
<td>MeOH</td>
<td>Methanol</td>
</tr>
<tr>
<td>min</td>
<td>Minute(s)</td>
</tr>
<tr>
<td>ML</td>
<td>Mucocutaneous leishmaniasis</td>
</tr>
<tr>
<td>MPLC</td>
<td>Medium pressure liquid chromatography</td>
</tr>
<tr>
<td>MsBr</td>
<td>Mesityl bromide</td>
</tr>
<tr>
<td>mw</td>
<td>microwave irradiation</td>
</tr>
<tr>
<td>NaNO₂</td>
<td>Sodium nitrite</td>
</tr>
<tr>
<td>NBS</td>
<td>N-bromosuccinimide</td>
</tr>
<tr>
<td>NF</td>
<td>Neurofibrillary tangles</td>
</tr>
<tr>
<td>NH₄OH</td>
<td>Ammonium hydroxide</td>
</tr>
<tr>
<td>NIS</td>
<td>N-iodosuccinimide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic spectroscopy</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>NTDs</td>
<td>Neglected tropical diseases</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>Phosphorus pentoxide</td>
</tr>
<tr>
<td>Pyr</td>
<td>Pyridine</td>
</tr>
<tr>
<td>Pd₂(dba)₃</td>
<td>Tris(dibenzylideneacetone)dipalladium(0)</td>
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<td>pNPP</td>
<td>para-Nitrophenyl phosphate</td>
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<td>SAR</td>
<td>Structure activity relationships</td>
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<td>Thionyl chloride</td>
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<tr>
<td>SSH1</td>
<td>Slingshot homology-1 protein</td>
</tr>
<tr>
<td>TBABr</td>
<td>Tetrabutyl ammonium bromide</td>
</tr>
<tr>
<td>TBAI</td>
<td>Tetrabutyl ammonium iodide</td>
</tr>
<tr>
<td>tBuOK</td>
<td>Potassium tert-butoxide</td>
</tr>
<tr>
<td>TEA</td>
<td>Triethylamine</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>TMBA</td>
<td>Trimethoxybenzylamine</td>
</tr>
<tr>
<td>TMSBr</td>
<td>Trimethylsilyl bromide</td>
</tr>
<tr>
<td>UHD</td>
<td>Ultra-high-definition</td>
</tr>
<tr>
<td>USF</td>
<td>University of South Florida</td>
</tr>
<tr>
<td>US FDA</td>
<td>United States food and drugs administration</td>
</tr>
<tr>
<td>VL</td>
<td>Visceral leishmaniasis</td>
</tr>
<tr>
<td>WHO</td>
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Abstract

Infectious and neurodegenerative diseases continue to be a major concern worldwide. In spite of the great advances in drug therapy for treating various infectious and neurodegenerative diseases, there is still an urgent need for new and improved drugs due to increasing drug resistance among pathogens, emergence of new pathogens, ease of transmission of infections, ineffective available treatments, toxicity associated with current standard of care, aging populations and the lack of better alternative treatment options.

The first part of this manuscript (chapters 1 - 5) describes the synthesis of novel agents active against *Leishmania donovani*. According to the World Health Organization (WHO), a significant number of deaths worldwide can be attributed to infectious diseases – particularly neglected tropical diseases (NTDs), one of which is leishmaniasis - a complex and clinically diverse disease transmitted through the bite of an infected female phlebotomine sand-fly. The pathogen that causes leishmaniasis develops through a complex life cycle via different morphological changes. Its clinical presentations range from the less severe (cutaneous) to lethal/fatal (visceral) forms depending upon the level of systemic involvement, infecting species and the endemic environment. Treatments (and vaccines) must be species-specific to be particularly effective since sensitivity to commonly used drugs is largely species-specific. Heat shock protein 90 (Hsp 90) has been shown to promote the differentiation of the protozoan parasite that causes leishmaniasis from the promastigote stage to the amastigote pathogenic stages. To this end a series of compounds were prepared based on known Hsp 90 inhibitors, SNX2112 and XL888. The synthetic approach allows
the probing of a hydrophobic pocket and rapid access to a collection of anti-leishmanial compounds. The most active compound, was found to be more than twice as active as the clinically used drug, miltefosine, in an infected J774 macrophage at IC\textsubscript{50} = 0.65 \mu M.

The second part of this manuscript (\textbf{chapters 6 - 9}) describes the synthesis of novel anti-Alzheimer’s agents. Alzheimer’s disease is a progressive neurodegenerative disease believed to be caused by tau hyperphosphorylation and plaque aggregation in the brain. It is known to affect about 44 million people worldwide and it is marked as the 6\textsuperscript{th} leading cause of death in the United States. Slingshot homology-1 (SSH1) proteins, important protein phosphatases, are promising targets for the discovery of a new generation of small molecule inhibitors as treatment for Alzheimer’s disease, since SSH1 is believed to contribute to both tau hyperphosphorylation and plaque aggregation in the brain. Through structure and activity relationships (SAR) studies, two (2) series of compounds were synthesized, thiazoles and pyridones, bearing a carboxylic acid or phosphonic acid functionality as inhibitors of SSH1 enzymes. In the preliminary screening efforts against SSH1 phosphatase activity, the thiazole series were found to be more potent at inhibiting the phosphatase activity than the pyridone series. Among the active thiazole series, eight (8) analogs exhibited significant inhibitory activity over the initial hit compound, observed via phosphatase inhibition curves (using a \textit{pNPP} phosphatase assay). Further investigations into the molecular target (SSH1) are currently underway.
Chapter 1

Introduction

1.1 The threat of infectious diseases

Chemotherapy continues to revolutionize medical practice. The discovery and development of different classes of medicaments have led to the transformation of deadly infectious diseases into curable ones – saving lives and relieving patient suffering. However, notwithstanding the great advances in drug therapy, infectious diseases continue to be a major concern worldwide with increasing threat to human health. The development of resistant strains of pathogens to available drugs, the emergence or the discovery of new pathogens, and the ease of transmission due to globalization, including the increasing numbers of immune-compromised patients and elderly populations, suggest that mankind may be fast approaching a post-antibiotic era.

The vast majority of infectious diseases affect the poorest of the poor and are prevalent in the tropical regions of the world, where there is limited access to both diagnosis and treatment. The lack of profitability in the discovery and development of essential medicaments, particularly the ones designated by the World Health Organization (WHO) as neglected tropical diseases (NTDs), has dried up the infectious disease pharmaceutical pipeline. There are twenty (20) NTDs that threaten human health and are currently affecting over 1 billion people in the tropics and subtropics, costing developing economies billions of dollars annually. Leishmaniasis, a zoonotic
parasitic infection caused by protozoa of the genus *Leishmania*, has been identified as one of the many NTDs.

1.2 Leishmaniasis

Leishmaniasis is a complex and clinically diverse disease transmitted through the bite of an infected female phlebotomine sand-fly, **Figure 1**. It is ranked among the most important poverty-related diseases\(^{14,15}\) and a major health problem in larger parts of the world, mainly in developing countries, causing enormous disability, death and economic loss.\(^{2,16}\)

![Figure 1.1: Female Phlebotomine sand fly](image)

The World Health Organization (WHO) has designated leishmaniasis as one of the world’s most neglected tropical diseases (NTDs).\(^{17}\) About twenty (20) different protozoan *Leishmania* species cause the disease,\(^{2,16}\) which is particularly worrying from the standpoint of vaccine development and drug discovery. It is estimated that about 12 million people are afflicted with the disease worldwide; 350 million people are considered at risk, with an estimated 2 million new cases
annually. It is considered to be endemic in 88 countries\textsuperscript{2,16,17,18} with 59,000 deaths per year from visceral leishmaniasis, despite the fact that leishmaniasis was among NTDs selected by the WHO for elimination by 2015.\textsuperscript{19}

1.2.1 \textit{Leishmania} Life Cycle

The genus \textit{Leishmania} belongs to the family Trypanosomatidae, order Kinetoplastida and the group Euglenozoa.\textsuperscript{14} The \textit{Leishmania} life cycle is dimorphic - human stage and insect stage – with a similar mode of transmission for all species,\textsuperscript{14} as depicted in Figure 1.2.\textsuperscript{20}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{leishmania_life_cycle.png}
\caption{Leishmania life cycle\textsuperscript{20}}
\end{figure}
During the human stage, an infected female phlebotomine sand-fly, genus *Phlebotomus* (Asia, Africa and Europe) or of the genus *Lutzomyia* (Latin America), goes for a blood meal, and in the process releases the slender, flagellated promastigote stages into the blood stream (Figure 1.3a). Upon transmission, the parasites are phagocytosed by tissue macrophages and neutrophilic granulocytes where they differentiate within the parasitophorous vacuoles of macrophages.\(^2,15\)

The parasite’s ability to block the fusion of phagosomes and lysosomes ensures their survival, and they establish themselves as round aflagelated non-motile amastigote stages as seen in Figure 1.3b, where they proliferate, leading to disruption of the macrophages. The released protozoan parasites then re-infect other macrophages, dendritic cells and blood monocytes.\(^15,21\)

![Figure 1.3: (a) L. major promastigote from culture, Giemsa staining, 100X. (b) amastigotes (arrows) in infected spleen tissue (VL), Giemsa staining.\(^14\)](image)
During the insect stage, a sandfly goes for a blood meal from an infected human, and takes up amastigote-infected monocytes, which disrupt and release the parasites into the lumen of the insect’s gut. The amastigotes then undergo stage differentiation into the flagellated promastigotes and attach themselves to the epithelial gut until they reach stationary growth stage.\textsuperscript{14} The promastigotes then detach from the epithelial gut and spread into the mouth parts and can be transmitted to healthy persons following another blood meal.

1.2.2 Forms of Leishmaniasis

The disease manifests itself in three main forms/types with a wide range of complex devastating clinical manifestations, depending upon the infecting species, endemic region and the level of systemic involvement:\textsuperscript{14,15,17,22}

i. **Cutaneous Leishmaniasis (CL):** the most common and least fatal localized and self-healing lesions. However sores can take months to years to heal as seen in Figure 1.4a. Symptoms include sores, papules or nodules on the skin, with or without swollen lymph nodes. Sores can leave significant permanent scars and can be disfiguring, especially for facial sores. These are caused by \textit{L. major}, \textit{L. tropica}, \textit{L. mexicana}, \textit{L. amazonensis}, \textit{L. panamensis}, \textit{L. braziliensis}, \textit{L. guyanensis} and \textit{L. peruviana}.

ii. **Mucocutaneous Leishmaniasis (ML):** the most destructive form. Occurs when untreated cutaneous lesions on the face spread to involve the mouth and nose as seen in Figure 1.4b. ML causes complete or partial destruction of mucus membranes in the nose, mouth and throat and can be very disfiguring. These are caused by \textit{L. braziliensis} and \textit{L. panamensis}.  

5
iii. **Visceral Leishmaniasis (VL):** also known as black fever or Kala Azar, occurs when the parasite disseminates into the entire reticuloendothelial system (spleen, liver and bone marrow) as seen in **Figure 1.4c.** VL can be fatal if left untreated. Symptoms include weight loss, persistent fever, anemia, massive hepatosplenomegaly (swelling of the liver and spleen) and deterioration of the host. In later stages of the disease, hemorrhages and edema are likely to develop. Mortality can reach >90% if efficient chemotherapy is not administered. These generalised infections are caused by *L. donovani* and *L. infantum.*

![Figure 1.4: (a) Cutaneous Leishmaniasis, (b) Mucocutaneous Leishmaniasis, (c) Visceral Leishmaniasis](image)

### 1.3 Current Treatment and Vaccine Development

New drug discovery efforts for leishmaniasis, particularly visceral leishmaniasis, come with a plethora of challenges which include: lack of sufficient investment, validated targets, *in vivo* models and very low hit rates from HTS due to the concealed location of the parasites within the acidic parasitophorous vacuoles of macrophages. Because of these challenges, there is a general lack of inexpensive and effective therapeutics for leishmaniasis. Current treatments for leishmaniasis, shown in **Figure 1.5,** are not ideal due to their high cost, toxicity, long duration of
treatment, complicated mode of administration and severe adverse reactions, which often lead to non-compliance and treatment abandonment.\textsuperscript{11}

\textbf{Figure 1.5: Current drugs used for the treatment of Leishmaniasis}

Moreover, these medications often do not completely eliminate the parasite. The past 15 years have witnessed novel formulations of conventional anti-leishmanial agents, such as liposomal amphotericin B, however these are too costly for practical use in developing countries. The clinical value of these medications have also been compromised by their associated irreversible toxicity and the rapid development of resistance.\textsuperscript{2,22} \textbf{Table 1.1} below summarizes the toxicity profile of the commonly used drugs.\textsuperscript{11}
<table>
<thead>
<tr>
<th>Number</th>
<th>Drugs</th>
<th>Mode of administration</th>
<th>Main limitations and Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Pentavalent antimonials</td>
<td>Parenteral</td>
<td>Prolonged treatment, poor compliance, severe cardiotoxicity, pancreatitis, nephrotoxicity, hepatotoxicity</td>
</tr>
<tr>
<td>2</td>
<td>Amphotericin B</td>
<td>Parenteral</td>
<td>Prolonged hospitalization, severe nephrotoxicity, infusion-related reactions, hypokalemia, metabolic acidosis, cardiotoxicity, high fever</td>
</tr>
<tr>
<td>3</td>
<td>Liposomal amphotericin B</td>
<td>Parenteral</td>
<td>Mild rigors and chills during infusion, mild nephrotoxicity (infrequent and mild)</td>
</tr>
<tr>
<td>4</td>
<td>Miltefosine</td>
<td>Oral</td>
<td>Vomiting and diarrhea, nephrotoxicity, hepatotoxicity, teratogenicity, ease of resistance</td>
</tr>
<tr>
<td>5</td>
<td>Aminosidine</td>
<td>Parenteral</td>
<td>Prolonged treatment, severe nephrotoxicity, ototoxicity, hepatotoxicity</td>
</tr>
<tr>
<td>6</td>
<td>Pentamidine</td>
<td>Parenteral</td>
<td>Prolonged treatment, high rate of hypoglycemia, as a result of pancreatic damage; hypotension, tachycardia, electrocardiographic changes, diabetes, poor response rate</td>
</tr>
</tbody>
</table>

Table 1.1: Toxicity profile of drugs used to treat leishmaniasis

Owing to the phase II clinical trials failure of sitamaquine,27,28 a promising 8-aminoquinoline (Figure 1.6) for the treatment of VL via oral administration, miltefosine remains the only orally applicable drug. The rest have complicated modes of administration. Another promising drug candidate for visceral leishmaniasis to fail in the clinic during its phase II clinical trial in Sudan29 is fexinidazole (Figure 1.6) - thus the clinical pipeline for visceral leishmaniasis is now empty.
Again, although synthetic vaccines have been developed against different protozoan parasites, there are currently no effective vaccines as well as no prophylactic treatments available for leishmaniasis. However, there is strong hope for developing vaccines because recovery from the disease is usually accompanied by strong immunity. Additionally, it is also possible to protect experimental animals against a live challenge. Thus, first generation vaccine candidates for leishmaniasis have been attempted with whole killed parasites/extracts, and among those tested include a *L. amazonensis*-based vaccine, *L. mexicana* (given with Bacille Calmette Guerin, BCG) and *L. major* (also given with BCG). Although results have been encouraging, they are largely inconclusive or negative for prophylaxis. Second generation vaccines on the other hand have been made with recombinant proteins and genetic vaccines. Leish-IIIf+MPL-SE is the only vaccine candidate that has made it to clinical trials.

Given the fact that developing protozoan vaccines is inherently difficult (based on results from available vaccines), chances of developing prophylactic vaccines within the next few years from any form of leishmaniasis are very slim. Hence chemotherapy is still the mainstay of both clinical management and disease control. Therefore pursuing, designing and developing new medications with different modes of action and that are highly effective with a good toxicity profile can lead to the development of novel anti-leishmanial compounds.
1.4 Compound Screening/High throughput screening

In an effort to identify new compounds with anti-leishmanial activity, our laboratory in collaboration with the laboratory of Prof. Dennis Kyle, formerly in the USF Department of Public Health, conducted a high throughput screening (HTS) campaign of a library of biologically active molecules for their antiprotozoal activity against the parasite that causes leishmaniasis using the *Leishmania donovani* axenic amastigote assay.\textsuperscript{31} Compound 1.001, shown in Figure 1.6, was found to exhibit reasonable activity in the assay (IC\textsubscript{50} = 420 nM) and was identified as SNX-2112.\textsuperscript{32,33} This compound and its related analog (SNX-5422) are currently being studied in clinical trials for different cancers,\textsuperscript{34,35} targeting the 90 KDa family of heat shock proteins (Hsp 90).

![SNX-2112 and SNX-5422](image)

Figure 1.7: SNX-2112 (initial hit) and its prodrug analog, SNX-5422

1.5 Heat Shock Proteins 90 (Hsp 90)

Hsp 90 are ubiquitous chaperone proteins that are expressed in all living cells (except Archaea)\textsuperscript{11} upon exposure to elevated temperatures/stress and are believed to play a critical role in acquiring thermotolerance.\textsuperscript{36,37} Heat shock proteins function in the regulation of cell fate and intracellular cell survival, facilitation of protein folding and avoiding miss-folding and protein...
aggregation. Hsp 90 is the target of known bioactive natural products such as geldanamycin (GA) and radicicol (RD), shown in Figure 1.7, and it is a well-known target for the development of small molecule inhibitors in cancer drug discovery. Thus, Hsp 90 or its related orthologs may serve as viable target in antiprotozoal drug discovery, owing to their expression in these cells.

![Figure 1.8: Radicicol (RD) and Geldanamycin (GA)](image)

1.5.1 **Heat shock proteins 90 (Hsp 90) as antiparasitic drug target**

The biological significance of Hsp 90 in a number of protozoan parasites such as *Plasmodium spp.*, *Toxoplasma gondii*, and trypanosomatids (*Trypanosoma* and *Leishmania spp.*) and as a drug target has been established. Both geldanamycin and radicicol are not stable in vivo and exhibit liver toxicity, hence they are not promising candidates for use in humans. However, analogs of geldanamycin, IPI-504, 17-AAG and 17-DMAG, shown in Figure 1.8, have been investigated as less toxic candidates. IPI-504 in particular contains a reduced hydroquinoline derivative, water (H₂O) soluble and can be formulated for parenteral administration. The compound 17-AAG (tanespimycin), an analog of geldanamycin and active against *L. infantum, L. panamensis* and *L. amazonensis*, has undergone clinical trials as an antiparasitic drug.
Other analogs of radicicol such as VER-52296, shown in Figure 1.9, and geldanamycin (19-methyl- and 19-phenylgeldanamycin) may find use as antiparasitic agents. Similarly, the non-radicicol drug, paclitaxel (taxol), has also proven effective on Leishmania Hsp 90.

1.5.2 Heat Shock Proteins 90 (Hsp 90) in Leishmania

Heat shock genes of Leishmania species have been cloned and sequenced and the results indicate that genes encoding the 90 KDa (83 KDa) form are organised in a multi-copy tandem clusters having up to fourteen (14) copies per haploid tandem. It is one of the most abundant proteins in the Leishmania parasite accounting for almost 3% of all cellular proteins. Their constitutive expression upon transmission to their mammalian hosts due to the rise in temperature.
suggests they are crucial for the survival of the parasite.\textsuperscript{51} Indeed Hsp 90 (Hsp 83) homeostasis is critical for proliferation – differentiation of the parasite from promastigote (insect stage) to amastigote (pathogenic stage).\textsuperscript{45,53,54} Thus, the rise in ambient temperature encountered upon transmission to a mammal can be viewed as a signal for cellular differentiation and not as stress.

Predictably, the pharmacological inhibition of \textit{Leishmania donovani} Hsp 90,\textsuperscript{45} as shown in Figure 1.10, or the related parasite \textit{Trypanosoma cruzi}\textsuperscript{55} using known inhibitors such as geldanamycin or radicicol leads to the arrest of proliferating promastigotes of the cell cycle, induction of Hsp 90 protein synthesis and conversion towards amastigote-like morphology.

![Figure 1.11: \textit{L. donovani} promastigotes following 24h incubation (a) at 25°C, pH 7; (b) at 37°C, pH 5.5; (c) at 25°C, pH 7 with 200 ng/mL GA.\textsuperscript{45}](image)

It was therefore hypothesized that compounds that can inhibit the protozoan ortholog of Hsp 90 (Hsp 83) could prove useful in disrupting amastigote growth. Thus, optimization of the initial hit compound, \textbf{1.001} (described in Chapter 2) was initiated, in our search for potential anti-leishmanial agents. To this end, several analogs were synthesized in order to improve upon the pharmacokinetic properties of the initial compound. Critical to the drug discovery efforts was the design of compounds that would not only be selective for the protozoan ortholog of Hsp 90 (Hsp 83), but also meet the following suggested criteria: orally bioavailable, safe, universally effective
over a short course (preferably no more than a 10 day regimen), affordable and capable of withstandng harsh environmental conditions.⁵⁶

1.6 Biological Assays

The anti-leishmanial activity of the synthesized compounds were evaluated in multiple assays including, the axenic amastigote assay (AA, initial screening assay), Figure 1.11, an infected macrophage assay (IM, high content screen assay) and a J774 cytotoxicity assay. Thus each of the analogs was evaluated in the initial screening assay (AA) as well in a high content screening (HCS) infected macrophage assay (with J774 murine macrophages), in order to investigate their anti-leishmanial activity inside of cells. The latter not only allowed the evaluation of amastigote growth inside these cells, but also evaluated the cytotoxicity of the compounds to ensure that the observed activity is not the result of killing the host cells.⁵⁷ Detailed procedures for these assays are provided in the appendices.

![Figure 1.12: 96 Well plate with Leishmania ready to be read in a spectrophotometer](image)
1.7 References


(19) Global plan to combat neglected tropical diseases, 2008–2015


(33) Compound 1 is commercially available from Ark Pharm Inc. in >98% purity and was used without further purification.


2.1 Drug design and Medicinal Chemistry Strategies

The identification of a novel target\textsuperscript{1} is primarily the initial step in designing and developing a new series of anti-leishmanial agents. The initial hit/lead compound, 1.001 (SNX-2112),\textsuperscript{2} shown in Figure 2.1, was identified via high throughput screen (HTS). This compound was not only active in inhibiting axenic amastigote growth of \textit{Leishmania donovani}, but was also found to be an active human Hsp 90 inhibitor, the primary biochemical target.\textsuperscript{3,4}

![Figure 2.1: Initial hit compound SNX-2112](image)

This compound possesses an indazole heterocyclic core and an anthranilamide portion. Molecules possessing the indazole heterocyclic core exhibit a variety of potent biological activities such as serotonin receptor antagonist,\textsuperscript{5} anti-cancer activity\textsuperscript{6,7} and anti-inflammatory activity.\textsuperscript{6} Others have been shown to be an active HIV protease inhibitor,\textsuperscript{8} or exhibit anti-hypertensive properties.\textsuperscript{9} Examples of indazole-based biologically active molecules are shown in Figure 2.2.
Docking\textsuperscript{2} and X-ray crystallographic\textsuperscript{10} studies on analogs of 1.001 depicted 3 critical hydrogen bonds upon binding to human Hsp 90, two of which are contributed by the anthranilamide portion, as shown in Figure 2.3. The protozoan ortholog of Hsp 90 has also been crystallized by Silva and coworkers\textsuperscript{11} and has been shown to exhibit similar binding characteristics.
In the initial discovery of **1.001**, SNX-2112, and its prodrug analog, **1.002**, SNX-5422, as discussed in Chapter 1, Huang and coworkers\(^2\) focused mainly on the anthranilamide portion in their attempt to optimize for pharmacokinetic properties. Thus, little attention was paid to the indazole heterocyclic core as well as the two (2) methyl substituents, but they noted that their presence was required for activity. The overall synthetic and structure and activity relationship (SAR) strategies adopted in our search for anti-leishmanial agents, fully incorporated the binding properties from the aforementioned models (docking) as well as modifications to the anthranilamide and indazole portions of the molecule. The necessity/importance of the two (2) methyl groups was also evaluated for activity.
Thus anti-leishmanial compounds based on the Hsp 90 inhibitor, SNX-2112, were synthesized, with the primary goal of designing compounds that would be selective for the protozoan ortholog of Hsp 90 (Hsp 83). In an attempt to confirm the initial hit, a simplified version of 1.001 was synthesized for a number of carefully considered reasons; not only was it feasible and synthetically less challenging to make compound 2.008, as shown in Scheme 2.1, as the first test compound, but a new data set to compare the anti-leishmanial activity would also prove useful. Furthermore, to optimize for pharmacokinetics and hopefully restrict the cytotoxicity that may arise when the compound binds to human Hsp 90, the -CF$_3$ group was deleted from the indazole heterocyclic core and a methyl (-CH$_3$) group was installed instead.$^{12} $

2.2 Synthesis of initial analog, 2.008

A variety of synthetic methods has been developed for indazole synthesis; however, the synthetic approach adopted has the advantage of being flexible enough to make a broad range of different modifications via robust synthetic chemistry, as shown in Scheme 2.1. This would enable the rapid preparation of several novel anti-leishmanial agents for SAR and structural optimization studies. Indazole heterocyclic rings can be achieved through condensation reaction involving a diketone and a hydrazinyl derivative.$^{15}$

2.2.1 Synthesis of initial test compound, 2.008: 2-((3-methoxypropyl)amino)-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzamide, 2.008.

Synthesis of the initial indazole analog 2.008, shown in Scheme 2.1, required a triketone 2.003 and an arylhydrazinyl derivative 2.005 for the key step. The triketone 2.003 was conveniently prepared from dimedone (2.001) and acetic anhydride (2.002) in the presence of N,N-diisopropylethylamine (DIPEA, Hunig’s base) and a catalytic amount of 4-dimethylamino-
pyridine (DMAP). Substitution of the fluorine of 2-bromo-4-fluorobenzonitrile, 2.004, with hydrazine in tetrahydrofuran (THF) under room temperature conditions afforded the known hydrazinyl compound 2.005. The fluoride substitution was feasible due to the presence of the cyano-group on the benzene ring which acts an activator in the reaction process, while itself being a convenient synthetic precursor for the carboxamide later in the synthesis.

Scheme 2.1: Synthesis of initial analog 2.008

Reagents and conditions: (a) iPrNEt, DMAP, DCM, rt, 89%; (b) H₂N-NH₂, rt, THF, 94%; (c) 2.003, EtOH, AcOH, rt, 59%; (d) H₂N(CH₂)₃OMe, Pd(OAc)₂, DPPF, NaOtBu, Toluene, mW, 120 °C, 64%; (e) H₂O₂, aqueous NaOH, EtOH, DMSO, mW, 100 °C, 72%.
The advanced intermediate condensation product, 2.006, was obtained by treating the triketone, 2-acetyl-5,5-dimethylcyclohexane-1,3-dione, 2.003, with 2-bromo-4-hydrazinylbenzonitrile, 2.005, in a mixture of ethanol (EtOH) and acetic acid (AcOH). This product was able to undergo the traditional Hartwig-Buchwald amination\(^\text{19}\) reaction using conditions involving palladium acetate \([\text{Pd(OAc)}_2]\), 1,1’-bis(dimethylphosphino)ferrocene (DPPF), and sodium tert-butoxide (NaOtBu) in toluene at 120 °C under microwave irradiation (mW) to install the methoxy propylamine side chain in compound 2.007. This procedure was followed by hydrogen peroxide (H\(_2\)O\(_2\)) catalysed hydration of the benzonitrile to the corresponding benzamide\(^\text{20}\) in a mixture of EtOH and dimethyl sulfoxide (DMSO) in the presence of aqueous sodium hydroxide (NaOH) to afford the target compound 2.008.

The addition of DMSO to the reaction system is known to both improve yields and increase the rate of the hydration process.\(^\text{2}\) Gratifyingly, the synthesized compound 2.008 retained its activity in the axenic amastigote assay with an IC\(_{50}\) of 0.65µM. As expected, the reaction conditions of the final hydration step also yielded the acid by-product compound, 2.009, Figure 2.4, which also showed good activity in the assays. The excellent \textit{in vitro} activity of the initial analog led to the explororation of closely related analogs based on Hsp 90 inhibitors, guided by computational modelling.

![Figure 2.4: Acid by-product of initial analog](image-url)
2.2.2 Computational modelling strategies

In order to optimize for activity and improve upon the pharmacokinetic properties and/or selectivity of the anti-leishmanial agents, a computational model for binding into the active site of human Hsp 90 was developed to guide the design, synthesis and development of further analogs. Publicly available coordinates (3D0B)\textsuperscript{10} were used to dock a related quinazoline analog \textit{2.010}, \textbf{Figure 2.5}, with the active site of Hsp 90 in Autodock 1.5.6.\textsuperscript{21}

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{initial_analog.png}
\caption{Initial analog 2.008 and quinazoline analog 2.010}
\end{figure}

The observations from the docking studies included a depiction of the methyl groups orienting towards a hydrophobic pocket,\textsuperscript{12} as shown in \textbf{Figure 2.6}. The choice of the quinazoline analog \textit{2.010} was based not only on its good activity in the assays, but it also restricts most of the rotatable bonds to facilitate computational analysis. Moreover, and perhaps most crucial, is the fact that it maintains the amide-like nature of the initial analog as well as all of the hydrogen-bonds that is expected to exist.
Based on the docking studies, the necessity of the dimethyl substituents in the initial lead compound was investigated, given the fact that their presence was the result of using 5,5-dimethylcyclohexane-1,3-dione (dimedone) as the starting material. Thus, compound 2.013 was prepared using 2-acetylcyclohexane-1,3-dione as the starting material, Scheme 2.2, and following similar synthetic steps as outlined above.

Scheme 2.2: Synthesis of desmethyl analog of 1.001

Reagents and conditions: (a) DIPEA, DMAP, DCM, rt, 67%; (b) i. 2.005, EtOH, AcOH, rt, 52%; (ii) H₂N(CH₂)₃OMe, Pd(OAc)₂, DPPF, NaOtBu, Toluene, mW, 120 °C, 74%; (iii) H₂O₂, aqueous NaOH, EtOH, DMSO, mW, 100 °C, 81%.
Predictably, preliminary SAR studies of this analog proved that, the geminal dimethyl groups are critical for antiprotozoal activity, since compound 2.013 showed no inhibitory activity in the axenic amastigote assay (IC$_{50}$ 25 µM), suggesting that the hydrophobic pocket where they were oriented could be exploited to gain additional activity and/or selectivity. Based upon this finding, a plan was put in place to maintain the methyl groups or diversify the entire indazoles bicyclic core while maintaining the anthranilamide portion of the molecule. In all of the synthetic efforts it was ensured that the compounds conformed to the traditional physicochemical parameters such as Lipinski’s Rule of 5, in order to maximize the likelihood of identifying compounds with optimal pharmacokinetic properties and acceptable oral bioavailability.

2.2.3 **Synthesis of methyl substituted analog, 2.017: 2-((3-methoxypropyl)amino)-5-methyl-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzamide**

A methyl substituted version, 2.017, of 1.001, **Figure 2.7**, was also prepared using similar synthetic routes as outlined previously. However, the condensation reaction between the methyl substituted arylhydrazinyl derivative, 2014, and the triketone, 2.003, was not possible under room temperature conditions, **Scheme 2.3**.

![Figure 2.7: Methyl substituted analog 2.017 and XL-888](image-url)
The rationale for the synthesis of 2.017 was based on the striking similarities between the anthranilamide portion of 1.001 and the known active Hsp 90 inhibitor, XL888, which is at various stages in clinical trials for different cancers. The methyl substituent on the anthranilamide of XL888 was designed to twist the phenyl ring out of planarity. Thus a methyl group was introduced into the first test compound in order to elicit a similar twisting effect and to evaluate its effect on anti-leishmanial activity as shown in Scheme 2.3.

Scheme 2.3: Synthesis of methyl substituted analog

Reagents and conditions: (a) i. 2.003, MeOH, AcOH, rt; ii. Reflux, 73%; (b) i) H₂N(CH₂)₃OMe, Pd(OAc)₂, DPPF, NaOtBu, Toluene, mW, 120 ºC, 66%; (ii) H₂O₂, aqueous NaOH, EtOH, DMSO, mW, 100 ºC, 70%.

The methylsubstituted arylhydrazinyl derivative 2.014 was obtained in 70% yield by treating 2-bromo-4-fluoro-5-methylbenzonitrile with hydrazine in THF. This compound was coupled to 2.003 under refluxing conditions to obtain the key intermediate 2.016. Ordinarily, this reaction
would go smoothly under room temperature conditions, however owing to the presence of sterics, the intermediate 2.015 (confirmed by LCMS) could not proceed to 2.016 until after being subjected to refluxing conditions. This was followed by the Hartwig-Buchwald amination and cyanide hydration to arrive at the target compound in 70% yield.
2.3 Results and discussion

The anti-leishmanial activity of the synthesized analogs were evaluated in the laboratory of Prof. Dennis Kyle by Dr. Brian Vesely, (formerly in the USF Department of Public Health), in the initial high throughput axenic amastigote assay as well as in a high content screening (HCS) infected-macrophage amastigote assay. The latter employed transformed J774 human macrophage cell\textsuperscript{24} lines, which allowed the evaluation of amastigote growth inside the cells as well as determined the cytotoxicity of the analogs to ensure that the observed activity was not a result of killing the host cells. Figure 2.8 depicts the test compounds and Table 2.1 displays the results of their corresponding biological evaluations. Miltefosine – a currently marketed anti-leishmanial drug was used as the standard molecule.

![Figure 2.8: Test compounds]

\[ R = \text{NH}_2, \text{OH} \]
\[ R_1 = \text{H}, \text{CH}_3; R_2 = \text{H}, \text{CH}_3 \]
Each of these analogs was active in inhibiting *L. donovani* amastigote growth, with **2.009** and **2.017** showing good selectivity at approximately 22 and 20 respectively. The initial synthetic analog **2.008** retained its anti-leishmanial activity in the sub-micromolar region with a four (4) fold selectivity over J774 cell lines, which compared very well to the cytotoxicity of the initial hit compound, **1.001**. Removal/deletion of the two (2) geminal dimethyl groups in compound **2.013** led to a complete loss of activity, signifying that both methyl groups are required for activity. Juxtaposition of this data with the docking studies suggests that this position can be exploited to gain additional selectivity, activity and/or improved pharmacokinetics.

Table 2.1: Biological data for indazole series

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
<th>Selectivity (Cytotox. IC₅₀/IM IC₅₀)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Axenic Amastigote</strong></td>
<td><strong>J774 Cytotoxicity</strong></td>
<td><strong>Infected Macrophage</strong></td>
</tr>
<tr>
<td>Miltefosine</td>
<td>3.26</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>1.001</td>
<td>0.42</td>
<td>0.06</td>
</tr>
<tr>
<td>2.008</td>
<td>NH₂</td>
<td>H</td>
</tr>
<tr>
<td>2.009</td>
<td>OH</td>
<td>H</td>
</tr>
<tr>
<td>2.013</td>
<td>NH₂</td>
<td>H</td>
</tr>
<tr>
<td>2.017</td>
<td>NH₂</td>
<td>CH₃</td>
</tr>
</tbody>
</table>

Compound **2.009** (acid-by-product) and **2.017** (methyl-substituted analog) exhibited considerably more activity in the infected macrophage assay with IC₅₀’s of 0.88 and 0.65 µM respectively, over
the initial compound 2.008 as well as the clinically used drug, miltefosine. More profound was that compound 2.017, the most potent analog thus far, had selectivity of almost 20.
2.4 Experimental section

All reagents and solvents, unless specifically stated, were used as obtained from their commercial sources without further purification. Air and moisture sensitive reactions were performed under an inert atmosphere using either argon or nitrogen in a previously oven-dried or flame-dried reaction flask, and addition of reagents were done using a syringe. Dry tetrahydrofuran (THF) was obtained via distillation from sodium benzophenone ketyl. All microwave (mW) reactions were carried out with an Anton Paar monowave 300 instrument. Thin layer chromatography (TLC) analyses were performed using 200 μM pre-coated Sorbtech fluorescent TLC plates and spots were visualized using UV light and/or by staining with different stains including iodine vapor, ceric ammonium nitrate, acidic vanillin or acidic anisaldehyde. Analytical LCMS data was obtained using an Agilent 1100 HPLC/MSD system equipped with a diode array detector running a MeOH/H$_2$O gradient. Purification of compounds was carried out using Sorbtech silica gel (60Å porosity, 40-63μM particle size) in fritted medium pressure liquid chromatography (MPLC) cartridges and eluted with Thomson Instrument SINGLE StEP pumps. Reverse phase preparative HPLC (prepHPLC) purification was achieved on an Agilent preparative 1200 LC/6120B equipped with a single quadrupole mass spectrometer detector.

High resolution mass spectral (HRMS) data was performed on an Agilent 6540 Ultra-High-Definition (UHD) Liquid chromatography quadrupole time-of-flight mass spectrometry (LC/QToF-MS) with electrospray ionization (ESI) detection. Nuclear magnetic resonance (NMR) spectrometry was run on a Varian Inova 500 MHz, Varian Inova 400 MHz or Varian Mercury 400 MHz spectrometer and data was processed using the ACD/NMR Processor program. Chemical shifts are reported in ppm with residual solvent peaks referenced as internal standard. All
compounds were determined to be >95% pure by HPLC analysis and further confirmed by NMR analysis.

**General synthetic procedures:**

\[ \text{2-Acetyl-5,5-dimethylcyclohexane-1,3-dione:} \]

To a solution of 5,5-dimethylcyclohexane-1,3-dione (10.0 g, 71.3 mmol), Hunig’s base (13.0 mL, 74.4 mmol) and DMAP (0.435 g, 3.560 mmol) in DCM (200 mL) was added acetic anhydride (7.0 mL, 74 mmol) and the reaction mixture was stirred overnight at room temperature (rt). The solution was then concentrated on a rotary evaporator, and the resultant yellow oil was partitioned between hexanes and 1 N HCl. The organic phase was then washed with brine, dried over MgSO\(_4\), filtered and concentrated on a rotary evaporator to give the product as a brown oil which was purified by flash column chromatography (hexanes/ethyl acetate 9:1 to 2:1) to afford **2.003** as a light yellow oil (11.6 g, 89%). \(^1H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 18.01 (s, 1H), 2.49 (s, 3H), 2.45 (s, 2H), 2.26 (s, 2H), 0.98 (s, 6H) ppm. \(^13C\) NMR (126 MHz, CDCl\(_3\)) \(\delta\) 202.3, 197.8, 195.0, 112.3, 52.4, 46.8, 30.5, 28.4, 28.1 ppm. LRMS (ESI) m/z calc’d for C\(_{10}\)H\(_{14}\)O\(_3\) [M+H]\(^+\): 183.10, found 183.10.

\[ \text{2-Acetylcyclohexane-1,3-dione:} \]

This compound was synthesized in the same manner as compound **2.003** (67%). \(^1H\) NMR (500 MHz, CDCl\(_3\)) \(\delta\) 18.04 (s, 1H), 2.59 (t, \(J = 6.5\) Hz, 2H),
2.51 (s, 3H), 2.38 - 2.44 (m, 2H), 1.91 (td, J = 13.1, 6.53 Hz, 2H) ppm. $^{13}$C NMR (126 MHz, CDCl$_3$) δ 203.0, 198.6, 195.3, 113.4, 38.5, 33.2, 28.7, 18.9 ppm. LRMS (ESI) m/z calc’d for C$_8$H$_{10}$O$_3$ [M+H]$^+$: 155.06, found 155.

2-Bromo-4-hydrazinylbenzonitrile: To a solution of 2-bromo-4-fluorobenzonitrile (2.51 g, 12.5 mmol) in dry THF (15 mL) was added anhydrous hydrazine (5.00 mL, 159 mmol) and the reaction mixture was stirred overnight at rt leading to the formation of an off-white solid precipitate. Additional THF (50 mL) was added to dissolve the solid which was then washed with saturated sodium bicarbonate solution. The isolated organic phase was concentrated under reduced pressure and the white solid was washed with H$_2$O, followed by diethyl ether (30 mL) and dried under reduced pressure to afford the title compound (2.5 g, 94%). $^1$H NMR (500 MHz, DMSO) δ 8.04 (s, 1H), 7.47 (d, J = 8.8 Hz, 1H), 7.06 (d, J = 1.5 Hz, 1H), 6.69 - 6.77 (m, 1H), 4.38 (s, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 156.7, 135.4, 125.9, 119.6, 113.6, 110.4, 98.5 ppm. LRMS (ESI) m/z calc’d for C$_7$H$_6$BrN$_3$ [M + H]$^+$: 211.97/213.97, found 212.08/214.08.

2-Bromo-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzonitrile: To a solution of 2-bromo-4-hydrazinylbenzonitrile, 2.005, (2.156 g, 10.17 mmol) and 2-acetyl-5,5-
dimethylcyclohexane-1,3-dione, 2.003, (1.86 g, 10.2 mmol) in EtOH (40 mL) was added acetic acid (1 mL), and the reaction mixture was stirred at room temperature for 72 hours. After formation of the product, monitored by TLC/LCMS, the mixture was concentrated on a rotary evaporator and purified by flash column chromatography eluting with hexanes and ethyl acetate (9:1 to 2:1 to 1:1) to obtain the target compound as an orange solid (2.2 g, 59%). ^1H NMR (500 MHz, DMSO) δ 8.08 - 8.13 (m, 2H), 7.80 (dd, J = 8.6, 2.20 Hz, 1H), 3.00 (s, 2H), 2.39 (s, 3H), 2.33 (s, 2H), 1.01 (s, 6H) ppm. ^13C NMR (126 MHz, DMSO) δ 193.3, 151.0, 150.0, 142.9, 136.4, 127.1, 125.9, 122.6, 117.7, 117.3, 113.3, 52.1, 36.6, 35.9, 28.2, 13.6 ppm. LRMS (ESI) m/z calc’d for C\textsubscript{17}H\textsubscript{16}N\textsubscript{3}OBr [M + H]^+: 358.0/360.0, found 357.8/359.8.

![Image](https://example.com/image.png)

2-bromo-4-(3-methyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzonitrile: This compound was synthesized in the same manner as compound 2.006 (52%). ^1H NMR (500 MHz, DMSO) δ 8.08 - 8.12 (m, 2H), 7.78 - 7.83 (m, 1H), 3.07 (t, J = 6.1 Hz, 2H), 2.37 - 2.44 (m, 5H), 2.05 (quin, J = 6.4 Hz, 2H) ppm. ^13C NMR (126 MHz, DMSO) δ 194.0, 152.2, 150.3, 143.0, 136.3, 127.0, 125.8, 122.6, 118.6, 117.3, 113.3, 38.2, 23.5, 23.4, 13.6 ppm. LRMS (ESI) m/z calc’d for C\textsubscript{15}H\textsubscript{12}BrN\textsubscript{3}O [M + H]^+: 330.0/332.0, found 330.2/332.2.
Synthesis of 2-((3-methoxypropyl)amino)-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzonitrile: A mixture of 2-bromo-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzonitrile, 2.006, (400.0 mg, 1.117 mmol), 1,1" bis(diphenylphosphino)ferrocene (61.50 mg, 0.741 mmol), sodium 2-methylpropan-2-olate (2150 mg, 2.233 mmol), Pd(OAc)$_2$ (15.04 mg, 0.067 mmol) and 3-methoxypropan-1-amine (199 mg, 2.233 mmol) in dry toluene was added to 30 mL microwave vial and the content was heated to 120 °C for 18 minutes in a microwave reactor. Upon cooling, DCM was added, transferred to a round bottomed flask, concentrated on a rotary evaporator and purified by flash column chromatography (eluting with 50% ethyl acetate in hexanes). The product was isolated as an off-white solid (524 mg, 64%). $^1$H NMR (500 MHz, DMSO) δ 7.62 (d, $J = 8.3$ Hz, 1H), 6.87 (s, 1H), 6.82 (d, $J = 8.3$ Hz, 1H), 6.45 - 6.53 (m, 1H), 3.41 (t, $J = 5.6$ Hz, 2H), 3.19 - 3.29 (m, 5H), 2.93 (s, 2H), 2.25 - 2.43 (m, 5H), 1.75 - 1.86 (m, 2H), 1.00 (s, 6H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 193.7, 151.7, 150.4, 149.2, 143.5, 135.1, 117.9, 117.1, 110.7, 105.4, 93.7, 70.4, 58.4, 52.1, 40.7, 36.9, 35.8, 28.6, 28.1, 13.5 ppm. LRMS (ESI) m/z calc’d for C$_{21}$H$_{26}$N$_4$O$_2$ [M + H]$^+$: 367.2, found 367.2.
2-((3-methoxypropyl)amino)-4-(3-methyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzonitrile: This compound was synthesized in the same manner as compound 2.007 (74%).

$^1$H NMR (500 MHz, CD$_3$OD) δ 7.54 (d, $J = 8.3$ Hz, 1H), 6.92 (d, $J = 2.0$ Hz, 1H), 6.82 (dd, $J = 8.3, 2.0$ Hz, 1H), 3.54 (t, $J = 5.6$ Hz, 2H), 3.36 - 3.39 (m, 2H), 3.35 (s, 3H), 3.04 (t, $J = 6.4$ Hz, 2H), 2.49 - 2.53 (m, 2H), 2.46 (s, 3H), 2.11 - 2.17 (m, 2H), 1.88 - 1.95 (m, 2H) ppm. $^{13}$C NMR (126 MHz, CD$_3$OD) δ 195.4, 151.6, 150.1, 143.2, 133.9, 117.6, 116.7, 110.4, 105.1, 94.3, 70.7, 57.6, 40.9, 37.6, 28.3, 23.4, 23.2, 12.0 ppm. LRMS (ESI) m/z calc’d for C$_{19}$H$_{22}$N$_4$O$_2$ [M + H]$^+$: 339.2, found 339.4.

2-((3-methoxypropyl)amino)-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzamide: A mixture of 2-((3-methoxypropyl)amino)-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzonitrile, 2.007, (88.00 mg, 0.246 mmol), 50% w/w NaOH (9.000 µL, 0.246 mmol), and 30% H$_2$O$_2$ (143 µL, 4.67 mmol) in EtOH (800 µL) and DMSO (200 µL) was added to a 2 mL microwave vial. The reaction mixture was heated to 100°C for 40 minutes in a microwave reactor. Upon cooling, the mixture was transferred into a separatory funnel followed
by the addition of H$_2$O and extracted with ethyl acetate and the organic solvent was removed in vacuo. The residue was purified by preparative HPLC using an acetonitrile (ACN)/H$_2$O gradient and lyophilized to give the product as a white fluffy solid (68 mg, 72%). $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.03 - 8.16 (m, 1H), 7.47 (d, $J$ = 8.3 Hz, 1H), 6.80 (d, $J$ = 1.5 Hz, 1H), 6.62 (dd, $J$ = 8.3, 1.5 Hz, 1H), 5.78 - 5.93 (m, 1H), 3.50 (t, $J$ = 6.0 Hz, 2H), 3.27 - 3.36 (m, 5H), 2.81 (s, 2H), 2.53 (s, 3H), 2.38 (s, 2H), 1.93 (quin, $J$ = 6.4 Hz, 2H), 1.77 - 1.87 (m, 1H), 1.09 (s, 6H) ppm. $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 193.5, 171.3, 150.6, 150.0, 149.1, 142.7, 129.6, 117.2, 112.6, 109.5, 106.8, 70.2, 58.7, 52.4, 40.7, 37.6, 35.8, 29.1, 28.4, 13.4 ppm. HRMS m/z: [M + H]$^+$ calc’d for C$_{21}$H$_{28}$N$_4$O$_3$ 385.2240; Found 385.2248.

2-((3-methoxypropyl)amino)-4-(3-methyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzamide: This compound was synthesized in the same manner as compound 2.008 and was obtained as a white fluffy solid (81%). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 7.70 (d, $J$ = 8.3 Hz, 1H), 6.84 (d, $J$ = 2.0 Hz, 1H), 6.70 (dd, $J$ = 8.3, 2.0 Hz, 1H), 3.52 (t, $J$ = 6.0 Hz, 2H), 3.27 - 3.34 (m, 8H), 3.03 (t, $J$ = 6.4 Hz, 2H), 2.45 - 2.53 (m, 5H), 2.11 - 2.17 (m, 2H), 1.89 - 1.94 (m, 2H) ppm. $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 195.5, 172.5, 151.4, 150.7, 149.7, 142.0, 130.0, 117.2, 113.3, 108.8, 105.7, 69.9, 57.5, 39.5, 37.7, 28.7, 23.4, 23.1, 12.0 ppm. HRMS m/z: [M + H]$^+$ calc’d for C$_{19}$H$_{24}$N$_4$O$_3$ 357.1927; Found 357.1926.
2-((3-methoxypropyl)amino)-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzoic acid: This compound was obtained as a side product during the final hydration step of 2.008 as an off-white solid. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.11 (d, $J = 8.8$ Hz, 1H), 7.10 (br. s., 1H), 6.87 (d, $J = 7.8$ Hz, 1H), 3.58 (t, $J = 5.6$ Hz, 2H), 3.32 - 3.47 (m, 5H), 2.86 - 2.94 (m, 2H), 2.56 (s, 3H), 2.40 - 2.46 (m, 2H), 2.00 - 2.10 (m, 2H), 1.22 - 1.31 (m, 1H), 1.09 (s, 6H) ppm. $^{13}$C NMR (101 MHz, CDCl$_3$) $\delta$ 193.5, 171.3, 150.6, 150.3, 149.3, 144.1, 133.9, 117.5, 110.9, 109.6, 107.5, 70.3, 58.7, 52.3, 41.9, 37.7, 35.9, 28.6, 28.4, 13.4 ppm. HRMS m/z: [M+H]$^+$ calc’d for C$_{21}$H$_{27}$N$_3$O$_4$ 386.2080.

2-bromo-4-hydrazinyl-5-methylbenzonitrile: Using conditions identical to those described above, 2.014 was obtained as white crystalline solid (70%). $^1$H NMR (500 MHz, DMSO) $\delta$ 7.53 (s, 1H), 7.33 (s, 1H), 7.28 (s, 1H), 4.36 (s, 2H), 1.99 (s, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO-d$_6$) $\delta$ 154.5, 134.7, 123.8, 120.2, 119.6, 112.7, 98.5, 17.0 ppm. LRMS (ESI) m/z calc’d for C$_8$H$_8$BrN$_3$ [M+H]$^+$: 226.08/228.08, found 225.90/227.90.
2-bromo-5-methyl-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzonitrile: Using conditions identical to those described above, 2.016, was obtained as an orange solid (73%). $^1$H NMR (500 MHz, DMSO) $\delta$ 8.11 (s, 1H), 7.97 (s, 1H), 2.57 (s, 2H), 2.38 (s, 3H), 2.31 (s, 2H), 2.07 (s, 3H), 1.00 (s, 6H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 193.1, 151.9, 148.9, 142.0, 137.7, 136.8, 132.0, 122.3, 117.1, 115.9, 115.8, 52.4, 35.9, 35.0, 28.1, 17.2, 13.5 ppm. LRMS (ESI) m/z calc’d for C$_{18}$H$_{18}$BrN$_3$0 [M+H]$^+$: 372.27/374.27, found 372.2/374.2

2-((3-methoxypropyl)amino)-5-methyl-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzonitrile: Using conditions identical to those above, 2.016 (b) i was obtained as a brownish solid (66%). $^1$H NMR (400 MHz, DMSO) $\delta$ 7.53 (s, 1H), 6.65 (s, 1H), 6.24-6.31 (m, 1H), 3.36 (d, $J$ = 1.0 Hz, 2H), 3.13-3.22 (m, 5H), 2.34 (s, 3H), 2.28 (s, 2H), 1.85 (s, 3H), 1.74 (quin, $J$ = 1.0 Hz, 2H), 0.97 (s, 6H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 192.9, 151.2, 150.0, 148.2, 142.5, 135.8, 122.1, 117.7, 115.5, 110.3, 95.8, 70.4, 58.3, 52.4, 40.7, 35.8, 35.3, 28.7, 28.1, 16.1, 13.5 ppm. LRMS (ESI) m/z calc’d for C$_{22}$H$_{28}$N$_4$O$_2$ [M+H]$^+$: 381.49, found 381.40.
Synthesis of 2-((3-methoxypropyl)amino)-5-methyl-4-(3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazol-1-yl)benzamide: Using conditions identical to those described above, 2.017 was obtained as a pale yellow solid (70%). $^1$H NMR (500 MHz, DMSO) $\delta$ 8.05 (t, $J = 5.5$ Hz, 1H), 7.84-8.00 (m, 1H), 7.64 (s, 1H), 7.30 (br s, 1H), 6.53 (s, 1H), 3.39 (t, $J = 6.1$ Hz, 2H), 3.21 (s, 3H), 3.07 – 3.16 (m, 2H), 2.52 (s, 2H), 2.38 (s, 3H), 2.30 (s, 2H), 1.89 (s, 3H), 1.75 (quin, $J = 6.5$ Hz, 2H), 1.00 (s, 6H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 193.1, 171.2, 151.1, 148.9, 147.9, 140.8, 132.1, 119.4, 115.5, 115.3, 109.9, 70.0, 58.4, 52.4, 39.9, 35.8, 35.4, 29.1, 28.2, 16.5, 13.6 ppm. HRMS m/z: [M+H]$^+$ calc’d for C$_{22}$H$_{30}$N$_4$O$_3$ 399.2396; Found 399.2407.
2.5 References

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(21) Trott, O.; Olson, A. AutoDock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of Computational Chemistry* 2009, NA-NA.


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Chapter 3
Tetrahydroquinoline Analog

3.1 Tetrahydroquinoline scaffold in biologically active molecules

The privileged scaffold, tetrahydroquinoline, is an important substructure of various natural products and pharmaceutical agents with a broad range of biological activities. For example, the incorporation of macrocyclic rings onto a tetrahydroquinoline scaffold led to the discovery of angiogenesis agents and inhibitors of an early embryonic development. Substituted tetrahydroquinolines are the core substructure of a number of key pharmacological agents and drug molecules including potent NF-κB inhibitors and cytotoxic agents, anti-tubercular agents, neurotropic agents, HIV protease inhibitors, and anti-tumor agents. The rest include, antibacterial, anti-malarial, antagonist of neuronal Na⁺ channels, inhibitors of Na⁺/H⁺ exchange, agonist of serotonin 5-HT₃ receptor, antagonist of histamine H₃ receptor, agonist of dopaminergic D₂ receptors, agonist of the large conductance calcium-activated potassium channel, agonist of β₃ adrenergic receptors and positive allosteric modulator of the α₇ nicotinic acetyl receptor agents. This subject has been thoroughly reviewed. Some selected tetrahydroquinoline compounds and their biological activities are shown in Figure 3.1. However, to our knowledge, this scaffold has not been previously explored as an anti-leishmanial agents.
Figure 3.1: Selected tetrahydroquinoline compounds and their biological activities

3.2 Rationale for designing tetrahydroquinoline analogs

The strategy to optimize for activity of the initial hit/lead compound was focused on the SAR studies in two main areas: The anthranilamide portion and the indazole bicyclic core, guided by docking studies. This led to the search for a novel scaffold that would be able to mimic the exact same binding orientation as 1.001, as shown in Figure 3.2, hence the choice for the privileged scaffold – tetrahydroquinoline.
It was rationalized that the nitrogen of tetrahydroquinoline analog 3.013 would serve as a viable mimic for the carbonyl in 1.001. Thus, synthesis which incorporated the tetrahydroquinoline substructure was initiated. Owing to the broad range of biological activities of the privileged scaffold, there has been considerable interest in the design and development of new and efficient synthetic methodologies aimed at obtaining tetrahydroquinoline derivatives in high yields and good purity.\textsuperscript{1,5,9,10,11} The approach to the synthesis of the tetrahydroquinoline scaffold in analog 3.013 followed a palladium-catalysed oxidation of a γ-hydroxyenaminone, as described in Scheme 3.1.

### 3.3 Synthesis of the first tetrahydroquiniline analog: 4-((7,7-dimethyl-5,6,7,8-tetrahydroquinolin-5-yl)amino)-2-((3-methoxypropyl)amino)benzamide.

Synthesis of the tetrahydroquinoline scaffold, Scheme 3.1, was initiated by a condensation reaction between 5,5-dimethylcyclohexane-1,3-dione, 3.001, and the corresponding 3-aminopropanol in the presence of molecular sieves to yield γ-hydroxyenaminone, 3.002.\textsuperscript{12}
Scheme 3.1: Synthesis of 7,7-dimethyl-5,6,7,8-tetrahydroquinolin-5-amine

Reagents and conditions: (a) H$_2$N(CH$_2$)$_3$OH, PhMe, reflux, 95%; (b) Pd(OAc)$_2$, PPh$_3$, K$_2$CO$_3$, mesityl bromide, DMF, 150 °C, 50%; (c) H$_2$NMe, HCl, DIPEA, 81%; Pd/C, H$_2$, TFA, Parr shaker.

Palladium-catalysed oxidation of 3.002 was followed by cyclization and spontaneous aromatization to provide the corresponding quinolin-5-one, 3.003, in 67% yield. The subsequent oxime intermediate 3.004 was obtained in decent yield (81%) by treating 3.003 with methoxyamine hydrochloride in the presence of Hunig’s base.$^{13,14}$ An attempt at hydrogenating the oxime in trifluoroacetic acid (TFA) to provide the free amine 3.005 yielded no product.$^{15}$ An alternative method was then sought for the synthesis of the tetrahydroquinolinamine, 3.005, Scheme 3.2.
Scheme 3.2: Preparation of advanced intermediate 3.008 via azide formation

Tetrahydroquinolinone 3.003 was subjected to sodium borohydride reduction in MeOH which provided the corresponding alcohol 3.006. The alcohol was converted to azide 3.007 using diphenylphosphoryl azide (DPPA) in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). The azide thus formed was able to undergo the Staudinger reduction with triphenylphosphine and H₂O to yield the free amine, tetrahydroquinolinamine, 3.005. Nucleophilic aromatic substitution of 3.005 with 2.004 yielded the key advanced intermediate 3.008, however the yield was very low. In an attempt to optimize the reaction and increase yield, the alcohol 3.006 was converted to a tosyl group, 3.009, in 80% yield. Functional group interconversion was also effected on 2.004 with a resulting 55% yield to the corresponding aminobromobenzonitrile 3.010, Scheme 3.3 - with the aim of coupling 3.009 and 3.010 together via S₂N₂ reaction, but this failed to provide the desired product.
Scheme 3.3: Preparation of advanced intermediate 3.008 via tosylation

Reagents and conditions: (a) TsCl, Et$_3$N, 80%; (b) 3,4,5-trimethoxybenzylamine (TMBA), TFA, DCM, 140 °C, 55%; (c) 3.009, pTSA

At this stage, the synthesis of quinazolin-5-one 3.003 as well as aminobromobenzonitrile 3.010 had been achieved – the perfect couple for a condensation reaction. Thus, condensation reaction involving 3.003 and 3.010 was initiated using the Dean-Stark trap apparatus leading to the formation of imine 3.011, and subsequent sodium borohydride reduction in MeOH afforded the advanced intermediate 3.008, as depicted in Scheme 3.4.
Scheme 3.4: Synthesis of tetrahydroquinoline analog

Reagents and conditions: (a) pTSA, PhMe, Dean-Stark, 175 °C, 59%; (b) NaBH₄, MeOH, 37%; (c) H₂N(CH₂)₃OCH₃, Pd(OAc)₂, DPPF, NaOtBu, mW, 120 °C, 52%; (d) aqueous NaOH, H₂O₂, EtOH, DMSO, mW, 100 °C, 72%.

With 3.008 bearing bromine as a synthetic handle, the nucleophilic replacement was achieved using the traditional Hartwig-Buchwald amination²⁰ conditions which allowed the installation of the methoxypropylamine side chain. This was followed by H₂O₂ catalysed hydration of the benzonitrile²¹ to afford the final product, 3.013, as the first tetrahydroquinoline analog.
3.4 Synthesis of N7-(7,7-dimethyl-5,6,7,8-tetrahydroquinolin-5-yl)quinazoline-4,7-diamine

Inspired by the activity of the quinazoline analog 2.010 used in the docking studies, preparation of a tetrahydroquinoline-quinazoline analog was initiated. Key to this synthesis was the avoidance of late stage structural modifications as shown in Scheme 3.5.

Scheme 3.5: Tetrahydroquinoline-Quinazoline analog

Reagents and conditions: (a) Formamidine acetate, NaH, DMF, rt; (b) NaH, Dioxane

However, reaction of the advanced intermediate 3.008 with formamidine acetate and a nucleophilic aromatic substitution reaction between 3.005 and 3.015 in the presence of sodium hydride (NaH) or Hunig’s base yielded no product. Failure of the above reactions led to the preparation of quinazoline-4,7-diamine 3.022, Scheme 3.6, targeted at coupling to 3.003. In the
course of this synthesis, an attempt was made to couple the various quinazoline-intermediates to different derivatives of the tetrahydroquinoline scaffold as shown in Scheme 3.6.

Scheme 3.6: Synthesis of quinazoline-4,7-diamine for tetrahydroquinoline-quinazoline analog

Reagents and conditions: CuI, NaN₃, CH₃NH(CH₂)₂NHCH₃, Cs₂CO₃, EtOH, 90 °C, 62%; (b) HCONH₂, 150 °C, mW, 70%; (c) Pd/C, H₂, MeOH, 82%; (d) 3.003, NaBH₃CN, MeOH Or 3.021, DIPEA, MeOH; (e) i. SOCl₂, DMF. ii. 7N NH₃/MeOH, rt, 50%; (f) (Boc)₂O, TEA, MeOH; (g) Pd/C, H₂, THF, MeOH; (h) Pd/C, H₂, DMF, THF/MeOH, rt, 93%; (i) 3.003, pTSA, rt, reflux.
In the above Scheme 3.6, copper-catalysed direct amination\(^{24}\) of 2-chloro-4-nitrobenzoic acid \(3.015\) using sodium azide (NaN\(_3\)) as the amino source under mild conditions provided 2-amino-4-nitrobenzoic acid \(3.016\). This compound was then treated with an excess amount of formamide\(^{25}\) and irradiated in a microwave reactor at 150 °C to obtain the quinazoline derivative \(3.017\). The nitro group in compound \(3.017\) was reduced to the amino derivative \(3.018\), and treated with \(3.003\) in the presence of sodium cyanoborohydride (NaBH\(_3\)CN) but yielded none of the desired product. The amino-derivative \(3.018\) underwent S\(_2\)N displacement reaction with \(3.020\), but the reaction did not produce the desired product.

The nitro-quinazoline derivative \(3.017\) was treated with SOCl\(_2\) in the presence of DMF (catalytic) followed by ammonia to provide aminonitro-quinazoline derivative \(3.021\). It was envisioned that, protecting the amine at the 4-position with an appropriate protecting group such as Boc\(^{26}\) and reducing the nitro-group to the corresponding amine in compound \(3.014\) could serve as a viable precursor to coupling to either \(3.003\) or \(3.020\). However, the Boc-protection reaction did not yield the desired product. The diaminoquinazoline compound \(3.022\) was also obtained, but again, coupling reactions failed. In the latter reaction, there was no anticipation of the amine at the 4-position interfering in the reaction process.

A lot of effort and labour went into designing a second tetrahydroquinoline analog, however data for \(3.013\) showed that the compound was not sufficiently active and also considerably cytotoxic (towards J774 cell lines). This did not warrant the pursuance of another analog of this series. Thus, attention was shifted from this scaffold to focus on other series that showed more promise as anti-leishmanial agents which will be discussed in Chapter 4.
3.5 Results and Discussion

Table 3.1 shows the biological evaluation of tetrahydroquinoline analog 3.013 in the axenic amastigote, J774 cytotoxicity and infected macrophage assays. This was performed in the laboratory of Prof. Dennis Kyle by Dr. Brian Vesely, formerly in the USF Department of Public Health.

<table>
<thead>
<tr>
<th>Compound ID</th>
<th>IC$_{50}$ (µM)</th>
<th>Selectivity (Cytotox. IC$<em>{50}$/IM IC$</em>{50}$)</th>
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<tbody>
<tr>
<td></td>
<td>Axenic Amastigote</td>
<td>J774 Cytotoxicity</td>
</tr>
<tr>
<td>Miltefosine</td>
<td>3.26</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>3.013</td>
<td>2.55</td>
<td>6.75</td>
</tr>
</tbody>
</table>

Table 3.1: Biological data for tetrahydroquinoline analog

The tetrahydroquinoline analog 3.013 showed activity in inhibiting amastigote growth in the assays, however there was no great improvement over the activities of the indazole series, as discussed in Chapter 2. Moreover, the level of selectivity depicted by this analog indicates poor selectivity in the infected macrophage assay compared to cytotoxicity (with a selectivity factor of 1.3).
3.6 Experimental

General synthetic procedures:

7,7-dimethyl-7,8-dihydroquinolin-5(6H)-one: A solution of 5,5-dimethylcyclohexane-1,3-dione (10.0 g, 71.3 mmol) and 3-aminopropan-1-ol (6.00 mL, 71.3 mmol) in toluene (250 mL) was heated to in a Dean-Stark trap until the separation of H₂O had finished. The solvent was removed in vacuo and the residual viscous oil was triturated with ethyl acetate to give a yellow crystalline solid, 3-((3-hydroxypropyl)amino)-5,5-dimethylcyclohex-2-enone (13.3 g, 95%). LRMS (ESI) m/z calculated for C₁₁H₁₉NO [M+H]⁺: 198.28, found 198.0. A portion of this material was used directly in the next step without further purification.

To a solution of 3-((3-hydroxypropyl)amino)-5,5-dimethylcyclohex-2-enone (10 g, 50.7 mmol) in DMF (60 mL) was added mesityl bromide (11.12 g, 55.90 mmol), triphenylphosphine (0.799 g, 3.040 mmol), palladium (II) acetate (0.342 g, 1.522 mmol) and potassium carbonate (8.52 g, 61.7 mmol). The resulting mixture was heated to 150°C for 2 hours. Upon cooling to room temperature, the mixture was filtered through a celite pad and evaporated in vacuo to give an oily residue, which was purified by column chromatography to yield 7,7-dimethyl-7,8-dihydroquinolin-5(6H)-one (4.4 g, 50%). ¹H NMR (400 MHz, CDCl₃) δ 8.68 (dd, J = 4.7, 1.6 Hz, 1H), 8.24 (dd, J = 7.8, 1.6 Hz, 1H), 7.27 (dd, J = 4.7, 7.8 Hz, 1H), 3.03 (s, 2H), 2.53 (s, 2H), 1.10 (s, 6H) ppm. ¹³C NMR (101 MHz, CDCl₃) δ 198.0, 162.2, 153.8, 134.6, 126.5, 122.1, 52.0, 46.4, 33.0, 28.2 ppm. LRMS (ESI) m/z calc’d for C₁₁H₁₃NO [M+H]⁺: 176.23, found 176.20.
**4-amino-2-bromobenzonitrile**: A mixture of 2-bromo-4-fluorobenzonitrile (3.00 g, 15.0 mmol) and (3,4,5-trimethoxyphenyl)methanamine (3.16 g, 16.0 mmol) was added to a 48 mL reaction vial, sealed and then stirred at 140 °C for 1 hour. Upon cooling to room temperature, DCM (7.5 mL) and trifluoroacetic acetic (7.5 mL) were sequentially added, sealed and allowed to stir at room temperature for 24 hours. The resulting mixture was concentrated, diluted with ethyl acetate (100 mL), washed with saturated aqueous sodium bicarbonate (2 x 50 mL) and dried over anhydrous sodium sulfate, filtered, concentrated and purified by gradient flash chromatography, eluting with 0% to 40% ethyl acetate in hexanes to yield the target compound as a pale yellow crystalline solid (1.734 g, 55%). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta 7.36\ (d, J = 8.2\ \text{Hz}, 1\text{H}), 6.87\ (d, J = 2.3\ \text{Hz}, 1\text{H}), 6.56\ (dd, J = 8.4, 2.2\ \text{Hz}, 1\text{H}), 4.19\ (\text{br s, 2H})\) ppm. \(^{13}\)C NMR (126 MHz, CDCl\(_3\)) \(\delta 154.1, 135.1, 126.5, 119.6, 116.7, 111, 104.2\) ppm. LRMS (ESI) m/z calc’d for C\(_7\)H\(_5\)BrN\(_2\) [M+H]^+: 198.04/200.04, found 198.08/200.08.

**2-bromo-4-((7,7-dimethyl-5,6,7,8-tetrahydroquinolin-5-yl)amino)benzonitrile**: Step 1: A mixture of 7,7-dimethyl-7,8-dihydroquinolin-5(6H)-one (0.50 g, 2.85 mmol) and 4-amino-2-
bromobenzonitrile (0.562 g, 2.850 mmol) in toluene (15.0 mL) was heated to reflux in a Dean Stark trap until the separation of H2O had finished. The reaction was monitored by LC/MS for 3-4 days until no starting material was seen. The solution was then concentrated on a rotary evaporator to give the product as a residual viscous oil which was purified by column chromatography using hexanes and ethyl acetate (1:1) as eluent, to give (E)-2-bromo-4-((7,7-dimethyl-7,8-dihydroquinolin-5(6H)-ylidene)amino)benzonitrile as a brownish yellow solid (0.6 g, 59%). LRMS (ESI) m/z calc’d for C18H16BrN3 [M+H]+: 355.25/357.25, found 355.20/357.20. This material was used in the next step.

**Step 2:** To a stirred solution of (E)-2-bromo-4-((7,7-dimethyl-7,8-dihydroquinolin-5(6H)-ylidene)amino)benzonitrile (900 mg, 2.54 mmol) in MeOH (11 mL) was added sodium borohydride (192 mg, 5.08 mmol) portionwise at 0°C. The reaction mixture was stirred at rt for > 5 h and monitored by LCMS. Upon completion of the reaction, the mixture was concentrated on a rotary evaporator and purified with flash column chromatography using hexanes and ethyl acetate (2:1) as the eluent to give the product as a pale white solid (675 mg, 37%). 1H NMR (400 MHz, CDCl3) δ 8.47 (br. s., 1H), 7.68 (d, J = 7.4 Hz, 1H), 7.40 (d, J = 8.6 Hz, 1H), 7.12 - 7.18 (m, 1H), 6.88 (s, 1H), 6.57 (d, J = 8.2 Hz, 1H), 4.74 (d, J = 7.4 Hz, 1H), 4.41 (d, J = 8.6 Hz, 1H), 2.81 (s, 2H), 2.01 - 2.09 (m, 1H), 1.52 - 1.58 (m, 1H), 1.13 (s, 3H), 1.07 (s, 3H) ppm. 13C NMR (101 MHz, CDCl3) δ 156.6, 151.5, 148.6, 135.3, 135.2, 131.3, 126.8, 121.7, 118.5, 115.7, 111.2, 102.0, 77.0, 49.5, 46.0, 42.7, 31.1, 30.6, 25.9 ppm. LRMS (ESI) m/z calc’d for C18H18BrN3 [M+H]+: 357.27/359.27, found 357.20/359.20.
4-((7,7-dimethyl-5,6,7,8-tetrahydroquinolin-5-yl)amino)-2-((3-methoxypropyl)amino)benzonitrile: To a mixture of 2-bromo-4-((7,7-dimethyl-5,6,7,8-tetrahydroquinolin-5-yl)amino)benzonitrile (100.0 mg, 0.281 mmol), palladium (II) acetate (3.200 mg, 0.014 mmol), 1,1'-bis(diphenylphosphino)ferrocene (15.50 mg, 0.187 mmol) and sodium tert-butoxide (54.00 mg, 0.562 mmol) in toluene (0.5 mL) was added 3-methoxypropan-1-amine (50.10 mg, 0.562 mmol) in a 10 mL microwave vial. The reaction mixture was heated to 120 °C in a microwave reactor for 16 minutes. Upon cooling, methylene chloride was added to the reaction mixture and concentrated on a rotary evaporator. The resulting solid was adsorbed onto silica gel using MeOH (3X) and purified by flash chromatography eluting with 100% ethyl acetate. Product was recovered as a brown oil (105.5 mg, 52%). $^1$H NMR (500 MHz, DMSO) δ 8.33-8.40 (m, 1H), 7.62 (d, $J = 7.6$ Hz, 1H), 7.19 (dd, $J = 7.8$, 4.7 Hz, 1H), 7.08-7.15 (m, 1H), 6.68 (d, $J = 8.8$ Hz, 1H), 6.04 (dd, $J = 1.7$, 8.6 Hz, 1H), 5.87 (s, 1H), 5.61 (t, $J = 5.5$ Hz, 1H), 4.65-4.78 (m, 1H), 3.29-3.40 (m, 4H), 3.06-3.20 (m, 3H), 2.61-2.73 (m, 2H), 1.83-1.92 (m, 1H), 1.68-1.79 (m, 2H), 1.54 (dd, $J = 11.3$, 12.4 Hz, 1H), 1.04-1.07 (s, 3H), 0.99 (s, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 156.5, 153.6, 152.3, 148.3, 135.3, 134.4, 132.9, 121.9, 120.2, 102.8, 92.6, 82.4, 70.7, 58.4, 48.6, 46.4, 42.4, 40.7, 31.5, 30.7, 28.7, 26.1 ppm. LCMS (ESI) m/z calc’d for C$_{22}$H$_{28}$N$_4$O [M+H]$^+$: 365.49, found 365.33.
4-((7,7-dimethyl-5,6,7,8-tetrahydroquinolin-5-yl)amino)-2-((3-methoxypropyl)amino)benzamide: A mixture of 4-((7,7-dimethyl-5,6,7,8-tetrahydroquinolin-5-yl)amino)-2-((3-methoxypropyl)amino)benzonitrile (95.00 mg, 0.261 mmol), 50% w/w NaOH (0.100 mL, 0.261 mmol), 30% H₂O₂ (0.15 mL, 4.89 mmol) in EtOH (1 mL) and DMSO (0.45 mL) was added to a 10 mL microwave vial. The reaction mixture was heated to 100°C for 45 minutes. The product was extracted with H₂O and ethyl acetate and following removal of the solvent on rotary evaporator, the product was purified by flash column chromatography to give the product as a pale brown solid (72 mg, 72%). ¹H NMR (500 MHz, CDCl₃) δ 8.48 (d, J = 3.9 Hz, 1H), 8.28 (br s, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.25-7.29 (m, 1H), 7.15 (dd, J = 7.8, 4.9 Hz, 1H), 5.89-5.97 (m, 2H), 5.49 (br s, 2H), 4.75-4.85 (m, 1H), 4.16 (br d, J = 8.3 Hz, 1H), 3.51 (t, J = 6.1 Hz, 2H), 3.35 (s, 3H), 3.23 (br t, J = 6.6 Hz, 2H), 2.83 (d, J = 3.4 Hz, 2H), 2.07-2.14 (m, 1H), 1.87-1.99 (m, 2H), 1.54-1.63 (m, 1H), 1.16 (s, 3H), 1.10 (s, 3H) ppm. ¹³C NMR (126 MHz, CDCl₃) δ 171.9, 156.6, 152.6, 152.1, 148.3, 135.5, 132.8, 130.5, 121.6, 103.1, 100.4, 93.2, 70.4, 58.7, 49.3, 46.2, 43.3, 39.8, 31.3, 30.6, 29.3, 26.0 ppm. HRMS m/z calc’d for C₂₂H₃₀N₄O₂ [M+H]⁺: 383.2447; Found 383.2450.
3.7 References


(8) Sridharan, V.; Suryavanshi, P.A.; Menendez, J.C. Advances in the chemistry of tetrahydroquinolines. *Chemical reviews* 2011, 111 (11), 7157-7259.


Chapter 4

Tropane Analogs

4.1 Tropanes

Tropanes are bicyclic amines possessing both pyrrolidine and piperidine rings that share a common nitrogen and two (2) carbon atoms. Thus, the typical structural element of all tropanes is the azabicyclo[3.2.1]octane skeleton. Tropanes occur naturally as part of esters in plant species, which are generally secondary metabolites of these plants, as shown in Figure 4.1.

![L-Hyoscyamine](image1.png)  ![L-Scopolamine](image2.png)  ![L-Cocaine](image3.png)

**Figure 4.1: Examples of some natural esters of tropanes**

Examples of plant species containing tropane alkaloids include *Datura species* (thorn apple, angel’s trumpet and jimson weed), *Hyoscyamus niger, Atropa belladonna* (deadly nightshade), *Mandragora officinarum, Brugmansia, Atropante, Duboisia, Scopolia, Anisodus* and
Anth thoroughly. Tropane alkaloids (ornithine-derived compounds) are extracted from the world’s oldest plant medicines and have a variety of pharmacological applications, including analgesics, hallucinations and poisons. Owing to their central nervous system (CNS) activity, tropane alkaloids are often abused – one such commonly abused tropane is cocaine.

Tropane derivatives are one of the most economically important pharmaceuticals, Figure 4.2, and over twenty (20) active pharmaceutical ingredients (API) possessing the tropane scaffold have found use as anti-emetics, anesthetics, bronchodilators, anti-spasmodics and mydriatics.

Many different synthetic protocols have been developed for the synthesis of tropane derivatives, due to their many useful applications. However, Robinson’s synthesis of tropinone...
developed in 1917 continues to be the ideal choice for the synthesis of tropanes and its derivatives.¹⁰

4.2 **XL-888 – potent Hsp 90 inhibitor: A novel tropane derivative**

Pursuant to our laboratory’s ongoing efforts to identify novel anti-leishmanial compounds based on Hsp 90 inhibitors and prior docking studies, the intriguing striking similarities shared by the anthranilamide portion of 1.001 with the potent Hsp 90 inhibitor, XL-888¹¹ and its analogs – (tropane derivatives used for the treatment of cancer), *Figure 4.3*, led to the exploration of a hybridized version of a compound that contained our anthranilamide. Aminopiperidine analogs (non-bridged) were also prepared simultaneously to allow us evaluate the necessity/importance of the bicyclic core.

![Chemical structures](image)

*Figure 4.3: Compound 1.001, XL-888 and its analog with proposed binding interactions*
4.3 Synthesis of piperidine analogs

Three (3) piperidine analogs were synthesized, Figure 4.4: benzylamide 4.014, amide 4.018, and an ester 4.021. A convergent synthetic approach was used, wherein the aminopiperidinyl nicotinamide/nicotinate and the anthranilamide portions were each synthesized separately, and then coupled together using an appropriate peptide coupling reagent.

![Figure 4.4: Piperidine analogs](image)

**4.3.1 Synthesis of piperidine benzylamide analog: N1-(1-(5-(benzylcarbamoyl)pyridin-2-yl)piperidin-4-yl)-3-((3-methoxypropyl)amino)terephthalamide:**

**Scheme 4.1: Synthesis of Boc-protected piperidine**

![Scheme 4.1: Synthesis of Boc-protected piperidine](image)

Reagents and conditions: (a) Boc₂O, TEA, DCM, rt, 85%; (b) Pd/C, H₂, MeOH, rt, 90%.

The primary amine functionality of commercially available benzyl-protected piperidine 4.001, **Scheme 4.1**, was reacted with di-tert-butyl-dicarbonate in DCM at room temperature to provide the respective Boc-protected intermediate, 4.002,¹² which subsequently underwent
hydrogenation\textsuperscript{13} to afford the target compound – Boc-protected piperidine, \textit{4.003}. This compound would subsequently undergo nucleophilic aromatic substitution (\textit{S\textsubscript{N}Ar}) with benzyl-6-chloronicotinamide, \textit{4.008}, as described in \textbf{Scheme 4.2}.

\textbf{Scheme 4.2: Synthesis of 6-(4-aminopiperidin-1-yl)-N-benzynicotinamide}

![Scheme 4.2: Synthesis of 6-(4-aminopiperidin-1-yl)-N-benzynicotinamide](image)

\textbf{Reagents and conditions:} (a) i. SOCl\textsubscript{2}, DMF, Toluene, 80 °C. ii. BnNH\textsubscript{2}, DCM, 0 °C - rt, 68%; (b) \textit{4.003}, DIPEA, ACN, reflux, 79%; (c) 1M HCl/Et\textsubscript{2}O, 0 °C - rt, 82%

Reaction of 6-chloronicotinic acid (\textit{4.004}) with thionyl chloride and then benzylamine yielded \textit{N}-benzyl-6-chloronicotinamide\textsuperscript{14} (\textit{4.005}) in an overall yield of 68%. This compound was able to undergo aromatic nucleophilic substitution reaction with \textit{4.003} to furnish compound \textit{4.006},\textsuperscript{15,16} which was subsequently deprotected under acidic conditions\textsuperscript{15} to arrive at the free amine in compound \textit{4.007}. This compound would subsequently be coupled to \textit{4.013} via peptide coupling. The synthesis of \textit{4.013} is provided in \textbf{Scheme 4.3}.
Scheme 4.3: Synthesis of 4-carbamoyl-3-((3-methoxypropyl)amino)benzoic acid

Ethyl 4-aminobenzoate was monobrominated\(^1\) with N-bromosuccinimide (NBS) to give compound 4.009 which was subsequently converted to ethyl 3-bromo-4-cyanobenzoate, 4.010, through cyanidation via diazonium salt formation. This functional group interconversion was initially attempted using sodium nitrite (NaNO\(_2\)) as described,\(^2\) however, what worked best was the use of tert-butyl nitrite and boron trifluoride diethyl etherate followed by the addition of copper (I) cyanide and sodium cyanide.\(^3\) An attempt at installing the methoxypropylamine side chain in compound 4.013 was not possible via the regular Hartwig-Buchwald amination conditions,\(^4\) thus a related reaction condition was employed that uses 4,5-bis(diphenylphosphino)-9,9-dimethylxanthene (XANTPHOS), cesium carbonate and tris(dibenzylideneacetone)-dipalladium (Pd\(_2\)(dba)\(_3\)),\(^5\) with a resulting yield of 81%. With compound 4.013 in hand, the nitrile group underwent H\(_2\)O\(_2\)-catalysed hydration\(^6\) and the carboxylate was hydrolysed under basic conditions (saponification) to yield the carboxylic acid 4.013. However, the presence of aqueous
NaOH in the reaction media resulted in a mixture of both the hydrated derivative and partly saponified product (confirmed by LC/MS). Thus, excess aqueous NaOH was added to force the reaction to the overall target compound **4.013** with a yield of 78%. This action also led to the formation of a diacid side product (verified via LCMS and NMR) as shown in Figure 4.4, which was difficult to separate via prepHPLC.

![Figure 4.5: Diacid acid resulting from addition of excess NaOH](image)

Compound **4.007** was added to **4.013** under appropriate reaction conditions using HATU$^{21}$ as the coupling reagent, which condensed and formed the amide bond as shown in compound **4.014**, Scheme 4.4.

**Scheme 4.4: Synthesis of piperidine-benzyl analog, 4.014**

![Scheme 4.4: Synthesis of piperidine-benzyl analog, 4.014](image)

Reagents and conditions: (a) HATU, DIPEA, DMF, 48%
Following the successful synthesis of the benzylamide analog, 4.014, synthesis of amide and ester analogs we initiated.

### 4.3.2 Synthesis of piperidine amide analog: N1-(1-(5-carbamoylpyridin-2-yl)piperidin-4-yl)-3-((3-methoxypropyl)amino)terephthalamide

**Scheme 4.5: Synthesis of piperidine-amide analog 4.018**

Reagents and conditions: (a) SOCl₂, NH₄OH, Toluene, 73%; (b) i. 4.003, DIPEA, ACN, reflux, 78%. ii. 1M HCl/Etherate, 87%; (c) 4.013, HATU, DIPEA, DMF, 48%

Reaction of chloronicotinic acid, 4.015, with thionyl chloride (SOCl₂) and aqueous ammonia (NH₄OH)²⁴ yielded 2-chloro-5-pyridinecarboxamide 4.016 in overall yield of 73%. The use of ammonia gas for this purpose has been reported,²⁵ however NH₄OH was found to be more convenient for this purpose. Following procedures described above, compound 4.017 was achieved over two (2) steps in 68% overall yield. To compound 4.017 was added 4.013 under appropriate reaction conditions using HATU²¹ as the coupling reagent which condensed and formed the amide bond as shown in compound 4.018.
4.3.3 Synthesis of piperidin-ester analog: ethyl 6-(4-(4-carbamoyl-3-((3-methoxypropyl)amino)benzamido)piperidin-1-yl)nicotinate

**Scheme 4.6: Synthesis of piperidine-ester analog 4.021**

Reagents and conditions: (a) Triethylorthoacetate, toluene, 95%; (b) i. 4.003, DIPEA, CAN, reflux, 78%. ii, 1M HCl/Etherate, 84%; (c) 4.013, HATU, DIPEA, 55%

6-chloronicotinic acid was treated with triethylorthoacetate to yield the corresponding ethyl-6-chloronicotinate.\textsuperscript{26,27} Using a ratio of 1:3 (halonicotinic acid: triethyl orthoacetate) led to an almost quantitative yield. Following procedures identical to the ones described above, \textbf{4.020} was achieved over two (2) steps in 66% overall yield. To compound \textbf{4.020} was added \textbf{4.013} under appropriate reaction conditions using HATU\textsuperscript{21} as the coupling reagent, which condensed and formed the amide bond as shown in compound \textbf{4.021}.
4.4 Synthesis of tropane/bridged analogs

Scheme 4.7 describes the synthesis of all three (3) bridged/tropane analogs 4.030, 4.031 and 4.032 (benzylamide, amide and ester) wherein R has been defined as -NHBn, -OEt or –NH₂.

Scheme 4.7: Synthesis of tropane analogs 4.030, 4.031 and 4.032

Reagents and conditions: (a) NaBH(AcO)₃, BnNH₂, AcOH, DCM, 45%; (b) Pd(OH)₂, (NH₄)₂HCO₂, EtOH, 92%; (c) DIPEA, CbzCl, DCM, rt, 69%; (d) HCl/Et₂O, rt, 84%; (e) R = -OEt: TEA, Dioxane, 51%, R = -NHBn: TEA, ACN, 48%, R = -NH₂: TEA, ACN, 61%; (f) Pd(OH)₂, H₂, MeOH, 72-78%; (g) 4.013, HATU, DIPEA, DMF, 48-55%.
In the above synthetic scheme, **Scheme 4.7**, the primary amine functionality in the known compound 4.024 was introduced stereoselectively under reductive amination conditions by treating Boc-protected tropinone, 4.022, with sodium triacetoxyborohydride in the presence of benzylamine to yield *N*-benzyl protective derivative 4.023. This was followed by debenzylation with Pearlman’s catalyst using ammonium formate as the hydrogen source to obtain 4.024. Reaction between tropane 4.024 and benzyl chloroformate (CbzCl) provided the *N*-Cbz protected derivative, 4.025. Deprotection of the Boc-group under acidic conditions, followed by aromatic nucleophilic substitution reaction with 4.027 furnished compound 4.028. This compound underwent deprotection using the Pearlman’s catalyst in the presence of hydrogen to arrive at the free amine in compound 4.029. To this compound was added 4.013 under appropriate reaction conditions using HATU as the coupling reagent which condensed to form the amide bond as shown in compounds 4.030, 4.031 and 4.032, **Scheme 7**.

The acid by-product, compound 4.033, shown in **Figure 4.5**, was isolated using preparative HPLC. 

![Figure 4.6: Tropane ester acid by product 4.033](image_url)
4.4.1 Alternative synthesis to tropane/bridged analogs

In order to optimize the reaction conditions with increased yields, alternative synthetic route to the tropane/bridged analogs was simultaneously pursued as shown below in Scheme 4.8. However, yields recorded were not superior to what was found in Scheme 4.7.

Scheme 4.8: Alternative synthesis to tropane/bridged analogs

Reagents and conditions: (a) NaBH(ACO)₃, BnNH₂, AcOH, DCM, 45%; (b) Pd(OH)₂, (NH₄)HCO₂, EtOH, 92%; (c) 4.013, HATU, TEA, DMF, 52%; (d) 1M HCl/Et₂O, 18-39%; (e) 4.027, TEA, ACN or Dioxane, 47-58%.
4.5 Results and Discussions

Results of the biological evaluations (performed in the laboratory of Prof. Dennis Kyle by Dr. Brian Vesely, formerly in the USF Department of Public Health) are displayed in Table 4.1. Evidently the apparent hydrophobic pocket can accommodate larger groups. Each of the synthesized bridged/non-bridged analogs showed inhibitory activity against amastigote growth, however compounds were less selective in the infected macrophage assay. In general terms, the compounds did not demonstrate significant cytotoxicity on the J774 cell lines, implying that the observed activity is specific to Leishmania amastigotes.

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC$_{50}$ (µM)</th>
<th>Selectivity (Cytotox. IC$<em>{50}$/IM IC$</em>{50}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Axenic Amastigote</td>
<td>J774 Cytotoxicity</td>
</tr>
<tr>
<td>miltefosine</td>
<td>3.26</td>
<td>&gt;50</td>
</tr>
<tr>
<td>4.014</td>
<td>8.19</td>
<td>24.19</td>
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<td>4.018</td>
<td>20.00</td>
<td>&gt;50.00</td>
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<td>4.032</td>
<td>1.80</td>
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</tr>
<tr>
<td>4.033</td>
<td>20.00</td>
<td>50.00</td>
</tr>
</tbody>
</table>

Table 4.1: Biological data for piperidine (non-bridged) and tropane (bridged) analogs

With the exception of compound 4.032, each compound showed considerably more activity in the HCS infected macrophage assay than the axenic amastigote assay, suggesting that these compounds may be exerting their anti-leishmanial activity via multiple targets, a phenomenon previously observed with opioid receptor antagonists. Compounds 4.021 (non-bridged) and 4.030 (bridged) were identified as being more potent in the infected macrophage assay than
miltefosine, which is currently being marketed as treatment for leishmaniasis. However, these compounds were less selective in the assays. The amide analogs, 4.018 and 4.031, which were not particularly soluble, compared to the ester or benzamide analogs, provided unusual results, which could be attributed to their solubility issues.
4.6 Experimental

General experimental procedures:

Tert-butyl piperidin-4-ylcarbamate: To a solution of 1-benzylpiperidin-4-amine (1.00 mL, 4.90 mmol) in DCM (20 mL) was added TEA (1.00 mL, 7.17 mmol), followed by di-tert-butyl dicarbonate (1.26 g, 5.78 mmol). The reaction mixture was stirred overnight at room temperature and then diluted with DCM (20 mL). The organic phase was washed with aqueous NaHCO₃ solution (15 mL), brine (15 mL), dried over anhydrous MgSO₄ and concentrated on a rotary evaporator. The resulting solid was dried in a lyophilizer to give tert-butyl (1-benzylpiperidin-4-yl)carbamate as a pale white solid (1.419 g, 85%). LRMS (ESI) m/z calc’d for C₁₇H₂₆N₂O₂ [M+H]⁺: 291.41, found 291.1. This material was used in the next step without further purification.

To a solution of tert-butyl (1-benzylpiperidin-4-yl)carbamate (1.40 g, 4.82 mmol) in MeOH (80 mL) was added 20% palladium on carbon (0.300 g, 0.282 mmol) at room temperature. After stirring overnight under H₂ atmosphere, the reaction mixture was filtered through a celite pad and evaporated to provide tert-butyl piperidin-4-ylcarbamate as a white powder (870 mg, 90%).¹H NMR (400 MHz, DMSO) δ ppm 6.69 - 6.77 (m, 1 H) 2.81 - 2.91 (m, 3 H) 2.35 - 2.46 (m, 3 H) 1.58 - 1.65 (m, 2 H) 1.35 - 1.37 (s, 9 H) 1.16 - 1.25 (m, 2 H) ppm. ¹³C NMR (101 MHz, DMSO-d₆) δ 155.2, 77.8, 48.4, 45.6, 33.6, 28.7 ppm. LRMS (ESI) m/z calc’d for C₁₀H₂₀N₂O₂ [M+H]⁺: 201.28, found 201.30.
**N-benzyl-6-chloronicotinamide**: Thionyl chloride (5.18 mL, 71.4 mmol) and DMF (77.00 µL, 0.999 mmol) were added to a stirred suspension of 6-chloronicotinic acid (7.50 g, 47.6 mmol) in toluene (75 mL) and heated to 80 °C for 2 hours. Upon cooling, the reaction mixture was concentrated under reduced pressure and the residue was dissolved in DCM (75 mL). Benzylamine (15.28 mL, 143.0 mmol) was then added at 0 °C over 20 minutes and allowed to stir overnight at room temperature. Saturated aqueous NaHCO₃ (100 mL) and DCM (50 mL) were added under vigorous stirring, the phases were separated and the aqueous phase was extracted with DCM (2 x 50 mL). The combined organic extracts were washed with saturated aqueous NaHCO₃, dried (Na₂SO₄) and concentrated on a rotary evaporator. The residue was dissolved in ethyl acetate (10 mL) at reflux, n-hexane (5.0 mL) was added and the solution was slowly cooled to room temperature and then over ice. Precipitation began on scratching with a glass rod. The precipitate was filtered off, washed with cold n-hexane/ethyl acetate 1:2 and dried in a lyophilizer to yield N-benzyl-6-chloronicotinamide as light brown crystals (11.92 g, 68%).

$^1$H NMR (500 MHz, DMSO) $\delta$ 9.30 (br t, $J = 5.7$ Hz, 1H), 8.89 (d, $J = 2.3$ Hz, 1H), 8.28 (dd, $J = 8.3$, 2.5 Hz, 1H), 7.65 (d, $J = 8.3$ Hz, 1H), 7.33 (d, $J = 4.6$ Hz, 4H), 7.22-7.36 (m, 1H), 4.50 (d, $J = 6.0$ Hz, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 164.2, 153.0, 149.5, 139.5, 139.1, 129.7, 128.8, 127.8, 127.4, 124.6, 43.2 ppm. LRMS (ESI) m/z calc’d for C₁₃H₁₁ClN₂O [M+H]+ = 247.49, found 247.40.
Tert-butyl (1-(5-(benzylcarbamoyl)pyridin-2-yl)piperidin-4-yl)carbamate: To a stirred solution of N-benzyl-6-chloronicotinamide (162.0 mg, 0.405 mmol) in ACN was added tert-butyl piperidin-4-ylcarbamate (158.0 mg, 0.809 mmol) and DIPEA (339 mg, 2.63 mmol). The reaction mixture was stirred at reflux for 48 h. Upon cooling to room temperature, the mixture was diluted with EtOAc and then washed with H₂O. The organic layer was washed with citric acid, brine and dried over anhydrous sodium sulphate. Filteration, concentration and purification by flash column chromatography (hexanes: EtOAc, 1:1) afforded tert-butyl (1-(5-(benzylcarbamoyl)pyridin-2-yl)piperidin-4-yl)carbamate as an off-white crystalline solid (213 mg, 79%). ¹H NMR (500 MHz, DMSO) δ 8.78 (t, J = 6.0 Hz, 1H), 8.63 (d, J = 2.4 Hz, 1H), 7.96 (dd, J = 9.0, 2.5 Hz, 1H), 7.27-7.34 (m, 4H), 7.19-7.27 (m, 1H), 6.85 (d, J = 9.1 Hz, 2H), 4.45 (d, J = 6.0 Hz, 2H), 4.31 (br d, J = 13.3 Hz, 2H), 3.40-3.59 (m, 1H), 2.91-3.04 (m, 2H), 1.77 (br d, J = 10.6 Hz, 2H), 1.38 (s, 9H), 1.28-1.33 (m, 2H) ppm. ¹³C NMR (126 MHz, DMSO) δ 165.4, 159.9, 155.2, 148.5, 140.4, 137.0, 128.7, 127.6, 127.1, 118.3, 105.9, 78.0, 48.0, 43.9, 42.8, 31.8, 28.7 ppm. LRMS (ESI) m/z calc’d for C₂₃H₃₀N₄O₃ [M+H]⁺: 411.52, found 411.32.

6-(4-Aminopiperidin-1-yl)-N-benzylnicotinamide: To a solution of tert-butyl (1-(5-(benzylcarbamoyl)pyridin-2-yl)piperidin-4-yl)carbamate (1.32 g, 3.22 mmol) in DCM was added
a 1.0M solution of HCl in diethyl ether (150 mL) at 0°C. After stirring for 20 minutes at 0 °C, the mixture was allowed to warm to room temperature and stirred for 2 days. The reaction mixture was concentrated to give 6-(4-aminopiperidin-1-yl)-N-benzynicotinamide as a pale white powder (820 mg, 82%). $^1$H NMR (500 MHz, DMSO) δ 9.29 (br s, 1H), 8.61 (s, 1H), 8.23-8.44 (m, 4H), 7.25-7.39 (m, 5H), 4.45 (s, 2H), 3.38 (q, $J = 7.0$ Hz, 2H), 3.26-3.31 (m, 1H), 2.06 (br d, $J = 10.8$ Hz, 2H), 1.53-1.71 (m, 2H), 1.03-1.37 (m, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 163.4, 154.7, 149.5, 140.3, 139.9, 128.8, 127.8, 127.3, 119.1, 110.6, 47.32, 44.8, 43.0, 29.4 ppm. LRMS (ESI) m/z calc’d for C$_{18}$H$_{22}$N$_{4}$O $[M+H]^+$: 311.40, found 311.20.

![4.009](image.png)

**Ethyl 4-amino-3-bromobenzoate:** To a solution of ethyl 4-aminobenzoate (1.850 g, 11.20 mmol) and DCM (10 mL) was added NBS (2.207 g, 12.40 mmol) and the mixture was allowed to stir at room temperature overnight. The resulting mixture was then extracted with H$_2$O and brine, dried over anhydrous MgSO$_4$ and concentrated. The residue was purified by column chromatography (n-hexane/ethyl acetate, 9:1) to afford ethyl 4-amino-3-bromobenzoate as a yellow solid (2.433 g, 89%). $^1$H NMR (500 MHz, DMSO) δ 7.88 (d, $J = 1.9$ Hz, 1H), 7.64 (dd, $J = 1.9, 8.5$ Hz, 1H), 6.80 (d, $J = 8.5$ Hz, 1H), 6.18 (s, 2H), 4.21 (q, $J = 7.1$ Hz, 2H), 1.27 (t, $J = 7.1$ Hz, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 165.2, 150.7, 134.1, 130.3, 118.3, 114.6, 106.3, 60.5, 14.7 ppm. LRMS (ESI) m/z calc’d for C$_{9}$H$_{10}$BrNO$_2$ $[M+H]^+$: 245.09/247.09, found 245.09/247.09.
Ethyl 3-bromo-4-cyanobenzoate: A solution of ethyl 4-amino-3-bromobenzoate (5.000 g, 20.48 mmol) in DCM (50 mL) was cooled to -10°C and treated dropwise with 90% tert-butyl nitrite (3.34 mL, 28.1 mmol) followed by boron trifluoride diethyl etherate (4.08 mL, 33.1 mmol) and the suspension was allowed to warm to room temperature and stirred for 4h.

Diethyl ether (20 mL) was then added, and the off-white solid was collected by filtration, washed with diethyl ether and dried briefly under high vacuum. The solid was then suspended in toluene (40 mL) and cooled to 0°C. A solution of copper (I) cyanide (2.6 g, 29 mmol) and sodium cyanide (3.55 g, 72.4 mmol) in H₂O (25 mL) was added dropwise over 10 min. The mixture was allowed to cool to room temperature, and ethyl acetate (50 mL) and H₂O (50 mL) were added. The aqueous phase was separated and extracted with ethyl acetate (2 x 50 mL). The organic phases were combined, washed with H₂O (2 x 30 mL) and brine, dried over Na₂SO₄, filtered and concentrated at reduced pressure. The solid residue obtained was adsorbed onto silica gel and chromatographed (hexanes/ethyl acetate 95:5 to 85:15) to afford ethyl 3-bromo-4-cyanobenzoate (4.3 g, 83%).¹H NMR (500 MHz, DMSO) δ 8.26 (d, J = 1.4 Hz, 1H), 8.03-8.13 (m, 2H), 4.35 (q, J = 7.1 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H) ppm.¹³C NMR (126 MHz, DMSO) δ 163.8, 135.9, 135.7, 133.4, 129.0, 125.4, 118.8, 117.1, 62.4, 14.4 ppm. LRMS (ESI) m/z calc’d for C₁₀H₈BrNO₂ [M+H]+: 255.08/257.08, found 255.20/257.20.
Ethyl 4-cyano-3-((3-methoxypropyl)amino)benzoate: A mixture of ethyl 3-bromo-4-cyanobenzoate (500.0 mg, 1.968 mmol), XANTPHOS (228.0 mg, 0.394 mmol), cesium carbonate (1.28 g, 3.93 mmol), 3-methoxypropan-1-amine (233 mg, 2.61 mmol), and Pd$_2$(dba)$_3$ (180.0 mg, 0.197 mmol) in dioxane (5 mL) was stirred at 100°C for 17 h. The cooled reaction mixture was partitioned between ethyl acetate (100 mL) and H$_2$O (50 mL), the organic phase was washed with brine (50 mL), dried over sodium sulfate, filtered and concentrated. Column chromatography on silica gel (hexanes:ethyl acetate 9:1) afforded ethyl 4-cyano-3-((3-methoxypropyl)amino)benzoate as a brown oil (418 mg, 81%). $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.41 (d, $J = 7.8$ Hz, 1H), 7.31 (d, $J = 1.2$ Hz, 1H), 7.23 - 7.29 (m, 1H), 4.36 (q, $J = 7.0$ Hz, 2H), 3.50 - 3.60 (m, 2H), 3.32 - 3.44 (m, 5H), 1.88 - 2.00 (m, 2H), 1.37 (t, $J = 7.0$ Hz, 3H) ppm. $^{13}$C NMR (126 MHz, CDCl$_3$) $\delta$ 165.8, 150.5, 135.5, 132.7, 117.2, 116.6, 111.2, 99.1, 71.2, 61.5, 58.8, 41.9, 28.7, 14.3 ppm. LRMS (ESI) m/z calc’d for C$_{14}$H$_{18}$N$_2$O$_3$ [M+H]$^+$: 263.31, found 263.21.

4-Carbamoyl-3-((3-methoxypropyl)amino)benzoic acid: A mixture of ethyl 4-cyano-3-((3-methoxypropyl)amino)benzoate (1.00 g, 3.81 mmol), 50% w/w NaOH (0.20 mL, 3.81 mmol), and H$_2$O$_2$ (2.22 mL, 72.4 mmol), in EtOH (15 mL) and DMSO (6.58 mL) was added to a 30 mL
microwave vial. The reaction was heated to 100°C in a microwave reactor for 45 minutes. Upon cooling, the mixture was extracted with H₂O and ethyl acetate, and the organic phase was concentrated on a rotary evaporator to give a solid substance which was purified with flash column chromatography to afford a mixture of ethyl 4-carbamoyl-3-((3-methoxypropyl)amino)benzoate and 4-cyano-3-((3-methoxypropyl)amino)benzoic acid as a yellow solid.

The mixture was then subjected to saponification conditions with excess aqueous NaOH in EtOH to yield a mixture of 4-carbamoyl-3-((3-methoxypropyl)amino)benzoic acid and 2-((3-methoxypropyl)amino)terephthalic acid (diacid, by product) as a light yellow solid (78%).

4.013: LRMS (ESI) m/z calc’d for C₁₂H₁₆N₂O₄ [M+H]⁺: 252.27, found 253.20.
4.013a: LRMS (ESI) m/z calc’d for C₁₂H₁₅NO₅ [M+H]⁺: 254.25, found 254.20.

\[
\text{N1-(1-(5-(benzylcarbamoyl)pyridin-2-yl)piperidin-4-yl)-3-((3-methoxypropyl)amino)terephthalamide:}\ A 15 \text{ mL vial was charged with a magnetic spin bar, 6-}
\] (4-aminopiperidin-1-yl)-N benzyl nicotinamide (320.0 mg, 1.031 mmol), 4-carbamoyl-3-((3-methoxypropyl)amino)benzoic acid (295.0 mg, 1.169 mmol), DMF (5.0 mL) and DIPEA (1.00 mL, 5.73 mmol). With stirring, HATU (730 mg, 1.92 mmol) was added and the reaction was warmed to 50 °C for 3 h. The reaction was then diluted with H₂O and extracted with ethyl acetate (3x). The combined organic extracts were dried with MgSO₄, filtered and concentrated in vacuo to yield the product which was purified via silica gel chromatography using 100% ethyl acetate as
eluent to provide the title compound. The title compound was further purified by preparative HPLC to give N1-(1-(5-(benzylcarbamoyl)pyridin-2-yl)piperidin-4-yl)-3-((3-methoxypropyl)amino)terephthalamide as a pale white solid (270 mg, 48%). $^1$H NMR (500 MHz, DMSO) δ 8.80 (t, $J = 6.0$ Hz, 1H), 8.66 (d, $J = 2.4$ Hz, 1H), 8.22 (d, $J = 7.9$ Hz, 1H), 8.16 (t, $J = 5.3$ Hz, 1H), 7.86-8.02 (m, 2H), 7.63 (d, $J = 8.2$ Hz, 1H), 7.27-7.48 (m, 4H), 7.02-7.27 (m, 3H), 6.88-6.98 (m, 2H), 4.40-4.49 (m, 4H), 3.99-4.23 (m, 1H), 3.42 (t, $J = 6.1$ Hz, 2H), 3.09-3.29 (m, 5H), 3.03 (br t, $J = 11.9$ Hz, 2H), 1.73-1.88 (m, 4H), 1.44-1.59 (m, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 171.5, 166.0, 165.4, 160.0, 150.1, 148.6, 140.3, 138.5, 137.0, 129.4, 128.7, 127.6, 127.1, 118.3, 116.0, 112.9, 110.2, 106.0, 70.0, 58.4, 47.4, 44.3, 42.8, 40.1, 31.3, 29.2 ppm. HRMS m/z: [M + H]$^+$ calc’d for C$_{30}$H$_{36}$N$_6$O$_5$ 545.2876; Found 545.2885.

![Structure](image)

A mixture of 6-chloronicotinic acid (8.31 g, 52.7 mmol) and thionyl chloride (5.78 mL, 79.0 mmol) in toluene (30 mL) was heated to reflux for 15 hrs and cooled to room temperature. The solvent was evaporated to dryness in vacuo and the residue thus obtained was dissolved in toluene (30 mL) and added to aqueous ammonia (50 mL). The reaction mixture was then stirred at room temperature for 8 hrs and filtered to give a brown solid which was recrystallized from distilled water (300 mL) to obtain **4.016** as a brownish crystalline solid (6.02 g 73%). LRMS (ESI) m/z calc’d for C$_5$H$_5$ClN$_2$O [M+H]$^+$: 157.57, found 157.60.
To a stirred solution of 6-chloronicotinamide (420 mg, 2.68 mmol) in ACN (10 mL) was added tert-butyl piperidin-4-ylcarbamate (540 mg, 2.70 mmol) and DIPEA (1.4 mL, 8.2 mmol). The reaction mixture was stirred at reflux for 2 days. The resulting mixture was cooled to rt and concentrated in vacuo to obtain a solid residue which was purified using column chromatography using DCM/MeOH as the eluent to obtain tert-butyl (1-(5-carbamoylpyridin-2-yl)piperidin-4-yl)carbamate as the title compound (421 mg, 49%). This compound underwent acidic deprotection in 1.0 M HCl in ether to obtain the title compound, **4.017** (190 mg, 69%). LRMS (ESI) m/z calc’d for C_{11}H_{16}N_{4}O [M+H]^+: 221.28, found 221.20.

**N1-(1-(5-carbamoylpyridin-2-yl)piperidin-4-yl)-3-((3-methoxypropyl)amino)terephthalamide**: Using conditions identical to those described above, the amide was obtained as an off white solid (48%). $^1$H NMR (500 MHz, DMSO) $\delta$ 8.60 (d, $J = 2.5$ Hz, 1H), 8.20 (d, $J = 7.8$ Hz, 1H), 8.15 (t, $J = 5.4$ Hz, 1H), 7.94 (dd, $J = 2.5$, 8.8 Hz, 1H), 7.85 - 7.90 (m, 1H), 7.68 - 7.75 (m, 1H), 7.62 (d, $J = 8.3$ Hz, 1H), 7.19 - 7.26 (m, 1H), 7.06 - 7.12 (m, 1H), 7.04 (d, $J = 1.0$ Hz, 1H), 6.93 (dd, $J = 8.3$, 1.5 Hz, 1H), 6.87 (d, $J = 8.8$ Hz, 1H), 4.41 (d, $J = 13.2$ Hz, 2H), 4.04 - 4.14 (m, 1H), 3.40 (t, $J = 6.4$ Hz, 2H), 3.22 (s, 3H), 3.18 (q, $J = 6.4$ Hz, 2H),
3.01 (t, $J = 11.7$ Hz, 2H), 1.74 - 1.87 (m, 4H), 1.50 (dd, $J = 12.0$, 3.67 Hz, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 171.5, 167.1, 165.9, 160.0, 150.0, 148.8, 138.6, 137.2, 129.3, 118.3, 116.1, 1 13.0, 110.2, 105.9, 70.0, 58.4, 47.4, 44.3, 31.2, 29.2 ppm. HRMS m/z: [M+H]$^+$ calc’d for C$_{23}$H$_{30}$N$_6$O$_4$ 455.2407; Found 455.2403.

To a slurry of 6-chloronicotinic acid (5.00 g, 31.7 mmol) in anhydrous toluene (35 mL) was added dropwise with stirring triethyl orthoacetate (17.6 mL, 95.0 mmol). The mixture was heated to reflux overnight and allowed to cool to room temperature and the resultant solution washed with sat NaHCO$_3$ (50 mL). The organic phase was dried with MgSO$_4$ and the solvent removed in vacuo to afford the title compound, ethyl 6-chloronicotinate, 4.019 (5.6 g 95%) as a brownish oil. LRMS (ESI) m/z calc’d for C$_8$H$_8$ClNO$_2$ [M+H]$^+$: 186.61, found 186.54.

To a solution of ethyl 6-chloronicotinate (200.0 mg, 1.078 mmol) in EtOH (2 mL) was added tert-butyl piperidin-4-ylcarbamate (256.0 mg, 1.278 mmol) and DIPEA (0.223 mL, 1.278 mmol). The mixture was heated to reflux overnight. The resulting mixture was cooled to rt and concentrated in vacuo to obtain a solid residue which was purified using column chromatography using hexane/EtOAc as the eluent to obtain ethyl 6-((tert-butoxycarbonyl)amino)piperidin-1-yl)nicotinate (339 mg, 76%). This compound underwent acidic deprotection in 1.0 M HCl in ether
to obtain the title compound, **4.020** (176 mg, 82%). LRMS (ESI) m/z calc’d for C₁₃H₁₉N₃O₂ [M+H]⁺: 250.31, found 250.30.

![Chemical Structure](image)

**Ethyl 6-(4-(4-carbamoyl-3-((3-methoxypropyl)amino)benzamido)piperidin-1-yl)nicotinate:**

Using conditions identical to those described above, the ester was obtained as a white fluffy solid (55%). ¹H NMR (500 MHz, DMSO) δ 8.63 (d, J = 2.3 Hz, 1H), 8.21 (d, J = 7.9 Hz, 1H), 8.15 (t, J = 5.3 Hz, 1H), 7.84-7.96 (m, 2H), 7.62 (d, J = 8.2 Hz, 1H), 7.23 (br s, 1H), 7.04 (s, 1H), 6.92 (t, J = 9.6 Hz, 2H), 4.45 (br d, J = 13.3 Hz, 2H), 4.23 (q, J = 7.1 Hz, 2H), 4.06-4.15 (m, 1H), 3.34-3.43 (m, 2H), 3.16-3.23 (m, 1H), 3.22 (s, 4H), 3.06 (br t, J = 11.8 Hz, 2H), 1.75-1.89 (m, 4H), 1.40-1.55 (m, 2H), 1.27 (t, J = 7.1 Hz, 3H) ppm. ¹³C NMR (126 MHz, DMSO) δ 171.5, 165.9, 165.5, 160.5, 150.8, 150.1, 138.5, 138.6, 129.4, 116.1, 113.9, 112.8, 110.2, 106.1, 70.0, 60.3, 58.4, 47.2, 44.1, 40.1, 31.4, 29.2, 14.7 ppm. HRMS m/z: [M+H]⁺ calc’d for C₂₅H₃₅N₅O₅ 484.2560; Found 484.2560.

![Chemical Structure](image)

**Tert-butyl 3-amino-8-azabicyclo[3.2.1]octane-8-carboxylate:** A solution of tert-butyl 3-oxo-8-azabicyclo[3.2.1]octane-8-carboxylate (2.500 g, 11.10 mmol), benzylamine (1.400 mL, 12.43 mmol) and sodium triacetoxyborohydride (3.530 g, 16.65 mmol) in a mixture of glacial acetic acid (7.5 mL) and methylene chloride (66 mL) was allowed to stir for 16 hours at room temperature.
The solvents were evaporated under reduced pressure and the residue was dissolved in ethyl acetate (50 mL) and washed with saturated aqueous sodium bicarbonate solution (13 mL) and H₂O (13 mL). The organic solution was dried (MgSO₄), filtered, and evaporated under reduced pressure. The residue was purified by column chromatography on silica gel (98:2:0.25 DCM: MeOH: conc. aq. NH₃) to afford tert-butyl 3-(benzylamino)-8-azabicyclo[3.2.1]octane-8-carboxylate as a white solid (1.77 g, 45%). LRMS (ESI) m/z calc’d for C₁₀H₂₈N₂O₂ [M+H]⁺: 317.48, found 317.40.

To a solution of tert-butyl 3-(benzylamino)-8-azabicyclo[3.2.1]octane-8-carboxylate (2.40 g, 7.58 mmol) in EtOH (15 mL) was added ammonium formate (2.39 g, 37.9 mmol) and 20% palladium hydroxide on carbon (0.320 g, 2.275 mmol). The resulting suspension was stirred at 50° until gas evolution had ceased. The mixture was then filtered through celite, concentrated and chromatographed on silica gel using MeOH and DCM to afford the target compound, tert-butyl 3-amino-8-azabicyclo[3.2.1]octane-8-carboxylate (1.58 g, 92%). ¹H NMR (500 MHz, CD₃OD) δ 4.13-4.20 (m, 2H), 3.09-3.18 (m, 1H), 2.16-2.29 (m, 2H), 1.94-2.03 (m, 4H), 1.40-1.59 (m, 13H). ¹³C NMR (126 MHz, CD₃OD-d₄) δ 153.8, 79.5, 52.5, 42.7, 37.1, 28.5, 27.3. LRMS (ESI) m/z calc’d for C₁₂H₂₂N₂O₂ [M+H]⁺: 227.32, found 227.40.

**Benzyl (8-(5-carbamoylpyridin-2-yl)-8-azabicyclo[3.2.1]octan-3-yl)carbamate:** To a solution of tert-butyl 3-amino-8-azabicyclo[3.2.1]octane-8-carboxylate (500.0 mg, 2.209 mmol) in DCM (5 mL) was added benzyl chloroformate (0.41 mL, 2.87 mmol) and DIPEA (0.60 mL, 3.31 mmol).
The solution was stirred vigorously overnight at room temperature, concentrated and purified on silica gel to provide tert-butyl 3-(((benzyloxy)carbonyl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate as a solid (549 mg, 69%). LRMS (ESI) m/z calc’d for C_{20}H_{28}N_{2}O_{4} [M+H]^+: 361.45, found 361.31. A portion of this material was used in the next step.

A suspension of tert-butyl 3-(((benzyloxy)carbonyl)amino)-8-azabicyclo[3.2.1]octane-8-carboxylate (200.0 mg, 0.555 mmol) in a 1M solution of HCl in diethyl ether was allowed to stir overnight. After completion of the reaction, monitored by LC/MS, the solution was concentrated on a rotary evaporator to give benzyl 8-azabicyclo[3.2.1]octan-3-ylcarbamate (121 mg, 84%). LRMS (ESI) m/z calc’d for C_{15}H_{20}N_{2}O_{2} [M+H]^+: 261.34, found 261.20. This material was used in the next step without further purification.

To a stirred solution of 6-chloronicotinamide (144.0 mg, 0.922 mmol) in ACN (5.0 mL) was added benzyl 8-azabicyclo[3.2.1]octan-3-ylcarbamate (200.0 mg, 0.768 mmol) and DIPEA (0.47 mL, 2.69 mmol). The reaction mixture was stirred at reflux for 48 h. The solid was collected by filtration and washed with ACN. After drying, the title compound, benzyl (8-(5-carbamoylpyridin-2-yl)-8-azabicyclo[3.2.1]octan-3-yl)carbamate was obtained as brownish solid (178 mg, 61%). \textsuperscript{1}H NMR (500 MHz, DMSO) δ 8.59 (d, \(J = 2.4\) Hz, 1H), 7.91 (dd, \(J = 2.5, 8.8\) Hz, 1H), 7.71 (br.s., 1H), 7.24-7.40 (m, 6H), 7.08 (br. S., 1H), 6.65-6.73 (m, 1H), 5.02 (s, 2H), 4.49 (br. S., 2H), 3.50 (d, \(J = 4.4\) Hz, 1H), 2.07-2.14 (m, 2H), 1.96-2.04 (m, 2H), 1.87-1.94 (m, 2H), 1.73-1.77 (m, 2H) ppm. \textsuperscript{13}C NMR (126 MHz, DMSO) δ 171.9, 167.3, 157.9, 156.3, 149.4, 137.7, 137.1, 128.8, 128.2, 118.0, 107.4, 65.6, 52.2, 43.6, 32.8, 27.6 ppm. LRMS (ESI) m/z calc’d for C_{21}H_{24}N_{4}O_{3} [M+H]^+: 381.45, found 381.4.
6-(3-amino-8-azabicyclo[3.2.1]octan-8-yl)nicotinamide: To a solution of benzyl (8-(5-carbamoylpyridin-2-yl)-8-azabicyclo[3.2.1]octan-3-yl)carbamate (400.0 mg, 1.051 mmol) in MeOH (10 mL) was added Pd(OH)$_2$/C (43.10 mg, 0.307 mmol). The resulting mixture was stirred under a hydrogen-filled balloon overnight, filtered through a celite pad, concentrated and purified by flash column chromatography (DCM:MeOH:NH$_4$OH) to afford the title compound (197 mg, 76%). $^1$H NMR (500 MHz, CD$_3$OD) $\delta$ 8.59 (d, $J = 2.5$ Hz, 1H), 7.95 (dd, $J = 9.1$, 2.48 Hz, 1H), 6.69 (d, $J = 9.0$ Hz, 1H), 4.56 (br s, 2H), 3.34 (m, 2H), 2.91-3.10 (m, 1H), 2.26 (td, $J = 13.9$, 6.0 Hz, 2H), 2.15-2.21 (m, 2H), 2.06-2.12 (m, 2H), 1.93-1.94 (m, 2H), 1.53-1.58 (m, 2H) ppm. $^{13}$C NMR (126 MHz, CD$_3$OD) $\delta$ 169.6, 158.0, 148.8, 136.8, 116.6, 107.1, 52.6, 42.9, 36.2, 28.1 ppm. LRMS (ESI) m/z calc’d for C$_{13}$H$_{18}$N$_4$O [M+H]$^+$: 247.31, found 247.30.

N1-(8-(5-(benzylcarbamoyl)pyridin-2-yl)-8-azabicyclo[3.2.1]octan-3-yl)-3-((3-methoxypropyl)amino)terephthalamide: Using conditions identical to those described above, compound 4.030 was obtained as an off white solid (48%). $^1$H NMR (500 MHz, DMSO) $\delta$ 8.77 (t, $J = 6.1$ Hz, 1H), 8.64 (d, $J = 2.0$ Hz, 1H), 8.20 (t, $J = 5.4$ Hz, 1H), 8.14 (d, $J = 4.4$ Hz, 1H), 7.97 (dd, $J = 8.8$, 2.5 Hz, 1H), 7.86 - 7.93 (m, 1H), 7.65 (d, $J = 8.3$ Hz, 1H), 7.18 - 7.34 (m, 6H), 6.97
(s, 1H), 6.86 (d, J = 7.8 Hz, 1H), 6.75 (d, J = 9.3 Hz, 1H), 4.56 (br. s., 2H), 4.44 (d, J = 5.9 Hz, 2H), 3.82 (br. s., 1H), 3.40 (t, J = 6.1 Hz, 2H), 3.17 - 3.25 (m, 5H), 2.19 (d, J = 7.3 Hz, 2H), 2.03 - 2.10 (m, 2H), 1.96 - 2.02 (m, 2H), 1.90 (d, J = 14.2 Hz, 2H), 1.80 (t, J = 6.4 Hz, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 171.5, 167.2, 165.5, 158.0, 149.9, 149.2, 140.4, 139.2, 136.8, 129.5, 128.7, 127.6, 127.1, 118.2, 116.1, 112.9, 110.3, 107.5, 70.1, 58.4, 52.4, 43.0, 42.8, 40.6, 32.4, 29.1, 27.9 ppm. HRMS m/z: [M+H]$^+$ calc’d for C$_32$H$_38$N$_6$O$_4$ 571.3033; Found 571.3042.

N1-(8-(5-carbamoylpyridin-2-yl)-8-azabicyclo[3.2.1]octan-3-yl)-3-((3-methoxypropyl)amino)terephthalamide: Using conditions identical to those described above, compound 4.031 was obtained as a light yellow solid (55%). $^1$H NMR (500 MHz, DMSO) δ 8.62 (d, J = 2.5 Hz, 1H), 8.21 (t, J = 5.4 Hz, 1H), 8.16 (d, J = 3.9 Hz, 1H), 7.95 (dd, J = 8.8, 2.0 Hz, 1H), 7.87 - 7.93 (m, 1H), 7.73 (br. s., 1H), 7.67 (d, J = 8.3 Hz, 1H), 7.25 - 7.30 (m, 1H), 7.09 (br. s., 1H), 6.99 (s, 1H), 6.86 - 6.90 (m, 1H), 6.75 (d, J = 8.8 Hz, 1H), 4.57 (br. s., 2H), 3.80 - 3.86 (m, 1H), 3.42 (t, J = 6.4 Hz, 3H), 3.25 (s, 3H), 3.19 - 3.24 (m, 2H), 2.17 - 2.23 (m, 2H), 2.08 (td, J = 14.2, 5.1 Hz, 3H), 1.98 - 2.04 (m, 2H), 1.91 (d, J = 14.2 Hz, 3H), 1.82 (quin, J = 6.5 Hz, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 171.5, 167.3, 167.2, 158.1, 150.0, 149.5, 139.2, 137.2, 129.5, 118.1, 116.1, 112.9, 110.3, 107.5, 70.1, 58.4, 52.4, 43.1, 39.8, 32.4, 29.1, 27.9 ppm. HRMS m/z: [M+H]$^+$ calc’d for C$_{25}$H$_{32}$N$_6$O$_4$ 481.2563; found 481.2571.
Ethyl 6-(3-(4-carbamoyl-3-((3-methoxypropyl)amino)benzamido)-8-azabicyclo[3.2.1]octan-8-yl)nicotinate: Using conditions identical to those described above, compound 4.032 was obtained as a white solid (55%). $^1$H NMR (500 MHz, DMSO) $\delta$ 8.79 (d, $J$ = 2.5 Hz, 1H), 8.35 (t, $J$ = 5.4 Hz, 1H), 8.31 (d, $J$ = 4.0 Hz, 1H), 8.06 (dd, $J$ = 9.1, 2.2 Hz, 2H), 7.80 (d, $J$ = 8.3 Hz, 1H), 7.36 - 7.47 (m, 1H), 7.12 (s, 1H), 7.02 (d, $J$ = 7.8 Hz, 1H), 6.91 (d, $J$ = 8.8 Hz, 1H), 4.66 - 4.82 (m, 2H), 4.39 (q, $J$ = 7.3 Hz, 2H), 3.98 (br. s., 1H), 3.55 (t, $J$ = 6.1 Hz, 2H), 3.32 - 3.40 (m, 5H), 2.35 (d, $J$ = 7.3 Hz, 2H), 2.13 - 2.25 (m, 4H), 2.08 (d, $J$ = 14.7 Hz, 2H), 1.91 - 2.00 (m, 2H), 1.43 (t, $J$ = 7.1 Hz, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 171.5, 167.2, 165.6, 158.5, 151.5, 149.9, 139.2, 138.3, 129.4, 116.1, 113.8, 112.9, 110.3, 107.6, 70.1, 60.3, 58.4, 52.5, 43.0, 39.5, 32.8, 29.1, 27.7, 14.8. HRMS m/z: [M+H]$^+$ calc’d for C$_{27}$H$_{35}$N$_5$O$_5$ 510.2716; Found 510.2727 ppm.

4-(((1R,3r,5S)-8-((5-ethoxycarbonyl)pyridin-2-yl)-8-azabicyclo[3.2.1]octan-3-yl)carbamoyl)-2-((3-methoxypropyl)amino)benzoic acid: Compound 4.033 was obtained in the manner described above. $^1$H NMR (500 MHz, CDCl$_3$) $\delta$ 8.84 (d, $J$ = 2.0 Hz, 1H), 8.09 (d, $J$ = 8.3 Hz, 1H), 8.01 (d, $J$ = 8.3 Hz, 1H), 7.18 (s, 1H), 6.76 (d, $J$ = 8.3 Hz, 1H), 6.67 (d, $J$ = 6.4 Hz, 1H), 6.59 (d,
$J = 7.3$ Hz, 1H), 4.74 (br. s., 2H), 4.36 (q, $J = 6.9$ Hz, 2H), 4.26 (q, $J = 6.0$ Hz, 1H), 3.48 - 3.57 (m, 3H), 3.31 - 3.43 (m, 5H), 2.26 - 2.42 (m, 4H), 2.09 - 2.20 (m, 2H), 1.88 - 2.03 (m, 4H), 1.38 (t, $J = 7.1$ Hz, 3H). HRMS m/z [M+H] calc’d for C$_{27}$H$_{34}$N$_{4}$O$_{6}$ 511.2478.
4.7 References


Chapter 5
Conclusions

5.1 Summary and conclusions

A novel series of compounds based on the known Hsp 90 inhibitor agents SNX-2112 and XL-888 that potentially inhibit *Leishmania donovani* amastigote growth has been discovered. These compounds have been tested in multiple assays for their anti-leishmanial activity, and the overall synthetic schemes developed allowed the preparation of several intermediates and different analogs.

Docking studies of a selected member compound clearly defined the probable binding interactions and available hydrophobic pocket that could be exploited, which assisted in the design of further analogs for evaluation. The synthesized analogs included indazole, tetrahydroquinoline, piperidine and tropane derivatives. Based on previous docking and x-ray crystallographic studies of analogs of 1.001, it was envisioned that the primary benzamide, indazole carbonyl, tetrahydroquinoline nitrogen or the piperidine/tropane nitrogen groups represented key structural features of these series of compounds that confer binding into the Hsp 90 active site.

The initial strategy provided a means of evaluating the necessity of the dimethyl groups at the 6-position on the indazole scaffold for activity. The impact a twisting effect would have on the overall Hsp 90 binding affinity was also anticipated with the introduction of a methyl group at the 5-
position on the benzene ring (anthranilamide portion). Additionally, the indazole scaffold was replaced with the privileged scaffold, tetrahydroquinoline, or piperidine/tropane scaffold. It was anticipated that such SAR strategies would enhance the activity of these molecules since docking studies revealed that the active site can accommodate considerably larger groups.

Clearly, the results (pages 34, 60 and 81) indicate that these changes resulted in a significant effect on the anti-leishmanial activities, suggesting significant binding affinities. Deletion of the geminal dimethyl groups led to a complete loss of activity in the assays. This result clearly defined the importance of the geminal dimethyl groups on the indazole bicyclic core and represents a crucial SAR discovery in the search for potent anti-leishmanial agents. For instance, the 6-position of the indazole bicyclic core can further be exploited to gain additional selectivity and/or improved pharmacokinetics.

Several of these compounds displayed more potent activity than the clinically used miltefosine in both assays (axenic amastigote and infected macrophage) and were found to have an excellent toxicity profile as well. Compound 2.017 exhibited superior inhibitory activity to all of the analogs including miltefosine. In fact, compound 2.017 was found to be more than twice as active as miltefosine in the infected macrophage assay and with selectivity of approximately 20-fold over human macrophage cell lines.

Given that some compounds displayed considerably more activity in the amastigote-infected macrophage assay than the initial axenic amastigote assay, it was investigated whether the observed activity is indeed the result of protozoan Hsp 90 inhibition. Thus, most of these
compounds were assayed using an Hsp 90 fluorescence polarization assay,¹ (Figure 5.1) run by Hardik Patel in the laboratory of Prof. Gabriela Chiosis.

![Figure 5.1: Hsp 90 fluorescence polarization assay. *Numberings are as received and not as used in manuscript](image)

As expected, compound 1.001 (shown in diagram as 1) exhibited inhibitory activity against human Hsp 90, but none of the other synthesized compounds displayed any significant inhibitory activity against the same. Remarkably, the desmethylated analog 2.013 (shown in Figure 5.1 as 10) which showed no inhibitory activity in the assays was more potent against human Hsp 90 than the rest of the compounds that displayed activity in the anti-leishmanial assays.

Based on these findings, it is proposed that these compounds may be exerting their anti-leishmanial activity through a different mechanism (such as having an allosteric effect) or that these compounds are selective for the protozoan ortholog of Hsp 90 (Hsp 83). The biochemical pathway is currently being investigated and it is targeted at understanding the observed activity.
In conclusion, SAR studies of Hsp 90 inhibitors can lead to the discovery of potent anti-leishmanial agents with an excellent toxicity profile and great selectivity over human macrophage cells.

References

Chapter 6

Introduction to Alzheimer’s diseases

6.1 Alzheimer’s disease

Alzheimer’s disease (AD) is a progressive and irreversible neurodegenerative disease and a complicated multi-factorial disorder characterized by the accumulation of neuritic plaques (senile plaques) consisting of the amyloid-β peptides (Aβ) in the brain, associated with hyperphosphorylated tau-based neurofibrillary tangles (NF), synapse loss, mitochondrial damage and ultimately cognitive decline.\(^1,2,3\) Figure 6.1. It is the most common form of dementia among elderly people and one of the major health problems in western societies,\(^4\) associated with tremendous cost to patients and their families - in both human and financial terms.

Figure 6.1: Cross sections of the brain show atrophy, or shrinking, of brain tissue caused by Alzheimer’s disease
Common symptoms of AD include memory loss with deficit in attention and language, disorientation, confusion and difficulty carrying on conversations or responding to their environment. The risk of developing AD increases with age and people who are aged 65 years and older are particularly at high risk. About thirty five (35) million people are afflicted with the disease worldwide and this number is projected to upsurge due to aging population – with current healthcare cost standing at many billions of dollars annually. In 2010, the global economic impact stood at an estimated $605 billion, but the social and emotional cost is incalculable.

In the United States alone nearly 5.5 million people are afflicted with the disease and the number is projected to rise to between 11 and 16 million by 2050 and this could potentially get worse if no effective intervention is found in time. It is currently the 6th leading cause of death in the United States. In 2012, $200 billion was spent for caring for AD patients and related dementia cases and this figure is projected to rise to $1.1 trillion by 2050. AD disease is indeed one of the most important economic, health and social crises of the current century.

**6.2 Etiology and pathology of Alzheimer’s disease**

Notwithstanding the extensive study on the etiology and pathology of the disease, the exact underlying mechanism is yet to be fully understood, due to its complex pathological characteristics. However, the two main pathological hallmarks of AD are the accumulation of Aβ peptide (Aβ plaques) within the extracellular space of the brain and tau accumulation (neurofibrillary tangles), which are intraneuronal inclusion of hyperphosphorylated tau. These have been recognized as being at the root of the disease pathogenesis. Albeit, designing effective new medicaments has been hampered by the lack of knowledge about the precise cause of the disease.
A number of hypotheses have been proposed in an attempt to explain the etiology and pathology of the disease, however no one hypothesis fully explains the precise mechanism of the disease progression. The three main hypotheses that currently lend credence to the molecular basis of the disease are the cholinergic hypothesis, the amyloid hypothesis and the tau hypotheses. These hypotheses currently serve as the basis for therapeutic development.

6.2.1 The cholinergic hypothesis

This first hypothesis postulates that the loss of cholinergic neurons that subsequently leads to dysfunction of cholinergic activity may play a role in the loss of memory and related cognitive impairment – (often observed in the brain of AD patients). Thus, restoring cholinergic function through the use of acetylcholinesterase inhibitors (AChEIs) or by modulating other cholinergic receptors (muscarinic and nicotinic ACh), can lead to a reduction of the serious lack of cognitive function. Acetylcholinesterase (AChE) plays a major role in nerve signal transmission, but AChEIs prevent the breakdown of acetylcholine, leading to an increase in the concentration of acetylcholine in the brain with improved cholinergic transmission, and ultimately improved cognitive function. This hypothesis led to the development of the only drugs currently approved by the FDA to treat AD (tacrine, donepezil, rivastigmine and galanthamine).

6.2.2 Amyloid hypothesis

This hypothesis postulates that intramembrane proteolysis of the transmembrane protein, β-amyloid precursor protein (APP), generates insoluble Aβ fragments (synaptotoxic/neurotoxic peptide), that leads to plaque aggregation and subsequent development of neurofibrillary tangles. This occurs due to imbalanced production of Aβ, clearance and aggregation, resulting in a series
of biochemical events that ultimately lead to AD. Aβ, typically a 42 amino acid peptide, is derived from the sequential proteolytic cleavage of APP, by three aspartic acid proteases called β-secretase (BACE-1), γ-secretase (presenilin) and α-secretase,\textsuperscript{5,12,13} as shown in Figure 6.2.

![Figure 6.2: Formation of Aβ and Aβ fibrils from APP by sequential cleavage by β- and γ-secretase\textsuperscript{12}](image)

The Aβ pathology has been the target of current drug discovery – the approach aims at modulating or inhibiting the proteolysis of APP to reduce the synaptotoxic Aβ formation, increase Aβ clearance, neutralize Aβ toxicity and/or remove existing Aβ aggregates.\textsuperscript{14,15} Inhibition of the proteolytic enzymes in preclinical studies have indeed demonstrated reduction in Aβ peptides in the brain, although BACE-1 inhibition is associated with a myriad of challenges. Aside from cleaving APP, BACE-1 also cleaves many substrates that are involved with important physiological roles, thus resulting in undesired side effects. Additionally, due to the elongated active site, developed inhibitors are usually bulky and do not readily cross the blood brain barrier (BBB).\textsuperscript{14,16} Conversely, in BACE-1 knockout mice, Aβ production was significantly reduced and
no apparent AD pathology was observed, but most crucial was the fact that there was no consistent phenotypic differences between the control.\textsuperscript{17,18,19} This establishes BACE-1 enzyme inhibition as a potential therapeutic target, however, no BACE-1 inhibitor has ever been approved by the FDA.

In spite of the current challenges, a number of BACE-1 inhibitors (such as AZD3293, CTS-21166, E2609, LYS281376, LY2886721, MK-8931, HPP854, PF-05297909, TAK-070 and VTP-37948) are currently in clinical trials,\textsuperscript{10,20} as a potential treatment for AD.

Additionally, inhibition of $\gamma$-secretase is also associated with challenges, due to the large numbers of transmembrane proteins cleaved by it, including Notch, which could lead to serious mechanism based side effects due to deficiency in selectivity.\textsuperscript{5} Notch inhibition has been the cause of premature termination of clinical trials owing to toxicity and deleterious effects on cognition and functionality of test subjects.\textsuperscript{21} Notwithstanding these challenges, there are a number of $\gamma$-secretase inhibitors and modulators currently being studied in clinical trials including Avagacestat (BMS-708163), Begacestat (GSI-953), NIC5-15, CHF5074 and E2012.\textsuperscript{10}

### 6.2.3 Tau hypothesis

This hypothesis postulates that the accumulation of neurotoxic neurofibrillary tangles in the brain is the ultimate pathway to AD, the result of atypical tau hyperphosphorylation.\textsuperscript{6} The current understanding is that the accumulation of Aβ in the pathogenic progression of the disease triggers a cascade of events, leading to tauopathy (tau hyperphosphorylation), mitochondrial dysfunction, synaptic and neuronal degeneration that eventually manifest in the symptoms of dementia,\textsuperscript{4} Figure 6.3.
There is a strong correlation between the formation of neurofibrillary tangles and the severity of AD.\textsuperscript{23,24} Under normal physiological conditions soluble tau peptides function to stabilize axonal microtubules, however, during early stages of AD (pathological conditions) they become hyperphosphorylated (which weakens their association to microtubules) and causes them to aggregate due to imbalanced activity of tau kinases and phosphatases.\textsuperscript{25,26,27}

### 6.3 Current treatment

Current treatment options for AD are all symptomatic and they do not alter the underlying biology of the disease process, but disease-modifying drugs are currently in development.\textsuperscript{28} Currently there are only five different drugs, Figure 6.4, approved by the United States Food and Drugs Administration (US FDA) for treating AD: One (1) glutamate N-methyl-D-aspartate
(NMDA) receptor antagonist, Memantine (Eli Lilly) and four (4) AChEIs, Tacrine (Warner-Lambert Co.), Donepezil (Pfizer), rivastigmine (Novartis) and galantamine (Janssen).\textsuperscript{10,16,29}

![Figure 6.4. Current drugs used to treat AD]

Tacrine, Donepezil and memantine are synthetic drugs whereas galanthamine and rivastigmine are AChEIs derived from plant alkaloids.\textsuperscript{30}

Many clinical trials have been conducted with the aim of reducing the accumulation of Aβ plaques between neurons and tau-based neurofibrillary tangles within neurons but these have failed to provide the desired result – cognitive improvement.\textsuperscript{6} One such compound to fail is semagacestat, an Elli Lilly drug. Notwithstanding the numerous failures in clinical trials, Figure 6.5 summarizes the biological and small molecule drug candidates currently being studied in clinical trials as of January, 2017.\textsuperscript{31}
Alternative approaches to combating AD include immunotherapy and tau-targeted therapy. The rest are stem cells, drugs modulating cholesterol and vascular-related risk factors, antioxidants, enhances of mitochondrial function, neurotropins and anti-inflammatory medicaments. Immunotherapy has been regarded as a promising approach to combating AD, although there have been reports of adverse side effects. Immunotherapy is administered as either active immunization (vaccination) or passive immunization (monoclonal antibodies), and several antibodies are currently being investigated in both preclinical and clinical trials. In spite of the enormous advances made so far, there are currently no new drugs that address the
underlying mechanisms of AD - either to slow the disease progression or stop it entirely. The lack of effective treatment for AD continues to be a major unmet medical need.

### 6.4 Virtual screening of Compounds

In an effort to identify new compounds as potential treatments for AD, our laboratory in collaboration with the laboratories of Professors David Kang (USF Byrd’s Alzheimer’s Institute) and Yu Chen (USF Morsani College of Medicine), conducted a virtual screen of >4 million compounds that could potentially be docked onto the catalytic pocket of the phosphatase slingshot homology-2 (SSH2), using the ZINC molecule library and the program (DOCK 3.5.5.4). This program was developed at the University of California, San Francisco, in the Shoichet laboratory. Among the compound candidates from the screen results, two (2) promising lead compounds were identified as shown in Figure 6.6, as slingshot homology-1 (SSH1) inhibitors, based on testing in cell-based assays.

![Figure 6.6: Lead compounds from virtual screen](image)

The lead compounds contained either a pyridone or a thiazole moiety with a carboxylic acid functionality, of which the catalytic residues of contact on SSH1 protein were obtained. These residues were 100% conserved between SSH1, SSH2 and SSH3 (homologs) and were found to
reduce Aβ-induced coflin dephosphorylation as well as significantly reduce Aβ secretion. Among the several studies conducted with these compounds, results indicated that Aβ oligomers led to rapid activation of SSH1 and Cofilin, both of which promote Aβ aggregation.  

6.5 Cofilin-Slingshot pathway in AD

The biochemical processes leading to the production of Aβ closely involve the actin cytoskeleton, which plays a crucial role both in the maturation and maintenance of synapses, by regulating synaptic plasticity, receptor anchoring and spine morphology. Of the many actin binding proteins, the Cofilin family and actin-depolymerizing factor (ADF) function to regulate actin filament dynamics and reorganization, which destabilizes F-Actin via severing and depolymerization of actin filaments. There is considerable evidence that support the role of Cofilin in Aβ induced dendritic spine changes, aggregation of Cofilin-Actin rods and increased Cofilin activity in the brains of AD patients. Cofilin is inactivated by phosphorylation on serine 3 (Ser3) by LIM kinase isoform 1 (LIMK1) and the same is reactivated by SSH1-mediated dephosphorylation (SSH1 activation), by the activity of calcineurin. SSH1 is inactivated via Ca^{2+} dependent protein kinase II and phosphoinositide-dependent protein kinase. LIMK1 and SSH1 show the highest substrate specificity among the many kinases and phosphatases that can affect Cofilin. LIMK1 is also a substrate for SSH1 activity and can control Cofilin activation via an upstream regulation of LIMK1, either directly or indirectly. Thus the SSH1-Cofilin activation molecular pathway is essential for both Aβ production and tau pathologies, which ultimately leads to mitochondrial and synaptic dysfunction, Figure 6.7.
Thus, compounds that can inhibit the SSH1 enzyme/pathway (leading to the inhibition of excessive Cofilin activation) represents a potentially viable therapeutic option in the fight against AD pathogenesis. It was therefore hypothesized that small molecules that can inhibit the SSH1 pathway can break the AD pathogenic pathway via inhibition of Aβ production and Aβ induced neurotoxicity.

\[\text{Figure 6.7: Progressive pathogenic cycle of AD}\]

6.6 \textit{In vitro phosphatase enzyme inhibitory assay}

Phosphatase inhibitory activity of the synthesized compounds was evaluated using the para-nitrophenyl phosphate (pNPP) phosphatase assay. Phosphatases control the removal of phosphate groups ($\text{PO}_4^{3-}$) from peptide molecules and this leads to the regulation of many important cellular processes, including differentiation, cell attachment, proliferation and apoptosis. pNPP is a chromogenic substrate for many phosphatases (alkaline, acid, protein tyrosine, and serine/threonine phosphatases), and the pNPP phosphatase assay has been optimized to detect phosphatase activity via the hydrolysis of pNPP to para-nitrophenol (pNP).
The pNP produced during the reaction of a phosphatase with pNPP has an intense absorption at 405 nm and can therefore be conveniently measured on a spectrophotometer. Compounds that inhibit a particular phosphatase should therefore be able to prevent the hydrolysis of pNPP to pNP – the chromogenic species. Each of the compounds was treated with SSH1-N461 enzyme followed by the addition of pNPP and incubated at 37 °C and the absorbances measured at intervals of 1 hour. Lower absorbances is an indication of phosphatase inhibitory activity.
6.7 References


Chapter 7

Pyridones Analogs

7.1 Pyridones

Nitrogen-containing heterocyclic molecules are of vital importance due to their abundance in various biologically active synthetic organic molecules and natural products. Among the nitrogen-containing heterocyclic molecules, 2-pyridones are recognized as an important structural motif and exhibit a broad range of biological properties. Synthetic 2-pyridone molecules have found many uses including as an anti-epileptic drug (Perampanel) and a phosphodiesterase 3 inhibitor (Amrinone), Figure 7.1. Other uses of pyridone containing compounds include anti-cancer, anti-fungal, anti-hepatitis B, anti-HIV, anti-Mycobacterium tuberculosis agents, human rinovirus 3C protease activities and MEK-1 inhibition.

![Perampanel and Amrinone](image)

**Figure 7.1: Biologically active compounds containing 2-pyridone**

Important to our laboratory’s drug discovery effort is the development of pyridones and related analogs as a potential treatment for Alzheimer’s disease. Synthesized anti-AD molecules
possessing the pyridone core that exhibit potent important biological activities include γ-secretase modulators\(^2\) and acetylcholinesterase inhibitor,\(^3\) Figure 7.2. Due to the complexity in etiology of AD, Sheng and colleagues\(^4\) have prepared a multifactorial 4-pyridone agent that integrates H\(_3\) receptor antagonist, metal chelation, anti-β-amyloid peptide aggregation and radical scavenging into a single molecule, Figure 7.2.

![Figure 7.2: Pyridones as treatment for AD](image)

A number of pyridone-related analogs such as pyradazinones (ABT-288, CEP-26401) and pyrimidinone (MK0249) are currently being evaluated at various stages in clinical trials targeting antagonism of the H\(_3\) receptor as therapeutic candidates for AD,\(^4\) Figure 7.3.
7.2 Pyridone alkaloids

Pyridone alkaloids have a pyridone nucleus and may contain either a tetracyclic or tricyclic ring. Many of these alkaloids exhibit profound biological neuroprotective and neurological properties. For instance, cytisine, shown in Figure 7.4, is a naturally occurring alkaloid possessing a pyridone nucleus with a variety of uses including smoke cessation. It acts as a partial agonist by binding to the nicotinic acetylcholine receptor and has been shown to have an affinity for the α4β2 receptor subtype to exert its activity.
Huperzine A, also a naturally occurring sesquiterpene alkaloid isolated from the Chinese herb *Huperzia serrata* by Liu and colleagues\(^7\) in the early 1980’s, is currently in various stages of clinical trials as a treatment for dementia, schizophrenia, traumatic brain injury, epilepsy, cognitive functioning, cocaine dependence and Alzheimer’s disease.\(^8\) As a natural selective inhibitor of acetylcholinesterase,\(^9\) huperzine A acts in a similar fashion to donepezil – an oral drug used to treat dementia and AD. Owing to its antioxidant and neuroprotective properties, huperzine A is currently available as a neutraceutical. The prodrug analog of huperzine A, ZT-1, also derived from natural sources\(^9\) is a potent selective inhibitor of acetylcholinesterase inhibitor. Data from phase I clinical trials indicates that ZT-1 has good pharmacokinetics properties. Militarinone A, produced by the entomogenous deuteromycete *Paecilomyces militaris* is also reported as having distinct neuritogenic properties in PC12 cells at 10 µM concentrations.\(^{10,11}\)
7.3 The Huprines

A class of compounds recognized as being perhaps the most promising drugs as AChEIs for the treatment of AD are the huprines, a (-)-huperzine A/tacrine hybrid.\textsuperscript{12,13} These are designed to span as much as possible the active site of AChE, Figure 7.5. These 4-aminoquinoline-based compounds share close resemblance to the cytisine alkaloid and are proven to inhibit brain acetylcholinesterase enzyme – both \textit{ex vivo} and \textit{in vivo} and have been shown to improve learning and memory in middle aged mice.\textsuperscript{14} This potent class of compounds is also known to interact with other cholinergic targets including nicotinic and muscarinic receptors.

\textbf{Figure 7.5: The huprines as AChE inhibitors}
7.4 Synthesis of pyridone analogs as SSH1 inhibitors

Owing to the profound biological importance of 2-pyridones and related analogs, several synthetic schemes have been devised for their synthesis.\textsuperscript{15,16,17} The synthetic approach adopted makes use of an α,β-unsaturated chromone to provide the target intermediate bearing the pyridone core,\textsuperscript{18} as shown in Scheme 7.1. This route allows for the introduction of a wide variety of different amines (less nucleophilic or sterically hindered) at the penultimate step of the synthesis, whilst ensuring quick determination of the most active analog for further development.

Scheme 7.1: Synthesis of 2-Pyridone analogs

Reagents and conditions: (a) DMF, POCl\textsubscript{3}, H\textsubscript{2}O, 0 °C – rt, 62-75%; (b) CH\textsubscript{2}(COOCH\textsubscript{3})\textsubscript{2}, TEA, EtOH, mW, 83%; (c) R-NH\textsubscript{2}, AcOH or CsF, EtOH, 75 °C, 55 - 75%; (d) Aqueous NaOH, TBAI, DMSO, mW, 30min, 55 - 88%.

Starting with commercially available hydroxyacetophenone, \textit{7.001}, the key starting compound, 3-formylchromone derivative, \textit{7.002}, was prepared in yields of up to 75% using the Vilsmeier-Haack
cyclization reaction\textsuperscript{19,20,21} employing dry DMF and POCl$_3$. The compound thus obtained, 7.002, is the key intermediate for the synthesis of many important heterocyclic systems owing to its three (3) electron-deficient sites, Figure 7.6, vis-à-vis C2 carbon for Michael addition; aldehyde carbon for Knoevenagel condensation and the C4 carbon of the carbonyl group.\textsuperscript{22}

![Figure 7.6: 3-Formylchromone and its various reactive sites](image)

Depending upon the type of substrate and reaction conditions, a number of different heterocyclic systems can be obtained from the chromone derivative, due to its ability to serve as a Michael acceptor. This compound, 7.002, was subjected to Knoevenagel reaction conditions\textsuperscript{23,24} using diethyl malonate in the presence of TEA to obtain the α,β-unsaturated derivative 7.003. Treatment of 7.003 with different primary amines (A-D) provided the corresponding pyridones in decent yields via a cascade reaction process, that is, aza-Michael addition, chromone ring opening and ring closure.\textsuperscript{18} Thus compounds 7.004 (A-D) were obtained in good yields, however, depending upon the type of amine used (thiazole-2-amine, benzylamine, aniline, or 4-(thiophen-2-yl)thiazol-2-amine), the catalyst (CsF or AcOH) and/or solvents (EtOH or DCM) needed to be varied for optimal yields.\textsuperscript{18} Compounds 7.004 (A-D) were then subjected to saponification conditions to afford the final compounds 7.005 (A-D) using aqueous NaOH in EtOH. The saponification procedure proved to be a much more difficult task than anticipated. The following reaction
conditions were attempted with no success: KOH/MeOH,\textsuperscript{25} FeCl\textsubscript{3}-promoted \textit{O}-alkylation cleavage of the esters;\textsuperscript{26} TMS-I/CCl\textsubscript{4}/H\textsubscript{2}O;\textsuperscript{27} TMS-Cl, NaI/CH\textsubscript{3}CN\textsuperscript{28} and LiOH/CHCl\textsubscript{3}. Recognizing that the lack of apparent reactivity was largely due to the poor solubility of this series of compounds, the saponification process was achieved by using DMSO as solvent, tetrabutylammonium iodide (TBAI) as a phase transfer reagent and an excess amount of aqueous NaOH under microwave irradiation conditions. Compounds \textbf{7.005 (A-D)} were achieved in yields ranging from 55 – 88%.

\textbf{7.5 Masking phenol: Intended mechanistic studies}

Computational modelling studies, as shown in \textbf{Figure 7.7}, suggested that the most crucial features involved in binding to the SSH1 active site are the thiazole nitrogen, which forms a hydrogen bond with SSH1 Arg 399, the carboxylate and the pyridone carbonyl. Neither the phenolic hydrogen nor the ketone functionality appeared to play any role in binding. Thus, in order to assess whether the phenolic functionality is involved in binding, the phenolic site was blocked via alkylation with a methyl group\textsuperscript{29} in order to assess this property in compound \textbf{7.007, Scheme 7.2}.

\begin{figure}
\centering
\includegraphics[width=0.5\textwidth]{compound7005a.png}
\caption{Compound 7.005A and its binding orientation in SSH2 active site}
\end{figure}
Scheme 7.2: *O*-Alkylation of the phenolic functionality

Reagents and conditions: (a) K$_2$CO$_3$, MeI, TBAI, ACN, H$_2$O, DMSO, 42%; (b) Aqueous NaOH, TBAI, DMSO, mW, 30 min, 51%.

Compound **7.004A** was able to undergo *O*-alkylation with methyl iodide (MeI) in the presence of K$_2$CO$_3$ to yield the ether derivative **7.006**, which was subsequently treated with aqueous NaOH to provide the target compound **7.007** as the *O*-methylated ether analog of the initial compound.

It was envisioned that if indeed the phenolic functionality is not involved in binding, it could represent an opportunity to be used as a handle for molecular probes$^{30}$ for elucidating the mechanism of action of these compound, **Figure 7.8**. That is, addition of fluorescein would potentially allow visualization in living systems;$^{31}$ addition of biotin would help pull down biological receptors$^{32,33}$ and the attachment of an azide would likely allow the formation of a covalent bond with a biomolecule under copper catalysis.$^{34}$
Figure 7.8: Compound 7.005A alkylated with proposed linkers for mechanistic studies.

7.6 Derivatization at the 5-position of compound 7.005A

In order to ensure that the compounds were not only active but have acceptable pharmacokinetic properties, derivatization at the 5-position via either carbon-carbon bond formation (Suzuki coupling)\textsuperscript{35,36} or carbon-nitrogen bond formation (Hartwig-Buchwald)\textsuperscript{37} was initiated. Given that these compounds have solubility issues, it was envisioned that this would also be a suitable site for the incorporation of solubilizing groups. A synthesis of the proposed analogs was initiated by first incorporating a synthetic handle, bromine, at the 5-position, which would be exploited using Suzuki methodology, where different boronic acids could be used for the rapid exploration of this portion of the molecule, Scheme 7.3. Additionally, functionality might also be achieved using the well-established Hartwig-Buchwald amination for the installation of amines at this position. The goal was to make additional changes to the molecule at the very end of the synthesis.
Scheme 7.3: Synthesis of bromo-substituted analog

Reagents and conditions: (a) i. DMF, POCl$_3$, H$_2$O, 0 °C – rt, 69%; ii. CH$_2$(COOCH$_3$)$_2$, TEA, EtOH, mW, 82%; iii. Thiazol-2-amine, AcOH, EtOH, 75 °C, 71%; (b). Aqueous NaOH, TBAI, DMSO, mW, 30min, 64%.

Following similar synthetic routes outlined previously, the bromo-analog, 7.010, was achieved in good yields. This compound and its precursor, 7.009, was subjected to Suzuki cross coupling$^{36,38}$ or the Hartwig-Buchwald$^{37}$ reaction conditions to introduce diversity at the 5-position. In order to test the viability of the coupling reactions on this substrate, phenylboronic acid was initially selected for Suzuki-coupling and benzylamine for the Hartwig-Buchwald amination reaction.

The Suzuki coupling on both 7.009 and 7.010 using potassium phosphate or potassium carbonate, bis(triphenylphosphine)palladium chloride [Pd$_2$(PPh$_3$)$_3$Cl$_2$], H$_2$O and DMF$^{38}$ did not produce the desired product. The Hartwig-Buchwald reaction also failed on the acid, 7.010, however an expected side-product, benzylamide, 7.011, was obtained in 11% yield as shown in Scheme 7.4, route a. In order to test the side product in the assays, this reaction was optimized and a yield of 89% - detailed procedure is provided under experimental. Thus, an alternative approach was sought to effect both the C-C coupling and C-N coupling on the compound.
Scheme 7.4: Hartwig-Buchwald reaction

Reagents and conditions: (a) BnNH₂, DPPF, NaOtBu, Pd(OAc)₂, 11%; (b) BnNH₂, CsCO₃, XANTPHOS, Pd²(dba)₃.

One such alternative approach was the use of XANTPHOS and Pd²(dba)₃ in the Hartwig-Buchwald amination reaction. The combination of XANTPHOS and Pd²(dba)₃ had proven to give excellent yields on substrates with an existing ester group. Indeed, this methodology has previously been used on a similar substrate with yield in excess of 80%, Figure 7.4, Chapter 4. However, in this case, the reaction with 4.009 failed to provide the desired product. The solubility issues associated with this series of analogs may account for the lack of reactivity.

Figure 7.9: Hartwig-Buchwald amination with XANTPHOS and Pd²(dba)₃
The C-N coupling was then attempted via nitration and anticipated reducing the nitro group, which would then be followed by S_N2 reaction with benzyl bromide to achieve the target analog, but this was accompanied by its own set of synthetic and purification challenges, Scheme 7.6. Neither 7.01 nor 7.015 could be reduced with SnCl2 and iron (Fe) reduction of 7.014 resulted in a product that could not be isolated. Compounds 7.017 and 7.018 also decomposed upon standing.

Scheme 7.5: Synthesis of nitro analog

Reagents and conditions: (a) Fuming HNO3, AcOH, 25%; (b) DMF, POCl3, H2O, 0 °C – rt, 88%; (c) CH3(COOCH3)2, TEA, EtOH, mW, 61%; (d) Thiazol-2-amine, AcOH, EtOH, 75 °C, 51%; (e) Aqueous NaOH, TBAI, DMSO, mW, 30 min, 59%.
7.6.1 Synthesis of a the phenyl analog via Suzuki coupling of starting material

Although undesirable, the phenyl group was installed at the beginning of the synthesis, using Suzuki methodology,\textsuperscript{36,38} as shown in Scheme 7.6. Compound 7.020 was obtained in the crude form and following similar synthetic routes outlined previously, compound 7.021 was obtained in good yields.

Scheme 7.6: Synthesis of phenyl analog

Reagents and conditions: (a) Phenylboronic acid, PdCl$_2$(PPh$_3$)$_2$, CH$_3$CHOHCH$_3$, H$_2$O; (b) i. DMF, POCl$_3$, H$_2$O, 0 °C – rt, 69%; ii. CH$_2$(COOCH$_3$)$_2$, TEA, EtOH, mW, 82%; iii. Thiazol-2-amine, AcOH, EtOH, 75 °C, 71%; iv. Aqueous NaOH, TBAI, DMSO, mW, 30min, 64%.

7.7 Modifications to the bottom portion of the molecule

Although computational modelling did not predict changes to the bottom portion of the molecule would result in significant impact on SSH1 activity, modern drug discovery hinges on the concept of parallel lead optimization - which ensures that most active drug candidates also possesses acceptable pharmacokinetic properties. Thus, modifications in this portion may lead to the identification of novel compounds with the greatest chance of success as a potential drug. The initial choice of an ideal candidate for replacing the bottom portion was a quinazoline scaffold (7.022), Figure 7.10, since the nitrogens in the quinazoline perfectly overlap the ketone and phenol oxygens in the original molecule, and may ultimately mimic any apparent binding interactions thereof.
Figure 7.10: Modification to bottom portion of molecule

Given that thiazol-2-ylboronic acid is expensive (1 g/$1435.00) for the proposed synthetic route for the top portion of the molecule, Scheme 7.6, a related isostere (phenylboronic acid) was pursued instead, Figure 7.11. To this end, it was proposed that a species such as 7.023 could be conveniently coupled to 7.024 via Suzuki cross coupling reaction.

Figure 7.11: Retrosynthetic scheme for the synthesis of 7.039

7.7.1 Synthesis of iodo and boronic ester derivatives: top portion of quinazoline analog

The top portion of 7.039 was synthesized according to the synthetic scheme outlined below, starting with commercially available 2-hydroxynicotinic acid, 7.025, Scheme 7.7. It was envisioned that either the iodo-derivative, 7.028, or the boronic ester, 7.029, could serve as an ideal
candidate for coupling to an appropriate boronic acid/ester or halo-derivative of quinazoline\textsuperscript{45} via Suzuki methodology.\textsuperscript{36,38}

**Scheme 7.7: Synthesis of iodo and boronic ester derivatives**

![Scheme 7.7: Synthesis of iodo and boronic ester derivatives](image_url)

**Reagents and conditions:** (a) SOCl\textsubscript{2}, DCM, THF, MeOH, 100\%; (b) Phenylboronic acid, Cu(OAc)\textsubscript{2}, Pyr, DCM, 56\%; (c) NIS, DCM, 89\%; (d) Cs\textsubscript{2}CO\textsubscript{3}, Pd(OAc)\textsubscript{2}, BISPIN, MeOH, 41\%

The commercially available 2-hydroxynicotinic acid, \textbf{7.025}, was treated with thionyl chloride, followed by MeOH to obtain methyl 2-hydroxynicotinate, \textbf{7.026}, in quantitative yield.\textsuperscript{46} This compound underwent \textit{N}-arylation (copper-catalyzed C-N coupling) in the presence of the corresponding phenylboronic acid, Cu(OAc)\textsubscript{2} and pyridine in DCM to yield \textbf{7.029}.\textsuperscript{46} Iodination of \textbf{7.029} with \textit{N}-iodosuccinimide (NIS) in DCM\textsuperscript{47} provided the iodo derivative \textbf{7.028}, which was subsequently converted to the corresponding boronic ester, \textbf{7.029}, using bis(pinacolato)diboron (BISPIN), Cs\textsubscript{2}CO\textsubscript{3} and Pd(OAc)\textsubscript{2} in MeOH.\textsuperscript{48} With compounds \textbf{7.028} and \textbf{7.029} in hand, compound \textbf{7.031} was synthesized to test the viability of the Suzuki reaction, **Scheme 7.8** (substituting the quinazoline for a phenyl group). Phenylboronic acid was added to \textbf{7.028} in the presence of Pd(PPh\textsubscript{3})Cl\textsubscript{2} and K\textsubscript{2}CO\textsubscript{3} in dioxane and H\textsubscript{2}O to provide \textbf{7.030} in 72\%. This compound
was able to undergo saponification using aqueous NaOH in dioxane and H2O to provide the acid product, **7.031**.

**Scheme 7.8: Synthesis of phenyl analog: Modification to bottom portion**

Reagents and conditions: (a) Phenylboronic acid, Pd(PPh3)Cl2, K2CO3, Dioxane, H2O, 73%; (b) Aqueous NaOH, Dioxane, H2O, 50 °C, 97%.

### 7.7.2 Synthesis of quinazoline analog bottom portion

Following the successful synthesis of **7.031**, synthesis of a series of quinazolines, **7.033**, **7.034**, **7.035** or **7.036** was initiated which would potentially be coupled to either **7.028** or **7.029** via Suzuki methodology.38,45

**Scheme 7.9: Synthesis of quinazoline derivatives**

Reagents and conditions: (a) HCONH2, 81%; (b) SOCl2, DMF, 50%
The anthranilic acid, **7.031**, was treated with an excess amount of formamide\(^{49}\) and heated to 120 °C under microwave irradiation to obtain compound **7.032**, which was subsequently treated with thionyl chloride and a catalytic amount of DMF\(^{50}\) to obtain compound **7.033**. However, because chlorides are not particularly great candidates for Suzuki cross coupling reactions, compound **7.032** was initially treated with CBr\(_4\) in the presence of PPh\(_3\) in toluene\(^{51}\) but could not achieve the desired product. A similar conversion was also attempted with TBABr, P\(_2\)O\(_5\) in toluene;\(^{52}\) PBr\(_3\), DMF; Appel type reaction on **7.033** with TMSBr in CH\(_3\)CN\(^{53}\) or with HBr-AcOH,\(^{54}\) however all these failed to yield the desired product – any formed bromo-quinazoline derivative quickly hydrolysed back to the keto form, **7.032**. The O-triflate quinazoline derivative, **7.035**\(^{55}\) was synthesized but this also hydrolysed to the keto form upon standing. An attempt to convert **7.033** to the corresponding boronic ester with BISPIN also resulted in **7.032**, even in the presence of molecular sieves and the use of dry solvents. Notwithstanding these challenges, the Suzuki cross coupling reaction was attempted using **7.033** and **7.29** as shown in Scheme 7.10, however, a trace amount of the desired product, **7.037**, was observed along with a significant amount of **7.032**.

**Scheme 7.10: Synthesis of quinazoline analog**

![Scheme 7.10](image)

Reagents and conditions:; (b) 7.033, Pd(OAc)\(_2\), Cs\(_2\)CO\(_3\), DMF, mW (c) Aqueous NaOH, Dioxane, H\(_2\)O, 60 °C.
7.8 Summary of synthesized pyridone analogs

A summary of the synthesized pyridones are provided in Figure 7.12, and their phosphatase inhibitory activities are discussed jointly with the thiazole series in Chapter 8.

Figure 7.12: Summary of pyridone test compounds
7.9 Experimental

General Experimental procedures:

![Chemical Structure](image)

**4-Oxo-4H-chromene-3-carbaldehyde:** A solution of 1-(2-hydroxyphenyl)ethanone (17.7 mL, 147 mmol) in DMF (68.0 mL, 881 mmol) was cooled over ice for 1 hour. POCl₃ (28.8 mL, 308 mmol) was slowly added to the solution and the mixture was heated to 45°C for 1 hour. While stirring, a mixture of ice and H₂O was added and the mixture was stirred for 4 hours. The resulting crystalline solid/precipitate was filtered off, washed thoroughly with H₂O and dried under vacuum. The acidic crude product was stirred in ACN for 10 minutes, filtered and washed with MeOH and dried under vacuum. The pure product, 4-oxo-4H-chromene-3-carbaldehyde, **7.002,** was isolated as a light yellow solid (16 g, 63% yield). ¹H NMR (500 MHz, DMSO) δ 10.11 (s, 1H), 8.91 (s, 1H), 8.13 (dd, J = 1.5, 7.8 Hz, 1H), 7.87 (ddd, J = 8.6, 7.1, 2.0 Hz, 1H), 7.74 (dd, J = 8.3, 1.0 Hz, 1H), 7.53 - 7.61 (m, 1H) ppm. ¹³C NMR (126 MHz, DMSO) δ 188.8, 175.3, 163.9, 156.1, 135.7, 127.2, 125.7, 125.1, 120.4, 119.4 ppm. LRMS (ESI) m/z calc’d for C₁₀H₆O₃ [M+H]+: 175.16, found 175.06.

**Diethyl 2-((4-oxo-4H-chromen-3-yl)methylene)malonate:** A mixture of **7.002** (2.000 g, 11.48 mmol), diethyl malonate (2.023 g, 12.63 mmol) and TEA (0.160 mL, 1.148 mmol) in EtOH (20.0 mL) in a 30 mL microwave vial was heated to 100 °C for 80 mins. The resulting mixture was added to H₂O and neutralized with HCl and extracted with EtOAc, washed with brine and dried.
over anhydrous sodium sulfate, and concentrated in vacuum. The crude product could either be purified by column chromatography (EtOAc:hexane 1:5) or recrystallized from hexane and EtOAc to obtain diethyl 2-((4-oxo-4H-chromen-3-yl)methylene)malonate, **7.003**, as a light brown solid (3.01 g, 9.53 mmol, 83% yield). $^1$H NMR (500 MHz, DMSO) $\delta$ 8.71 (d, $J = 1.0$ Hz, 1H), 8.08 (dd, $J = 8.1$, 1.7 Hz, 1H), 7.85 (ddd, $J = 8.6$, 7.1, 1.5 Hz, 1H), 7.70 (d, $J = 7.8$ Hz, 1H), 7.50 - 7.57 (m, 2H), 4.15 - 4.26 (m, 4H), 1.24 (t, $J = 7.1$ Hz, 3H), 1.14 (t, $J = 7.1$ Hz, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 174.4, 164.9, 164.3, 159.8, 155.7, 135.3, 134.3, 128.1, 126.8, 125.9, 123.5, 119.0, 118.6, 61.8, 61.3, 14.5, 14.2 ppm. LRMS (ESI) m/z calc’d for C$_{17}$H$_{16}$O$_6$ [M+H]$^+$: 317.31, found 317.20.

**Ethyl 1-benzyl-5-(2-hydroxybenzoyl)-2-oxo-1,2-dihydropyridine-3-carboxylate:** To a 50 mL round bottomed flask was added **7.003** (500.0 mg, 1.581 mmol), benzylamine (129.0 µL, 1.179 mmol) and acetic acid (45.00 µL, 0.786 mmol) in EtOH (5 mL). The reaction was stirred at 75 °C for 24 - 36 hours and monitored by TLC. After completion of the reaction, the mixture was evaporated under vacuum and purified by flash column chromatography (hexane and EtOAc) to afford the desired product, **7.004B** (453 mg, 75%). $^1$H NMR (500 MHz, DMSO) $\delta$ 10.25 (s, 1H), 8.69 (d, $J = 2.8$ Hz, 1H), 8.30 (d, $J = 2.8$ Hz, 1H), 7.24 - 7.44 (m, 7H), 6.96 (d, $J = 7.9$ Hz, 1H), 6.92 (t, $J = 7.5$ Hz, 1H), 5.23 (s, 2H), 4.20 (q, $J = 7.2$ Hz, 2H), 1.22 (t, $J = 7.0$ Hz, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 191.4, 164.4, 158.3, 156.2, 149.5, 143.4, 136.6, 133.4, 130.5, 129.1,
128.3, 128.2, 125.1, 119.7, 119.3, 117.1, 116.2, 61.2, 53.2, 14.5 ppm. LRMS (ESI) m/z calc’d for 
C_{22}H_{19}NO_5 [M+H]^+ : 378.40, found 378.38.

1-benzyl-5-(2-hydroxybenzoyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid: A mixture of 
7.004B (200.0 mg, 0.530 mmol), 50% w/w NaOH (70.00 µL, 1.325 mmol) and 
tetrabutylammonium iodide (9.790 mg, 0.026 mmol) in DMSO (5 mL) in a 30 mL microwave vial 
was heated to 75°C in a microwave reactor for 30 minutes. The resulting mixture was allowed to 
cool to room temperature, acidified with aqueous HCl to a pH of 3 and the solid was collected 
using vacuum filtration, rinsed with ethyl acetate and ACN and identified as 1-benzyl-5-(2-
hydroxybenzoyl)-2-oxo-1,2-dihydropyridine-3-carboxylic acid, 7.005B, (163 mg, 0.466 mmol, 
88% yield). ^1H NMR (500 MHz, DMSO) δ 13.57 (br. s., 1H), 10.33 (s, 1H), 8.89 (d, J = 2.9 Hz, 
1H), 8.47 (d, J = 2.9 Hz, 1H), 7.41 - 7.47 (m, 1H), 7.34 - 7.40 (m, 5H), 7.29 - 7.34 (m, 1H), 6.93 - 
7.01 (m, 2H), 5.37 (s, 2H) ppm. ^13C NMR (126 MHz, DMSO) δ 191.4, 164.8, 162.9, 156.2, 149.0, 
144.7, 135.9, 133.9, 130.7, 130.7, 129.2, 128.5, 128.5, 124.8, 119.9, 118.8, 117.1, 53.6 ppm. 
HRMS m/z: [M + H]^+ calc’d for C_{20}H_{15}NO_3 350.1028; Found 350.1026.
**Ethyl 5-(2-hydroxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylate:**

Using conditions identical to those described above, **7.004A** was obtained as a yellow powder (55%). $^1$H NMR (500 MHz, DMSO) $\delta$ 10.34 (s, 1H), 9.35 (d, $J = 2.9$ Hz, 1H), 8.49 (d, $J = 2.5$ Hz, 1H), 7.80 (d, $J = 3.4$ Hz, 1H), 7.75 (d, $J = 3.4$ Hz, 1H), 7.40 - 7.49 (m, 2H), 6.95 - 7.04 (m, 2H), 4.30 (q, $J = 6.9$ Hz, 2H), 1.30 (t, $J = 7.1$ Hz, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 191.4, 163.6, 156.9, 156.2, 155.1, 142.5, 141.8, 138.6, 133.9, 130.6, 124.7, 121.7, 121.4, 119.9, 117.7, 117.1, 61.7, 14.5 ppm. LRMS (ESI) m/z calc’d for C$_{18}$H$_{14}$N$_2$O$_5$S [M+H]$^+$: 371.38, found 371.20.

**5-(2-Hydroxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylic acid:** Using conditions identical to those described above, **7.005A** was obtained as a light brown solid (68%). $^1$H NMR (500 MHz, DMSO) $\delta$ 13.23 (br. s., 1H), 10.35 (s, 1H), 9.37 (d, $J = 2.5$ Hz, 1H), 8.49 (d, $J = 2.5$ Hz, 1H), 7.81 (d, $J = 3.4$ Hz, 1H), 7.77 (d, $J = 3.4$ Hz, 1H), 7.41 - 7.48 (m, 2H), 6.95 - 7.03 (m, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 191.5, 164.8, 157.9, 156.2, 155.1, 142.8, 141.4, 138.6, 133.9, 130.6, 124.7, 121.8, 121.5, 119.9, 118.1, 117.1 ppm. HRMS m/z: [M + H]$^+$ calc’d for C$_{16}$H$_{10}$N$_2$O$_5$S 343.0389; Found 343.0379.
Ethyl 5-(2-hydroxybenzoyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate: Using conditions identical to those described above, 7.004C was obtained as a yellow solid (61%). $^1$H NMR (400 MHz, CDCl$_3$) δ 11.36 (s, 1H), 8.59 (d, $J = 1.2$ Hz, 1H), 8.18 (br. s., 1H), 7.58 (d, $J = 8.2$ Hz, 1H), 7.45 - 7.55 (m, 4H), 7.39 (d, $J = 7.4$ Hz, 2H), 7.07 (d, $J = 8.6$ Hz, 1H), 6.94 (t, $J = 7.6$ Hz, 1H), 4.38 (q, $J = 7.0$ Hz, 2H), 1.36 (t, $J = 7.0$ Hz, 3H) ppm. $^{13}$C NMR (101 MHz, CDCl$_3$) δ 194.2, 164.1, 162.2, 158.4, 147.0, 144.1, 139.6, 136.4, 131.4, 129.5, 129.4, 126.4, 126.3, 120.9, 119.2, 118.7, 115.9, 61.6, 14.2 ppm. LRMS (ESI) m/z calc’d for C$_{21}$H$_{17}$NO$_5$ [M+H]$^+$: 364.37, found 364.27.

5-(2-Hydroxybenzoyl)-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylic acid: Using conditions identical to those above, 7.005C was obtained as a brownish solid (77%). $^1$H NMR (500 MHz, DMSO) δ 13.47 (br. s., 1H), 10.41 (s, 1H), 8.57 (d, $J = 2.9$ Hz, 1H), 8.37 (d, $J = 2.5$ Hz, 1H), 7.49 - 7.61 (m, 5H), 7.37 - 7.45 (m, 2H), 6.91 - 7.01 (m, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 191.1, 164.8, 162.6, 156.3, 149.0, 144.7, 139.7, 134.0, 130.8, 130.0, 129.8, 127.2, 124.7, 120.0, 118.2, 118.1, 117.2 ppm. HRMS m/z: [M + H]$^+$ calc’d for C$_{19}$H$_{13}$NO$_5$ 336.0872; Found 336.0868.
Ethyl 5-(2-hydroxybenzoyl)-2-oxo-1-(4-(thiophen-2-yl)thiazol-2-yl)-1,2-dihydropyridine-3-carboxylate: Using conditions identical to those described above, **7.005D** was obtained as a solid (66%). $^1$H NMR (500 MHz, DMSO) $\delta$ 10.37 (s, 1H), 9.42 (d, $J = 2.5$ Hz, 1H), 8.48 (d, $J = 2.5$ Hz, 1H), 7.96 (s, 1H), 7.56 (d, $J = 3.9$ Hz, 2H), 7.47 - 7.52 (m, 1H), 7.45 (dd, $J = 7.8$, 1.5 Hz, 1H), 7.12 (t, $J = 4.2$ Hz, 1H), 7.05 (d, $J = 8.3$ Hz, 1H), 6.98 - 7.02 (m, 1H), 4.31 (q, $J = 7.3$ Hz, 2H), 1.30 (t, $J = 7.09$ Hz, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 191.4, 163.6, 157.0, 156.3, 154.8, 144.4, 142.6, 141.8, 137.7, 133.8, 130.5, 128.7, 127.0, 125.2, 124.6, 121.2, 119.8, 117.7, 117.1, 114.1, 61.7, 14.5 ppm. LRMS (ESI) m/z calc’d for C$_{22}$H$_{16}$N$_{2}$O$_5$S$_2$ [M + H]$^+$: 453.50, found 453.42.

5-(2-hydroxybenzoyl)-2-oxo-1-(4-(thiophen-2-yl)thiazol-2-yl)-1,2-dihydropyridine-3-carboxylic acid: Using conditions identical to those described above, **7.005D** was obtained as a solid (76%). $^1$H NMR (500 MHz, DMSO) $\delta$ 13.25 (br. s., 1H), 10.38 (s, 1H), 9.43 (d, $J = 2.5$ Hz, 1H), 8.48 (d, $J = 2.5$ Hz, 1H), 7.97 (s, 1H), 7.55 - 7.59 (m, 2H), 7.47 - 7.52 (m, 1H), 7.45 (dd, $J = 7.6$, 1.7 Hz, 1H), 7.12 (dd, $J = 5.1$, 3.7 Hz, 1H), 7.04 (d, $J = 8.3$ Hz, 1H), 6.98 - 7.02 (m, 1H) ppm.
$^{13}$C NMR (126 MHz, DMSO) δ 191.5, 164.8, 157.9, 156.3, 154.8, 144.5, 142.9, 141.4, 137.7, 133.9, 130.5, 128.7, 127.0, 125.2, 124.7, 121.4, 119.9, 118.0, 117.1, 114.2 ppm. HRMS m/z: [M + H]$^+$ calc’d for C$_{20}$H$_{12}$N$_2$O$_5$S$_2$ 425.0266; Found 425.0248.

Ethyl 5-(2-methoxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylate: To a mixture of 7.004A (100.0 mg, 0.270 mmol), iodomethane (57.50 mg, 0.405 mmol), K$_2$CO$_3$ (56.00 mg, 0.405 mmol) and tetrabutylammonium iodide (9.970 mg, 0.027 mmol) in ACN (1.5 mL) was added a few drops of DMSO and H$_2$O and heated to 70°C overnight. The resulting mixture was concentrated and purified using DCM/MeOH as the eluent to afford ethyl 5-(2-methoxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylate, 7.006, (43.6 mg, 0.113 mmol, 42% yield). $^1$H NMR (500 MHz, DMSO) δ 9.27 (d, $J = 2.5$ Hz, 1H), 8.46 (d, $J = 2.9$ Hz, 1H), 7.79 (d, $J = 3.4$ Hz, 1H), 7.76 (d, $J = 3.4$ Hz, 1H), 7.59 - 7.65 (m, 1H), 7.44 (dd, $J = 7.3$, 2.0 Hz, 1H), 7.26 (d, $J = 8.3$ Hz, 1H), 7.11 - 7.16 (m, 1H), 4.30 (q, $J = 6.9$ Hz, 2H), 3.75 (s, 3H), 1.30 (t, $J = 7.1$ Hz, 3H) ppm. $^{13}$C NMR (101 MHz, DMSO) δ 190.6, 163.5, 157.1, 156.8, 155.0, 142.0, 141.8, 138.5, 133.5, 129.9, 126.7, 121.6, 121.5, 121.4, 117.6, 112.8, 61.6, 56.3, 14.5 ppm. LRMS (ESI) m/z calc’d for C$_{19}$H$_{16}$N$_2$O$_5$S [M + H]$^+$: 385.41, found 385.36.
5-(2-Methoxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylic acid: Using conditions identical to those above, 7.007 was obtained as a solid (51%). $^1$H NMR (500 MHz, DMSO) $\delta$ 13.24 (br. s., 1H), 9.29 (d, $J = 2.5$ Hz, 1H), 8.46 (d, $J = 2.5$ Hz, 1H), 7.80 (d, $J = 3.4$ Hz, 1H), 7.76 (d, $J = 3.4$ Hz, 1H), 7.61 (dt, $J = 8.0$, 1.7 Hz, 1H), 7.45 (dd, $J = 7.3$, 1.5 Hz, 1H), 7.26 (d, $J = 8.3$ Hz, 1H), 7.13 (t, $J = 7.3$ Hz, 1H), 3.74 (s, 3H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 190.7, 164.7, 158.9, 157.9, 157.2, 155.0, 142.2, 141.5, 138.6, 133.6, 129.9, 126.7, 121.8, 121.3, 117.9, 112.7, 56.1 ppm. HRMS m/z [M + H]$^\dagger$ calc’d for C$_{17}$H$_{12}$N$_2$O$_5$S: 357.0545; Found 357.0545.

6-bromo-4-oxo-4H-chromene-3-carbaldehyde: Using conditions identical to those described above, 7.008 (a) i was obtained as a tan solid (69%). $^1$H NMR (500 MHz, DMSO) $\delta$ 10.08 (s, 1H), 8.94 - 8.95 (m, 1H), 8.19 (d, $J = 2.5$ Hz, 1H), 8.04 (dd, $J = 8.8$, 2.5 Hz, 1H), 7.76 (d, $J = 8.8$ Hz, 1H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 188.5, 174.2, 164.2, 155.0, 138.2, 127.8, 126.7, 122.0, 120.5, 119.7 ppm. LRMS (ESI) m/z calc’d for C$_{10}$H$_3$BrO$_3$ [M + H]$^\dagger$: 254.05/256.05, found 254.10/256.10.
**Diethyl 2-((6-bromo-4-oxo-4H-chromen-3-yl)methylene)malonate:** Using conditions identical to those described above, 7.008 (a) ii was obtained as a brown solid (82%). $^1$H NMR (500 MHz, DMSO) δ 8.74 (s, 1H), 8.13 (d, $J = 2.5$ Hz, 1H), 8.02 (dd, $J = 8.8, 2.5$ Hz, 1H), 7.72 (d, $J = 8.8$ Hz, 1H), 7.49 (d, $J = 1.0$ Hz, 1H), 4.15 - 4.26 (m, 4H), 1.24 (t, $J = 7.1$ Hz, 3H), 1.11 - 1.19 (m, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 173.2, 164.8, 164.2, 160.1, 154.7, 137.9, 133.8, 128.5, 127.9, 125.0, 121.8, 119.2, 118.6, 61.9, 61.4, 14.4, 14.2 ppm. LRMS (ESI) m/z calc’d for C$_{17}$H$_{13}$BrO$_6$ [M + H]+: 395.20/397.20, found 395.28/397.28.

**Ethyl 5-(5-bromo-2-hydroxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylate:** Using conditions identical to those described above, 7.009 was obtained as a solid (71%). $^1$H NMR (500 MHz, DMSO) δ 10.63 (s, 1H), 9.33 (d, $J = 2.5$ Hz, 1H), 8.47 (d, $J = 2.5$ Hz, 1H), 7.81 (d, $J = 3.4$ Hz, 1H), 7.77 (d, $J = 3.4$ Hz, 1H), 7.60 (dd, $J = 8.6, 2.7$ Hz, 1H), 7.54 (d, $J = 2.5$ Hz, 1H), 6.98 (d, $J = 8.8$ Hz, 1H), 4.30 (q, $J = 6.9$ Hz, 2H), 1.30 (t, $J = 7.1$ Hz, 3H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 189.7, 163.5, 156.8, 155.1, 155.0, 142.2, 142.0, 138.6, 136.0, 132.4, 127.0, 121.7, 121.5, 119.4, 117.4, 110.9, 61.7, 14.5 ppm. LRMS (ESI) m/z calc’d for C$_{18}$H$_{13}$BrN$_2$O$_5$S [M + H]$^+$: 448.28/450.28, found 448.17/450.17.
5-(5-Bromo-2-hydroxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylic acid: Using conditions identical to those described above, 7.010 was obtained as a solid (64%).

$^1$H NMR (500 MHz, DMSO) $\delta$ 13.26 (br. s., 1H), 10.64 (s, 1H), 9.35 (d, $J = 2.9$ Hz, 1H), 8.47 (d, $J = 2.9$ Hz, 1H), 7.83 (d, $J = 3.4$ Hz, 1H), 7.78 (d, $J = 3.4$ Hz, 1H), 7.60 (dd, $J = 8.6$, 2.7 Hz, 1H), 7.54 (d, $J = 2.9$ Hz, 1H), 6.98 (d, $J = 8.8$ Hz, 1H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 189.8, 164.8, 157.8, 155.1, 155.0, 142.5, 141.7, 138.6, 136.0, 132.4, 127.8, 121.7, 121.1, 119.4, 117.7, 110.9 ppm. HRMS m/z calculated for C$_{16}$H$_9$BrN$_2$O$_5$S [M + H]$^+$ 420.9494; Found 422.9459.

N-benzyl-5-(5-bromo-2-hydroxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxamide: A mixture of ethyl 5-(5-bromo-2-hydroxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylate (200.0 mg, 0.445 mmol), phenylmethanamine (57.20 mg, 0.534 mmol) and potassium 2-methylpropan-2-olate (100 mg, 0.89 mmol) in toluene (1.0 mL) in a microwave vial was heated to 65°C for 1 hour. After completion of the reaction, the crude was purified in hexane/ethyl acetate to afford N-benzyl-5-(5-bromo-2-hydroxybenzoyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxamide (202 mg, 89%). $^1$H NMR (500 MHz, DMSO) $\delta$ 12.77 (s, 1H), 10.59 (s, 1H), 8.97 (d, $J = 2.5$ Hz, 1H), 8.68 (d, $J = 2.5$ Hz, 1H), 7.59 (dd, $J = 8.8$, 2.7 Hz, 1H) ppm.
2.5 Hz, 1H), 7.53 (d, J = 3.4 Hz, 1H), 7.50 (d, J = 2.5 Hz, 1H), 7.35 - 7.41 (m, 4H), 7.30 - 7.34 (m, 2H), 6.97 (d, J = 8.8 Hz, 1H), 5.41 (s, 2H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 183.8, 172.4, 162.0, 160.8, 157.3, 155.3, 149.5, 144.0, 138.1, 136.0, 135.8, 132.5, 129.2, 128.5, 127.3, 119.5, 118.3, 117.3, 115.2, 110.9, 53.8 ppm. HRMS m/z [M + H]$^+$ calcd for C$_{23}$H$_{16}$BrN$_3$O$_4$S 510.0123; found 512.0114.

1-(4-Hydroxy-[1,1'-biphenyl]-3-yl)ethan-1-one: To a stirred solution of 1-(5-bromo-2-hydroxyphenyl)ethanone (1.00 g, 4.65 mmol), phenylboronic acid (1.14 g, 9.30 mmol), K$_2$CO$_3$ (1.29 g, 9.30 mmol) in 2-propanol and H$_2$O (14 mL: 3.5 mL) under argon atmosphere was added Pd(PPh$_3$)$_2$Cl$_2$ (0.07 g, 0.10 mmol). The contents were stirred at room temperature for 20 minutes and slowly heated to reflux for 12 hours. The solvents were removed under reduced pressure, the reaction mixture was extracted with ethyl acetate (2X) and washed with H$_2$O, dried over sodium sulfate and concentrated under pressure to obtain the crude product which was used directly in the next step without further purification.

4-oxo-6-phenyl-4H-chromene-3-carbaldehyde: Using conditions identical to those described above, 7.020 (b) i, was obtained as a solid (58%). $^1$H NMR (500 MHz, DMSO) δ 10.12 - 10.15 (m, 1H), 8.94 - 8.96 (m, 1H), 8.30 (d, J = 2.0 Hz, 1H), 8.16 - 8.20 (m, 1H), 7.83 - 7.87 (m, 1H), 7.76 (d, J = 7.3 Hz, 2H), 7.52 (t, J = 7.6 Hz, 2H), 7.40 - 7.46 (m, 1H) ppm. $^{13}$C NMR (101 MHz,
DMSO) $\delta$ 188.9, 175.3, 163.9, 155.5, 139.0, 138.5, 134.0, 129.7, 128.7, 127.4, 125.4, 122.9, 120.4, 120.2 ppm. LRMS (ESI) m/z calc’d for C$_{16}$H$_{10}$O$_{3}$ [M + H]$^+$: 251.25, found 251.20.

Dimethyl 2-((4-oxo-6-phenyl-4H-chromen-3-yl)methylene)malonate: Using conditions identical to those above, 7.020 (b) ii was obtained as a solid and used in the next step without further purification (63%). LRMS (ESI) m/z calc’d for C$_{21}$H$_{16}$O$_{6}$ [M + H]$^+$: 365.35, found 365.25.

Methyl 5-(4-hydroxy-[1,1'-biphenyl]-3-carbonyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylate:

Using conditions identical to those described above, 7.020 (b) iii was obtained as a solid (70%). $^1$H NMR (500 MHz, DMSO) $\delta$ 10.50 (s, 1H), 9.43 (d, $J$ = 2.5 Hz, 1H), 8.54 (d, $J$ = 2.5 Hz, 1H), 7.76 - 7.81 (m, 3H), 7.70 (d, $J$ = 2.5 Hz, 1H), 7.62 - 7.65 (m, 2H), 7.39 - 7.44 (m, 2H), 7.28 - 7.33 (m, 1H), 7.11 (d, $J$ = 8.8 Hz, 1H), 3.84 (s, 3H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 191.1, 164.1, 156.8, 155.8, 155.1, 142.9, 142.0, 139.5, 138.6, 132.0, 131.9, 129.3, 128.5, 127.4, 126.6, 125.1, 121.7, 121.0, 117.8, 52.9 ppm. LRMS (ESI) m/z calc’d for C$_{23}$H$_{16}$N$_{2}$O$_{5}$S [M + H]$^+$: 433.45, found 433.32.
5-(4-hydroxy-[1,1'-biphenyl]-3-carbonyl)-2-oxo-1-(thiazol-2-yl)-1,2-dihydropyridine-3-carboxylic acid: Using conditions identical to those described above, 7.021 was obtained as a solid (89%). $^1$H NMR (500 MHz, DMSO) $\delta$ 13.26 (br. s., 1H), 10.51 (s, 1H), 9.43 (d, $J = 2.5$ Hz, 1H), 8.54 (d, $J = 2.5$ Hz, 1H), 7.76 - 7.82 (m, 3H), 7.70 (d, $J = 2.5$ Hz, 1H), 7.62 - 7.65 (m, 2H), 7.40 - 7.44 (m, 2H), 7.28 - 7.33 (m, 1H), 7.11 (d, $J = 8.3$ Hz, 1H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 191.2, 164.8, 157.9, 155.8, 155.1, 142.8, 141.6, 193.5, 138.6, 132.0, 131.9, 129.4, 128.5, 127.4, 126.6, 125.2, 121.8, 121.5, 118.1, 117.8 ppm. HRMS m/z [M + H]$^+$ calc’d for C$_{22}$H$_{14}$N$_2$O$_5$S 419.0702; Found 419.0690.

Methyl 2-hydroxynicotinate: To a suspension of 2-hydroxynicotinic acid (10.0 g, 71.9 mmol) in anhydrous DCM (150 mL) was added thionyl chloride (26.0 mL, 356 mmol) followed by anhydrous THF (150 mL). The reaction mixture was stirred at room temperature for 1 hr., and then excess MeOH (300 mL) was added to the suspension until a homogenous solution was obtained. The reaction mixture was filtered and the filtrate was concentrated under reduced pressure to give a pale yellow oil which solidified to an off white solid (11 g, 100%) under vacuum. A portion of the crude material was used directly in subsequent step without further purification. LRMS (ESI) m/z calculated for C$_7$H$_7$NO$_3$ [M + H]$^+$: 154.14, found 154.10.
Methyl 5-iodo-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate.

**Step 1:** To a mixture of methyl 2-hydroxynicotinate (6.00 g, 39.2 mmol), phenylboronic acid (14.3 g, 118 mmol), copper (II) acetate (14.23 g, 78.00 mmol) and pyridine (12.68 mL, 157.0 mmol) in DCM was added 5 g of 4 Å molecular sieves. The reaction mixture was stirred at room temperature overnight and open to the atmosphere. The solvent was removed in vacuo and extracted 3 times with aqueous ammonium chloride solution and DCM and the organic phase was purified using column chromatography (hexane:ethyl acetate=1:2) to obtain methyl 2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate (5 g, 21.81 mmol, 56% yield), which was used in the next step.

**Step 2:** To a solution of methyl 2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate (3.000 g, 13.09 mmol) in DCM (19.45 mL) and TFA (16.21 mL) was added N-iodosuccinimide (2.940 g, 13.09 mmol) under an argon atmosphere. After stirring for 19 h at ambient temperature the solvent was concentrated in vacuo. To the residue were added ethyl acetate (200 mL) and saturated aq. sodium hydrogen carbonate (150 mL) and the organic phase was collected, washed with H₂O and brine, dried over sodium sulfate, and concentrated in vacuo. The residue was the subtitled compound, methyl 5-iodo-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate (4.12 g, 89% yield) as a pale yellow solid. ¹H NMR (500 MHz, DMSO) δ 8.20 (d, J = 2.9 Hz, 1H), 8.16 (d, J = 2.9 Hz, 1H), 7.43 - 7.53 (m, 3H), 7.38 - 7.43 (m, 2H), 3.73 (s, 3H) ppm. ¹³C NMR (126 MHz, DMSO) δ 164.3, 157.3, 151.4, 148.5, 140.1, 129.5, 129.2, 127.4, 123.0, 63.9, 52.5 ppm. LRMS (ESI) m/z calc’d for C₁₃H₁₀INO₃ [M + H]⁺: 356.13, found 356.0.
A flame dried round bottomed flask was charged with cesium carbonate (1.84 g, 5.63 mmol), BISPIN (2.860 g, 11.26 mmol), palladium(II) acetate (0.063 g, 0.282 mmol) and methyl 5-iodo-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate (1.00 g, 2.82 mmol) and MeOH (20 mL) under argon atmosphere. The reaction mixture was heated to reflux for 42 h. After completion of the reaction, the mixture was passed over a short column of neutral Al₂O₃ with diethyl ether. The solvent was evaporated under reduced pressure to obtain the crude material, methyl 2-oxo-1-phenyl-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-dihydropyridine-3-carboxylate (0.550 g, 55%). LRMS (ESI) m/z calc’d for C₁₉H₂₂BNO₅ [M + H]⁺: 356.20, found 356.01.

2-oxo-1,5-diphenyl-1,2-dihydropyridine-3-carboxylic acid: Methyl 5-iodo-2-oxo-1-phenyl-1,2-dihydropyridine-3-carboxylate (300.0 mg, 0.845 mmol), potassium carbonate (292.0 mg, 2.112 mmol), phenylboronic acid (155.0 mg, 1.267 mmol) and bis(triphenylphosphine)palladium(II) chloride (29.60 mg, 0.042 mmol) in a round bottom flask was added dioxane (6.80 mL) and H₂O (1.70 mL). The resulting mixture was stirred under argon...
at 80 °C for 24 hours and allowed to cool to rt. The cooled reaction mixture was filtered through alumina and concentrated on a rotary evaporator to obtain methyl 2-oxo-1,5-diphenyl-1,2-dihydropyridine-3-carboxylate (187 mg, 73%).

**Step 2:** To a solution of methyl 2-oxo-1,5-diphenyl-1,2-dihydropyridine-3-carboxylate (300.0 mg, 0.983 mmol) in dioxane (2 mL) and H₂O (1 mL) was added 50% w/w NaOH (104 µL, 1.965 mmol) and the mixture was heated to 50 °C for 3 hr. The resulting solution was concentrated on a rotary evaporator and purified using column chromatography (hexanes and EtOAc, 4:1, as eluent) to obtain compound 7.031 as a brown solid, 97%. ¹H NMR (400 MHz, DMSO) δ 14.28 (s, 1H), 8.70 - 8.73 (s, 1H), 8.49 - 8.53 (s, 1H), 7.35 - 7.76 (m, 10H) ppm. ¹³C NMR (126 MHz, DMSO) δ 165.1, 163.2, 144.6, 142.5, 139.7, 134.7, 129.8, 129.7, 129.5, 128.4, 127.4, 126.6, 121.2, 117.9 ppm. HRMS m/z: [M + H]⁺ calc’d for C₁₈H₁₃NO₃ [M + H]⁺ 292.0974; Found 292.0972.

![7.032](image)

A mixture of 2-aminobenzoic acid (10.0 g, 72.9 mmol) and formamide (21.3 mL, 535 mmol) in a microwave vessel was heated in a microwave reactor to 150 °C for 1 hour. The slurry was cooled to rt and stirred in NaHCO₃ and the resulting solid was filtered and washed with water followed by with diethyl ether and dried on a lyophilizer to provide quinazolin-4(3H)-one, 7.032 (7.51 g, 71%). ¹H NMR (500 MHz, DMSO) δ 12.22 (br. s., 1H), 8.11 (dd, J = 8.1, 1.2 Hz, 1H), 8.08 (s, 1H), 7.77 - 7.82 (m, 1H), 7.65 (d, J = 8.3 Hz, 1H), 7.48 - 7.53 (m, 1H) ppm. ¹³C NMR (126 MHz, DMSO) δ 161.2, 149.2, 145.9, 134.7, 127.6, 127.2, 126.3, 123.1 ppm. LRMS (ESI) m/z calc’d for C₈H₆N₂O [M + H]⁺: 147.15, found 147.1.
To a solution of quinazolin-4(3H)-one (2.000 g, 13.68 mmol) in thionyl chloride (52.3 mL, 717 mmol) was added DMF (0.8 mL) and the mixture was heated to reflux overnight. The reaction mixture was cooled to rt and evaporated to dryness to provide crude compound which was purification by column chromatography to provide 4-chloroquinazoline, 7.033 (0.650 g 29%). $^1$H NMR (500 MHz, DMSO) $\delta$ 8.65 (s, 1H), 8.14 (dd $J = 8.1$, 1.2 Hz, 1H), 7.87 - 7.91 (m, 1H), 7.77 (d, $J = 7.8$ Hz, 1H), 7.58 - 7.62 (m, 1H) ppm. $^{13}$C NMR (126 MHz, DMSO) $\delta$ 160.3, 147.6, 144.3, 135.6, 128.2, 126.8, 124.4, 122.3 ppm. LRMS (ESI) m/z calc’d for C$_8$H$_5$ClN$_2$ [M + H]$^+$: 164.59, found 164.48.
7.10 References


(8) Huperzine A - List Results - ClinicalTrials.gov
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Chapter 8

Thiazole Analogs

8.1 Thiazoles

Thiazoles are aromatic heterocyclic organic compounds with a five membered molecular ring structure (C₃H₃NS), having nitrogen and sulfur heteroatoms as part of the ring, Figure 8.1. The aromaticity of the thiazole ring is based on the delocalization of a lone pair electrons of the sulfur heteroatom which completes the 6π electrons that satisfy the Hückel’s rule. Thiazole was discovered in 1887 by Hantzsch and Wader and its structure was confirmed by Popp two (2) years later.2

![Figure 8.1: Structure of thiazole](image)

8.1.1 Thiazole based Therapeutics

The thiazole ring is present in many different natural products and medicinally important compounds, Figure 8.2, including vitamin B₃ (thiamine),3 the β-lactam antibiotic, penicillin G;4 the potent anti-fungal, ruvaconazole;5 the anti-retroviral drug, ritonavir;6 the respiratory stimulant and antidote for barbiturate/opiate overdose, amiphenazole;7 the anti-microbial agent, sulfathiazole;8 broad spectrum anti-fungal agent, abafungin;9 the fungicide, angiogenesis inhibitor, chelating agent for lead, mercury and antimony poisoning, thiabendazole;10 the nonsteroidal anti-inflammatory drug (NSAID) for joint and muscular pain, fentiazac11 and the schistosomicide and
periodontitis, niridazole.\textsuperscript{12,13} Among others are the monobactam antibiotic, aztreonam;\textsuperscript{14,15} xanthine oxidase inhibitor, feboxostat;\textsuperscript{16} the dopamine D3 receptor agonist used to treat Parkinson’s disease, pramipexazole;\textsuperscript{17} treatment for type II diabetes mellitus, teneligliptin\textsuperscript{18} and the antipsychotic agent used for the treatment of schizophrenia, lurasidone.\textsuperscript{19} All of the above mentioned drugs are currently in use in the clinic.

Figure 8.2: Some thiazole based drugs in clinical use.
There are a number of thiazole derivatives currently under clinical investigation and they include anticancer agents\textsuperscript{20} CNS modulators,\textsuperscript{21} anticonvulsants,\textsuperscript{22} acetylcholine receptor ligands\textsuperscript{23} opioid receptor antagonists,\textsuperscript{24} anti-tubercular agents,\textsuperscript{25} anti-diabetic agents,\textsuperscript{26} anti-obesity agents,\textsuperscript{27} anti-inflammatory agents,\textsuperscript{28} anti-viral agents,\textsuperscript{29} antimicrobial agents,\textsuperscript{30} and anti-Alzheimer’s agents.

Development of thiazole-based anti-Alzheimer’s agents, \textbf{Figure 8.3}, include compounds that inhibit the dual specific tyrosine phosphorylation regulated kinase 1A phosphorylation on Thr 212, \textit{8.001},\textsuperscript{31} AD related glycogen synthase kinase 3β inhibitor, \textit{8.002},\textsuperscript{32} A\textsubscript{2A}R antagonists, \textit{8.003},\textsuperscript{33} strong inhibitor of plaque deposition, \textit{8.004},\textsuperscript{34} potent A\textsubscript{3}R antagonist, \textit{8.005},\textsuperscript{35} (112) and dopamine d\textsubscript{4} receptor antagonists, \textit{8.006}.\textsuperscript{36}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure83.png}
\caption{Some thiazole based compounds as anti-AD agents in development}
\end{figure}

Thiazoles have established themselves as an important scaffold in medicinal chemistry through their profound therapeutic effects. Owing to their therapeutic profile in organic and biological
chemistry, various synthetic methods have been developed for the construction of the thiazole ring. These include the Hantzch, Tchernic, Gabriel and Cook-Heilborn thiazole synthesis.\textsuperscript{37} The Hantzsch synthesis, which involves the reaction between thioamides and haloketones, was used for the synthesis of all the thiazole analogs.

### 8.2 Synthesis of thiazole analogs

Thiazoles can be synthesised via the Hantzsch thiazole synthesis\textsuperscript{37,38} involving the reaction between an α-haloketone species and a thiourea derivative. The retrosynthetic scheme, Figure 8.4, for the Hantzsch thiazole synthesis is shown below.

![Retrosynthetic scheme of thiazole synthesis](image)

The required α-haloketone derivative (α-bromothiophene), 8.008, Scheme 8.1, was initially prepared by treating commercially available 2-acetylthiophene (2-AT) with two equivalents of copper (II) bromide in DCM,\textsuperscript{39} but the best results came from treating 2-AT with an equimolar quantity of bromine in MeOH and H\textsubscript{2}O at 0 °C to rt\textsuperscript{40} or by using DCM as the solvent at rt\textsuperscript{41} or with AcOH as the solvent\textsuperscript{38} to provide a mixture of the desired bromide, 8.008, and the corresponding dibromide, which was easily separable via column chromatography.
Reagents and conditions: (a) Br₂, MeOH, H₂O, 72%; (b) HCl/Ether, rt, 82%; (c) NH₃SCN, NaHSO₃, 52%; (d) EtOH, 79 - 87%; (e) Aqueous NaOH, EtOH, 69 - 86%.

The thiourea derivative, 8.011, for the Hantzch thiazole synthesis was prepared from the corresponding HCl-salt of ethyl aminobenzoate (EAB), 8.009, in H₂O. Alternatively, the thiourea, 8.011, can be synthesized in aqueous HCl (2N) using the free amine form, 8.009. Thus, the addition of 2.1 equivalents of ammonium thiocyanate to the amine solution of ethyl aminobenzoate, 8.010, in H₂O in the presence of catalytic amounts of sodium bisulfite, 42 readily afforded the thiourea, 8.011, which was isolated as a solid. The ester thiazoles, 8.012 (A-E), were
consistently obtained in yields greater than 78% when the respective $\alpha$-bromothiophenes were coupled together with the thiourea, 8.011, in the Hanszch thiazole synthesis.\textsuperscript{40} The products thus obtained were able to undergo saponification with aqueous NaOH in DMSO to obtain compounds 8.013 (A – E). A methyl substituent was introduced in compound 8.013 B for a twisting effect and to observe how this might affect the overall activity. Compound 8.014 was synthesized in the same manner starting with 3-acetylthiophene.

8.2.1 Phenyl analogs of thiazoles

In the pursuit of active SSH1 inhibitor that could potentially be used as a treatment for AD, a systematic exploration of the thiazole series was carried out by first substituting the thiophene for benzene (phenyl) or methoxy-substituted phenyl derivatives, and employing similar synthetic routes as outlined previously. Benzene (phenyl group) is a known thiophene isostere\textsuperscript{43} and could offer superior binding orientation. Thus, compounds 8.015 – 8.019 were prepared (Figure 6.2), and subsequently tested in the biochemical assay. The para-methoxy analog, 8.019, was initially obtained from commercial sources, which showed good SSH1 inhibitory activity in the assay and was synthesized to confirm its activity. The meta- and ortho-methoxy substituted analogs (8.017, 8.018) were also synthesized in an attempt to improve upon the observed activity.
8.2.2 Pyridine analogs of thiazoles

Three pyridine analogs were prepared according to the synthetic procedures described previously. However, α-bromination of the pyridinylethanones were carried out using HBr and Br₂ in acetic acid,⁴⁴,⁴⁵ which gave the corresponding α-bromoketones with yields consistently greater than 84%. Since pyridine derivatives are known to exhibit excellent activities against a wide range of biological targets,⁴⁶ it was envisioned that pyridine analog could improve upon the activity and exhibit improved pharmacokinetics. The synthesized pyridine analogs are provided in Figure 8.6.
8.2.3 tert-butyl analog of thiazoles

A tert-butyl analog, 8.023, was also prepared to investigate the effect removal of aromaticity would have on the SSH1 inhibitory activity, Figure 8.7.

8.023

Figure 8.7: tert-butyl analog

8.2.4 Phosphonic Acid analog

A recently obtained crystal structure of the active site of SSH1 indicated the presence of a number of inorganic phosphate groups. Thus, it was envisioned that compounds that could compete with the inorganic phosphate groups might offer superior binding and may lead to the development of potent compounds as SSH1 inhibitors. To this end a phosphonate version of the thiazole analog was prepared to evaluate its activity. The synthetic scheme for the phosphonic acid analog is provided below, Scheme 8.2.
Scheme 8.2: Synthesis of phosphonic acid analog 8.029

Reagents and conditions: (a) HCl/Ether, 94%; (b) NH₃SCN, NaHSO₃, 89%; (c) 8.008A, EtOH, 76%; (d) Diethyl phosphonate, TEA, Pd(PPh₃)₄, 62%; (e) Conc. HCl, 32%.

The bromo-thiazole derivative, 8.027, was synthesized according to the previously described procedure, and was able to undergo palladium-catalyzed cross coupling reaction with diethyl phosphonate⁴⁷ to give the aryl phosphonate product, 8.028, in 62% yield. This product was then hydrolyzed in concentrated HCl⁴⁸ to give the corresponding acid, 8.029.
8.3 Summary of synthesized thiazole compounds

Figure 8.8: Summary of thiazole test compounds
Figure 8.9: Pyridone analogs
8.4 Results and discussion

The SSH1 inhibitory activity of the synthesized compounds was evaluated in the pNPP phosphatase assay by Dr. Zao Xingyu in the laboratory of Prof. David Kang, USF Byrd’s Alzheimer’s Institute. Graphs 1, shows the SAR results observed with analogs 7.005 (A-D) and 8.013A.

Graph 1: SSH1 inhibitory activity of analogs 8.013A, 7.005A-D

The activity of the thiazole lead compound, 8.013A, was confirmed, but the initial pyridone hit/lead compound, 7.005A, did not show activity. Among the compounds tested, only the thiophene-thiazole analog, 7.005D, showed some activity. It is worth noting that this compound shares striking similarities with 8.013A.
With the activity of \textbf{8.013A} confirmed, the activity of the remaining pyridone analogs were evaluated, using \textbf{8.013A} as the standard compound. Graph 2 shows the SAR studies results observed with analogs \textbf{7.007}, \textbf{7.010}, \textbf{7.011} and \textbf{7.021}.

![Graph 2: SSH1 inhibitory activity of analogs 8.013A, 7.007, 7.010, 7.011, 7.021.](image)

\textbf{8.013A} maintained its SSH1 inhibitory activity, however, there was no inhibitory activity observed from the remaining pyridone analogs. Strikingly, the amide analog, \textbf{7.011}, obtained as a by-product, appeared to show increased activity and enhanced the enzyme’s ability to catalyze the dephosphorylation from para-nitrophenyl phosphate to para-nitrophenol, the chromogenic species.

Following the successful confirmation of SSH1 inhibitory activity of \textbf{8.013A}, a series of compounds were acquired from commercial sources (Chembridge) and their activities compared with \textbf{8.013A}. Graphs 3, 4 and 5 show the results of this SSH1 inhibitory comparison studies.
Graph 3: Comparison of 8.013A to E1-series of analogs.
[Chembridge IDs: E1B 5976776; E1E 6748322; E1C 7425371; E1D 7369557]

Clearly, 8.013A is more potent than the set of E-series of compounds shown above. E1B, E1C and E1E exhibit comparable activity, which can be distinguished by running multiple concentrations.

Graph 4: Comparison of 8.013A to C2-series of analogs
[Chembridge IDs: C2B 5935324; C2C 7905874; C2D 5663386; C2E 7984560; C2F 5807736; C2G 7412943]
C2B is a paramethoxyphenyl thiazole analog and appears to be the only C2 analog with good SSH1 inhibitory activity. Other related analogs were synthesized in an attempt to improve upon the activity of the methoxyphenyl thiazole analogs.

Graph 5: Comparison of 8.013A to E-series analogs
[IDs: E1 7965015; E2 7992372; E3 7958228; E4 7114024;E5 7970657]

E1, E2 and E3 are structurally similar and exhibit nearly identical activity. E1 – E4 showed superior activity to 8.013A. In an attempt to improve upon the observed activity, SAR studies of 8.013A was explored and a range of diverse analogs was prepared and screened in the pNPP assay for their inhibitory effects, Graph 6.
Although several (10) of these synthesized compounds (10) showed good activity as SSH1 inhibitor in the pNPP assay, eight (8) showed superior inhibitory activity at 8 hours (Graph 6), over the initial hit/lead compound, 8.013A. Analogs that showed better inhibitory activity over 8.013A include 8.013B (methyl thiazole), 8.013C (5-methyl thiophene), 8.013D (4-methyl thiophene), 8.013E (3-methyl thiophene), 8.014 (3-thiophene analog), 8.019 (para-methoxyphenyl analog), 8.021 (3-pyridine analog) and 8.022 (4-pyridine analog).
8.5 Experimental section

General synthetic procedures:

2-bromo-1-(thiophen-2-yl)ethan-1-one: To a solution of 2-acetylthiophene (5.00 g, 39.6 mmol) in MeOH (20 mL) at 0°C was added bromine (2.2 mL, 42 mmol) dropwise. The mixture was allowed to stir for 45 min and H₂O (20 mL) was added to the resulting mixture and stirred overnight at room temperature. At the completion of the reaction H₂O (20 mL) was added and the resulting mixture was extracted twice with EtOAc. The organic layer was washed with sodium bicarbonate and H₂O, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to obtain 2-bromo-1-(thiophen-2-yl)ethanone, 8.007A, (5.85 g, 72%) as a brown semi-solid. LRMS (ESI) m/z calc’d for C₆H₅BrOS [M+H]⁺: 204.07/206.07, found 205.17/207.17. A portion of the crude material was used in a subsequent step without further purification.

Ethyl 4-thioureidoanbezoate: Ethyl 4-aminobenzoate (20.0 g, 121 mmol) was stirred in 1N HCl in diethyl ether (200 mL) overnight and the resulting off-white precipitate was filtered off, dried and identified as ethyl 4-aminobenzoate hydrochloride (23.9 g, 98%). This compound (23.0 g, 114 mmol) was dissolved in H₂O (160 mL), followed by the addition of ammonium thiocyanate (18.0 g, 240 mmol) and sodium bisulfite (0.60 g, 5.70 mmol). The reaction mixture was heated at 85 °C for 15 h and then refluxed for 24 hours. The solution was cooled to room temperature and the precipitate filtered off and washed with H₂O and 2N HCl. The product was dried under vacuum.
and identified as mixture of the thiourea, 8.011, and ethyl amnobenzoate, 8.009, as a light yellow solid (6.50 g, 25.4%). LRMS (ESI) m/z calc’d for C_{10}H_{12}N_{2}O_{2}S [M+H]^+: 225.28, found 225.08.

![8.012A](image)

**Ethyl 4-((4-(thiophen-2-yl)thiazol-2-yl)amino)benzoate**: To a solution of 2-bromo-1-(thiophen-2-yl)ethanone (3.60 g, 17.6 mmol) in EtOH (37 mL) was added ethyl 4-thioureidobenzoate (4.330 g, 19.31 mmol). The mixture was allowed to stir at 60 °C for 3.5 hours and cooled to room temperature. Aqueous sodium bicarbonate was added to the resulting mixture, which was extracted twice with ethyl acetate. The combined organic phase was washed with H₂O and brine, dried over anhydrous magnesium sulfate and concentrated under reduced pressure. The resulting residue thus obtained was purified by column chromatography (EtOAc/hexane = 1/2) to obtain the title compound, 8.012A, (4.58 g, 79%). \(^1\)H NMR (500 MHz, DMSO) \(\delta\) 10.74 (s, 1H), 7.92 (d, \(J = 8.7\) Hz, 2H), 7.78 (d, \(J = 8.8\) Hz, 2H), 7.56 - 7.44 (m, 2H), 7.25 (s, 1H), 7.09 (dd, \(J = 5.0, 3.7\) Hz, 1H), 4.26 (q, \(J = 7.1\) Hz, 2H), 1.30 (t, \(J = 7.1\) Hz, 3H) ppm. \(^13\)C NMR (126 MHz, DMSO) \(\delta\) 165.8, 162.7, 145.5, 145.3, 138.8, 131.1, 128.4, 125.9, 124.1, 122.5, 116.5, 103.0, 60.7, 14.7 ppm. LRMS (ESI) m/z calc’d for C_{16}H_{14}N_{2}O_{2}S_{2} [M+H]^+: 331.42, found 331.40.
4-((4-(Thiophen-2-yl)thiazol-2-yl)amino)benzoic acid: To 8.012A (4.000 g, 12.11 mmol) in EtOH (15 mL) was added 50% w/w sodium hydroxide (2.00 g, 24.2 mmol). The mixture was heated to 75 °C in a microwave reactor for 35 minutes. The resulting mixture was acidified with 2N HCl and the solid collected via suction filtration, washed with EtOH and EtOAc and dried under vacuum to obtain the title compound as a solid, 8.013A, (3.15 g, 86%). $^1$H NMR (500 MHz, DMSO) δ 12.56 (br. s., 1H), 10.71 (s, 1H), 7.91 (d, $J = 8.8$ Hz, 2H), 7.75 (d, $J = 8.8$ Hz, 2H), 7.50 (m, 2H), 7.25 (s, 1H), 7.09 (dd, $J = 5.0$, 3.6 Hz, 1H) ppm. $^{13}$C NMR (126 MHz, DMSO) δ 167.4, 162.7, 145.3, 145.2, 138.9, 131.2, 128.4, 125.9, 124.1, 123.4, 116.4, 102.9 ppm. HRMS m/z: [M+H]$^+$ calc’d for C$_{14}$H$_{10}$N$_2$O$_2$S$_2$ 303.0262; Found 303.0242.

2-bromo-1-(thiophen-2-yl)propan-1-one: To a stirred solution of 1-(thiophen-2-yl)propan-1-one (5.00 g, 35.7 mmol) in DCM (95 mL) was added bromine (1.80 mL, 35.7 mmol) dropwise. The solution was stirred for 1 hour, then neutralized by the addition of aqueous sodium carbonate. The aqueous phase was extracted three times with DCM, and the combined organic extracts were concentrated under reduced pressure to afford 2-bromo-1-(thiophen-2-yl)propan-1-one, 8.007B, in nearly quantitative yield (7.5 g, 96%). LRMS (ESI) m/z calc’d for C$_7$H$_7$BrOS [M+H]$^+$:
218.10/220.10, found 218.23/220.23. A portion of this material was used in the next step without further purification.

![8.012B](image)

**Ethyl 4-((5-methyl-4-(thiophen-2-yl)thiazol-2-yl)amino)benzoate:** Using conditions identical to those described above, the coupling reaction between 8.007B and 8.011 in EtOH yielded the target compound, 8.012B, as a solid (87%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.57 (s, 1H), 7.86 (d, $J = 8.8$ Hz, 2H), 7.73 (d, $J = 8.8$ Hz, 2H), 7.49 (d, $J = 4.6$ Hz, 1H), 7.34 (d, $J = 3.2$ Hz, 1H), 7.10 (dd, $J = 5.1$, 3.5 Hz, 1H), 4.23 (q, $J = 7.0$ Hz, 2H), 2.45 (s, 3H), 1.27 (t, $J = 7.0$ Hz, 3H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 165.8, 158.8, 158.6, 145.6, 140.8, 140.0, 138.8, 130.9, 128.2, 122.2, 116.7, 116.3, 60.6, 14.7, 12.2 ppm. LRMS (ESI) m/z calc’d for C$_{17}$H$_{16}$N$_2$O$_2$S$_2$ [M+H]$^+$: 345.45, found 345.40.

![8.013B](image)

**4-((5-Methyl-4-(thiophen-2-yl)thiazol-2-yl)amino)benzoic acid:** Using conditions identical to those described above, 8.012B was converted into 8.013B and obtained as a solid (77%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.67 (s, 1H), 7.85 (d, $J = 8.8$ Hz, 2H), 7.72 (d, $J = 8.8$ Hz, 2H), 7.49 (d, $J = 4.6$ Hz, 1H), 7.33 (d, $J = 3.1$ Hz, 1H), 7.10 (dd, $J = 5.1$, 3.5 Hz, 1H), 2.44 (s, 3H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 167.5, 158.7, 145.4, 140.0, 138.9, 131.1, 128.3, 125.7, 124.9, 123.1, 116.6, 116.3, 12.3 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{15}$H$_{12}$N$_2$O$_2$S$_2$ 315.0262; Found 315.0261.
4-((4-(5-Methylthiophen-2-yl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, 8.012C was converted into 8.013C and obtained as a solid (82%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.95 (s, 1H), 7.86 (d, $J = 8.8$ Hz, 2H), 7.77 (d, $J = 8.8$ Hz, 2H), 7.27 (d, $J = 3.5$ Hz, 1H), 7.09 (s, 1H), 6.73 - 6.76 (m, 1H), 2.50 (s, 1H), 2.42 (s, 3H) ppm. $^{13}$C NMR (101MHz, DMSO) $\delta$ 167.5, 162.7, 145.4, 145.3, 139.1, 136.6, 131.1, 126.7, 123.9, 123.2, 116.4, 102.0, 15.5 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{15}$H$_{12}$N$_2$O$_2$S$_2$ 315.0262; Found 315.0257.

4-((4-(5-Methylthiophen-2-yl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, 8.012D was converted into 8.013D and obtained as a solid (69%). $^1$H NMR (400 MHz, DMSO) $\delta$ 11.02 (s, 1H), 7.86 (d, $J = 8.8$ Hz, 2H), 7.75 (d, $J = 8.8$ Hz, 2H), 7.31 (s, 1H), 7.15 (s, 1H), 7.02 (s, 1H), 2.50 (s, 1H), 2.19 (s, 3H) ppm. $^{13}$C NMR (101MHz, DMSO) $\delta$ 167.5, 162.7, 145.4, 145.2, 138.6, 138.3, 131.1, 126.1, 123.2, 121.0, 116.4, 102.7, 16.0 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{15}$H$_{12}$N$_2$O$_2$S$_2$ 315.0262; Found 315.0260.
4-((4-(3-methylthiophen-2-yl)thiazol-2-yl)amino)benzoic acid. Using conditions identical to those described above, 8.012E was converted into 8.013E and obtained as a solid (73%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.91 (s, 1H), 7.86 (d, $J = 8.8$ Hz, 2H), 7.77 (d, $J = 8.8$ Hz, 2H), 7.35 (d, $J = 5.1$ Hz, 1H), 6.96 (s, 1H), 6.92 (d, $J = 5.1$ Hz, 1H), 2.50 (s, 1H), 2.38 (s, 3H) ppm. $^{13}$C NMR (101 MHz, DMSO-d$_6$) $\delta$ 167.4, 162.0, 145.3, 145.0, 133.9, 132.8, 132.1, 131.1, 124.4, 123.3, 116.4, 103.9, 16.2 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{15}$H$_{12}$N$_2$O$_2$S$_2$ 317.0262; Found 315.0260.

4-((4-(Thiophen-3-yl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, 8.014 was obtained as a solid (69%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.81 (br. s., 1H), 7.84 - 7.90 (m, 3H), 7.79 - 7.84 (m, 2H), 7.54 - 7.57 (m, 2H), 7.22 (s, 1H). $^{13}$C NMR (101 MHz, DMSO) $\delta$ 167.5, 162.7, 146.9, 145.5, 139.1, 131.2, 127.3, 126.4, 123.2, 122.3, 116.5, 104.0 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{14}$H$_{10}$N$_2$O$_2$S$_2$ 301.0105; Found 301.0105.
4-((4-Phenylthiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, and starting with acetophenone, **8.015** was obtained as a solid (68%). $^1$H NMR (400 MHz, DMSO) $\delta$ 11.03 (br. s., 1H), 7.83 - 7.93 (m, 7H), 7.37 - 7.43 (m, 3H), 7.25 - 7.31 (m, 1H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 167.5, 162.8, 150.4, 145.5, 134.8, 131.2, 129.1, 128.1, 126.2, 123.2, 116.4, 104.7 ppm. HRMS m/z: [M-H] calc’d for C$_{16}$H$_{12}$N$_2$O$_2$S 295.0541; Found 295.0543.

4-((5-Methyl-4-phenylthiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, and starting with propiophenone, **8.016** was obtained as a solid (71%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.62 (br. s., 1H), 7.85 (d, $J$ = 8.7 Hz, 2H), 7.71 (d, $J$ = 8.8 Hz, 2H), 7.64 (d, $J$ = 8.7 Hz, 2H), 7.40 - 7.45 (m, 2H), 7.29 - 7.34 (m, 1H), 2.40 (s, 3H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 167.4, 159.4, 145.3, 144.8, 134.8, 131.2, 128.8, 128.5, 127.8, 123.3, 118.0, 116.6, 12.3 ppm. HRMS m/z: [M-H] calc’d for C$_{17}$H$_{14}$N$_2$O$_2$S 309.0698; Found 309.0698.
4-((4-(2-Methoxyphenyl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, and starting with 2-methoxy-substituted-acetophenone, 8.017 was obtained as a solid (88%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.75 (br. s., 1H), 8.12 (dd, $J = 7.8, 1.56$ Hz, 1H), 7.88 (d, $J = 8.7$ Hz, 2H), 7.79 (d, $J = 8.7$ Hz, 2H), 7.45 (s, 1H), 7.28 (ddd, $J = 8.5, 7.1, 2.0$ Hz, 1H), 7.00 - 7.11 (m, 2H), 3.89 (s, 3H) ppm. $^{13}$C NMR (101MHz, DMSO) $\delta$ 167.5, 161.2, 157.0, 146.2, 145.4, 131.2, 129.8, 129.2, 123.2, 122.8, 121.0, 116.4, 112.0, 108.5, 55.9 ppm. HRMS m/z: [M+H]$^+$ calc’d for C$_{17}$H$_{14}$N$_2$O$_3$S 327.0803; Found 327.0804.

4-((4-(3-Methoxyphenyl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, and starting with 3-methoxy-substituted-acetophenone, 8.018 was obtained as a solid (89%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.82 (br. s., 1H), 7.89 (d, $J = 8.7$ Hz, 2H), 7.78 - 7.82 (d, $J = 8.8$ Hz, 2H), 7.43 - 7.50 (m, 3H), 7.31 (t, $J = 8.0$ Hz, 1H), 6.87 (td, $J = 1.4, 8.2$ Hz, 1H), 3.79 (s, 3H), 2.50 (s, 1H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 167.5, 162.6, 160.0, 150.4, 145.5, 136.1, 131.2, 130.2, 123.2, 118.6, 116.4, 113.7, 111.6, 105.0, 55.5 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{17}$H$_{14}$N$_2$O$_3$S 325.0647; Found 325.0646.
4-((4-Methoxyphenyl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, and starting with 4-methoxy-substituted-acetophenone, **8.019** was obtained as a solid (89%). $^1$H NMR (400 MHz, DMSO-d$_6$) $\delta$ 10.91 (s, 1H), 7.89 - 7.81 (m, 7H), 7.22 (s, 1H), 6.96 (br d, $J = 8.2$ Hz, 2H), 3.75 (s, 3H). $^{13}$C NMR (101MHz, DMSO-d$_6$) $\delta$ 167.5, 162.7, 159.3, 150.2, 145.6, 131.2, 127.7, 127.5, 123.1, 116.4, 114.5, 102.5, 55.6. HRMS m/z: [M-H]$^-$ calc’d for C$_{17}$H$_{14}$N$_2$O$_3$S 325.0647; Found 325.0640.

2-Bromo-1-(pyridin-2-yl)ethan-1-one: To a solution of 2-acetylpyridine (5.00 g, 41.3 mmol) and conc. HBr (48% in acetic acid; 13.9 g, 83.0 mmol) was added bromine (6.60 g, 41.3 mmol) dropwise at room temperature with stirring. The reaction mixture was heated to 60 °C for 5 hr. The solvents were then evaporated to afford a yellowish solid, which was washed with diethyl ether via filtration (89%). The bromoketone was used in the next step without further purification. LRMS (ESI) m/z calc’d for C$_7$H$_6$BrNO [M + H]$^+$: 199.04/201.04, found 199.21/201.21.
**Ethyl 4-((4-(pyridin-2-yl)thiazol-2-yl)amino)benzoate:** Using conditions identical to those described above, 8.020 was obtained as a solid (74%). $^1$H NMR (400 MHz, DMSO) $\delta$ 11.02 (s, 1H), 8.75 (d, $J = 5.5$ Hz, 1H), 8.38 - 8.49 (m, 2H), 8.19 (s, 1H), 7.88 - 7.98 (m, 4H), 7.74 - 7.81 (m, 1H), 4.25 (q, $J = 7.0$ Hz, 2H), 1.28 (t, $J = 7.0$ Hz, 3H) ppm. $^{13}$C NMR (101 MHz, DMSO) 165.8, 163.9, 146.0, 145.6, 144.9, 143.3, 143.2, 131.0, 125.3, 124.0, 123.0, 117.2, 115.9, 60.7, 14.7 ppm. LRMS (ESI) m/z calc’d for C$_{17}$H$_{15}$N$_3$O$_2$S [M + H]$^+$: 326.39, found 326.40.

**4-((4-(Pyridin-2-yl)thiazol-2-yl)amino)benzoic acid:** Using conditions identical to those described above, 8.020 was obtained as a solid (91%). $^1$H NMR (400 MHz, DMSO) $\delta$ 11.25 (s, 1H), 8.74 (br d, $J = 5.4$ Hz, 1H), 8.47 - 8.38 (m, 2H), 8.31 (s, 1H), 7.95 - 7.88 (m, 5H), 7.79 - 7.73 (m, 1H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 167.4, 163.8, 145.0, 144.4, 141.6, 140.7, 139.5, 133.5, 131.2, 127.7, 123.6, 116.8, 110.5 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{15}$H$_{11}$N$_3$O$_2$S 296.0494; Found 296.0495.
4-((4-(Pyridin-3-yl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, and starting with 3-acetylpyridine, 8.021 was obtained as a solid (87%). $^1$H NMR (400 MHz, DMSO) $\delta$ 11.13 (s, 1H), 9.36 (d, $J = 1.6$ Hz, 1H), 8.95 (br d, $J = 8.2$ Hz, 1H), 8.77 (br d, $J = 5.3$ Hz, 1H), 8.02 (dd, $J = 8.1$, 5.6 Hz, 1H), 7.96 (s, 1H), 7.90 (dd, $J = 8.1$, 5.6 Hz, 2H), 7.85 (m, $J = 5.6$, 8.1 Hz, 2H), 2.50 (s, 1H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 167.44, 163.77, 145.02, 144.42, 141.62, 140.73, 139.51, 133.49, 131.18, 127.69, 123.61, 116.76, 110.45 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{15}$H$_{11}$N$_3$O$_2$S 296.0494; Found 296.0497.

4-((4-(Pyridin-4-yl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, and starting with 4-acetylpyridine, 8.022 was obtained as a solid (85%). $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 11.28 (s, 1H), 8.86 (d, $J = 6.6$ Hz, 2H), 8.45 (d, $J = 6.7$ Hz, 2H), 8.36 (s, 1H), 7.92 (d, $J = 8.8$ Hz, 2H), 7.86 (d, $J = 8.8$ Hz, 2H), 2.50 (s, 1H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ = 167.42, 163.68, 148.90, 145.78, 144.90, 142.36, 131.18, 123.78, 122.84, 116.96, 116.84. HRMS m/z: [M-H]$^-$ calc’d for C$_{15}$H$_{11}$N$_3$O$_2$S 296.0494; Found 296.0504.
Ethyl 4-((4-(tert-butyl)thiazol-2-yl)amino)benzoate: Using conditions identical to those described above, and starting with commercially available 1-bromo-3,3-dimethylbutan-2-one, 8.023 i was obtained as a solid (83%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.49 (s, 1H), 7.87 (d, $J = 8.7$ Hz, 2H), 7.60 (d, $J = 8.7$ Hz, 2H), 6.50 (s, 1H), 4.23 (q, $J = 7.03$ Hz, 2H), 1.23 - 1.28 (m, 12H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 165.8, 162.2, 162.0, 145.9, 131.0, 121.9, 116.1, 101.2, 60.5, 34.9, 30.0, 14.7 ppm. LRMS (ESI) m/z calc’d for C$_{16}$H$_{20}$N$_2$O$_2$S [M+H]$^+$: 305.41, found 305.39.

4-((4-(tert-butyl)thiazol-2-yl)amino)benzoic acid: Using conditions identical to those described above, 8.023 was obtained as a solid (92%). $^1$H NMR (400 MHz, DMSO) $\delta$ 10.51 - 10.70 (m, 1H), 7.84 (d, $J = 8.7$ Hz, 2H), 7.69 (d, $J = 8.7$ Hz, 2H), 6.48 (s, 1H), 1.24 (s, 9H) ppm. $^{13}$C NMR (101 MHz, DMSO) $\delta$ 167.4, 162.6, 160.8, 145.4, 131.2, 123.3, 116.5, 101.0, 34.7, 29.9 ppm. HRMS m/z: [M-H]$^-$ calc’d for C$_{14}$H$_{16}$N$_2$O$_2$S 275.0854; Found 275.0855.
**N-(4-bromophenyl)-4-(thiophen-2-yl)thiazol-2-amine**: Using conditions identical to those described above, **8.027** was obtained as a light brown solid (76%). LRMS (ESI) m/z calc’d for C_{13}H_{9}BrN_{2}S_{2} [M+H]^+: 336.25/338.25, found 336.40/338.40.

\[
\text{8.027}
\]

**8.027**

**13C NMR (101 MHz, DMSO) δ 163.0, 145.1, 143.7, 139.0, 132.2, 128.4, 125.8, 124.0, 116.5, 116.3, 102.5 ppm. HRMS m/z: [M-H]^- calc’d for C_{13}H_{11}N_{2}O_{3}PS_{2} 336.9870; Found 336.9880.**

**4-((4-(Thiophen-2-yl)thiazol-2-yl)amino)phenyl)phosphonic acid**: A mixture of **8.029** (1.000 g, 2.97 mmol), diethyl phosphonate (1.640 g, 11.86 mmol), TEA (1.080 g, 10.67 mmol) and tetrakis(triphenylphosphine)palladium(0) [Pd(PPh_3)_4] (0.514 g, 0.445 mmol) was irradiated under microwave conditions for 4 hours to obtain the phosphonate ester which was then treated with concentrated HCl to give **8.029** as a dark brown solid. \(^1\text{H} \text{NMR (400 MHz, DMSO) δ 10.53 (s, 1H), 7.66 (dd, J = 8.5, 2.8 Hz, 2H), 7.55 - 7.52 (m, 3H), 7.44 (d, J = 3.2 Hz, 1H), 7.40 (d, J = 5.0 Hz, 1H), 7.14 (s, 1H), 7.02 (d, J = 3.9 Hz, 1H) ppm.} \(^{13}\text{C} \text{NMR (101 MHz, DMSO) δ 163.0, 145.1, 143.7, 139.0, 132.2, 128.4, 125.8, 124.0, 116.5, 116.3, 102.5 ppm. HRMS m/z: [M-H]^- calc’d for C_{13}H_{11}N_{2}O_{3}PS_{2} 336.9870; Found 336.9880.**
8.6 References


Chapter 9
Conclusions

9.1 Summary and conclusions

Current treatment for AD only temporarily relieves symptoms, but does not alter the underlying biology of the disease. Patients who receive the current standard of care ultimately progress to the advanced form of the disease. In spite of the tenacious efforts to identify compounds for treating AD, the lack of effective treatment for the disease continues to be a major unmet medical need.

Novel pyridones and thiazoles that potentially inhibit the SSH1 phosphatase enzyme pathway, which could lead to inhibition of excessive Cofilin activation have been successfully synthesized. The pyridone analogs were obtained via a aza-bicyclo-Michael type reaction, and the thiazoles were prepared through a Hantzsch thiazole synthesis. The synthetic approaches allowed for the quick preparation of several different analogs for evaluation. Each synthesized compound was evaluated for in vitro SSH1 inhibitory activity employing the pNPP phosphatase assay. As a non-proteinaceous and non-specific substrate, pNPP was employed to evaluate protein phosphatases using a spectrophotometric assay. This assay is based on the ability of the enzymes (phosphatases) to catalyze the hydrolysis of pNPP to pNP (para-nitrophenol). The pNP is a chromogenic species with an absorbance at 405 nm. The majority of the synthesized compounds showed moderate to high SSH1 inhibitory activity.
The initial plan was to confirm the activity of the lead compounds by first synthesizing compounds 7.005A and 8.013A, together with other pyridone analogs. The SAR studies were initially centered on the pyridone nitrogen where varying choices of amines were used. This allowed the quick synthesis of different analogs and effortlessly determine the most active candidate for further development. Modelling suggested that the crucial features of the pyridone series were the carboxylic acid functionality, pyridone carbonyl and the thiazole nitrogen. As a result, compound 7.007, was prepared by masking the phenolic hydrogen to confirm the findings. Moreover, the thiazole nitrogen was believed to bind to Arg 399, thus it was envisioned that decreasing or increasing this binding interaction may have a clear impact on the activity. To this end, compound 7.005C, of which the thiazole ring was substituted for a phenyl group was prepared. Hence the sulfur was replaced with a carbon-carbon double bond and the nitrogen with a carbon atom. Compound 7.005D - a thiazole-thiophene N-substituted analog was also prepared aimed, at evaluating the importance of the thiophene ring. This compound proved to be the only analog among the pyridone series that showed activity in the assay.

Surprisingly, data from the initial screening results showed that the pyridones series were not particularly active as SSH1 inhibitors, but the activity of the thiazole analog, 8.013A, was confirmed. Among the pyridone series, compound 7.011 exhibited an increased activity, that is, it appeared to enhance the phosphatase ability at catalyzing the hydrolysis of pNPP to pNP. Attention was thus shifted to focus on the thiazole series for SAR studies, since compound 8.013A exhibited superior and comparable SSH1 phosphatase inhibitory activity over a number of compounds presumed to be SSH1 inhibitors (from commercial sources).
A lead optimization campaign was initiated with 8.013A to identify compounds that would not only be more potent and selective, but also possess acceptable pharmacokinetic properties. Firstly, a methyl substituted thiazole analog, 8.013B, was prepared in order to investigate the twisting effect of the methyl substituent on the activity of this analog. Additionally, to investigate the effect of substituents on this series, compounds 8.013C-D was prepared via a ‘methyl walk’ on the thiophene ring. Subsequently the thiophene ring was substituted for the known thiophene-isostere, phenyl group, as well phenyl-substituted analogs in compounds 8.015, 8.016, 8.017, 8.018 and 8.019. The thiophene ring was replaced with 2-,3- and 4-substituted pyridine rings (8.020, 8.021, 8.022). Finally, one drastic change to the lead molecule was made by replacing the thiphene ring with a tert-butyl group. This was intended to remove aromaticity from the molecule and to observe the effect this will have on the SSH1 inhibitory activity.

Following the revelation of the presence of inorganic phosphate groups within the active site of SSH1 via crystal structure, it was envisioned that compounds bearing the phosphonic acid functionality may achieve superior binding and consequently better activity by competing with the existing inorganic phosphate groups. To this end, a phosphonic acid analog 8.029 was prepared.

Compound 8.013C possessing a methyl group on the thiazole ring was one of the most potent compounds in this series. Similarly, compounds which differ from 8.013C by having the methyl substituent on the thiophene rings (8.013E, 8.013D and 8.013B) demonstrated potent SSH1 inhibitory activity. These findings suggest that methyl substitutions on either the thiazole or thiophene rings are important for anti-SSH1 activity. Compound 8.021, the 3-pyridine analog, exhibited unusual activity – its profound inhibition was only seen at 6 – 7 hours after incubation.
Prior to those stated hours, compound 8.021 showed no inhibitory effect. The 4-pyridine analog, 8.022, was equipotent to 8.013A, however the 2-pyridone analog, 8.020, showed no activity. This suggests that the position of the nitrogen on the pyridine ring is important. Moving the sulfur in the thiophene ring was also shown to enhance activity and compound 8.014 was observed to be slightly better than 8.013A. Multiple concentrations have to be tested in order to identify the best compound.

The exchange of the thiophene ring for a phenyl group was shown to be deleterious for SSH1 inhibitory activity, however, the methoxy-substituted phenyl analogs exhibited SSH1 inhibitory activity, with the best compound being the para-methoxy analog, 8.019. No SSH1 inhibition was observed for the tert-butyl, 8.023, and phosphonic acid analogs, 8.029.

Data obtained from this series further establishes the importance of the thiazole scaffold in medicinal chemistry. The introduction of a methyl group increased the SSH1 inhibitory activity of the series, giving rise to the most potent compound seen to date. The methyl groups probably twist the compound in the right orientation to facilitate binding. Further investigations into the molecular target and mechanistic studies of these compounds are currently underway.
Appendices

(Assays, NMR Spectra and Copyright permission)
Appendix – I

Biological Assays

1. Leishmaniasis

All data were obtained using 96-well plates. Control compounds were used in order to ensure proper assay behaviour. All reported assay data are the average of three consecutive readings (N=3) and variability were typically in the range of less than +/- 10%. Miltefosine was obtained from commercial sources (Sigma-Aldrich) and was used as acquired.

1.a. L. donovani axenic amastigote assay

Cell Line

L. donovani axenic amastigotes were cultured in RPMI 1640 at a pH of 5.5 with 7.5 g/L Hepes (Invitrogen Corp., Carlsbad, CA), 5.86 g/L MES (Sigma-Aldrich, St Louis, MO), 2 g/L sodium bicarbonate (Fisher Scientific, Pittsburgh, PA), 10 mg/L Hemin (Sigma-Aldrich, St Louis, MO), 100 μM Xanthine (Sigma-Aldrich, St Louis, MO), 40 mg/L Tween-80 (Sigma-Aldrich, St Louis, MO), 1% Penicillin-Streptomycin, 5 g/L Trypton-Peptone (BD Bioscience, Sparks, MD), and 20% 16 hours heat inactivated FBS. L. donovani was incubated at 37 °C. All culturing was done using non-vented 25 cm² tissue culture flasks (Coning, Lowell, MA)

Cell viability assay

The viability of amastigotes was determined by the Cell Titer 96 Aqueous Assay (Promega, Madison, WI) that employs a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium; MTS] and electron-coupling reagent, phenazine methosulphate (PMS). Test compounds were serially diluted in 100 μL of PBS
in 96 cell plates using a Biomeck 3000 (Beckman Coulter, Miami, FL). Ten μl of each well was transferred to another 96 well plate and then receives 90 μl or parasites in media. The parasites were in a concentration to have 6,600 parasites per well. After 72 hours 20 μl of MTS solution is added to each well in the 96 well plates. The plates were then incubated 37 °C for four hours to achieve optimal color development. After 4 h of incubation, the OD values were determined at 490 nm using a Spectra Max M2 (Molecular Devices, Sunnyvale, CA). The results were presented as the percentage of survivors (OD value with test compound divided by that of untreated control). Curve fitting using non-linear regression was done using trifox to obtain IC50 values.

1.b. Cytotoxicity assay

Cell Line

J774A.1 (ATCC, Manassas, VA) macrophages were cultured in RPMI 1640 (Invitrogen Corp., Carlsbad, CA) at a pH of 7.2 containing 10% fetal bovine serum (Invitrogen Corp., Carlsbad, CA) and 1% penicillin-streptomycin (Invitrogen Corp., Carlsbad, CA) The cells were incubated at 37 °C in a humidified atmosphere containing 5% CO2 and serially passaged biweekly.

Cell viability assay

The viability of the macrophages was determined by the Cell Titer 96 Aqueous Assay (Promega, Madison, WI) that employs a tetrazolium compound [3-(4,5-dimethylthiazol-2-yl)-5(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium; MTS] and electron-coupling reagent, phenazine methosulphate (PMS). Test compounds were serially diluted in 100 μl of PBS in 96 cell plates using a Biomeck 3000 (Beckman Coulter, Miami, FL). Ten μl of each well was transferred to another 96 well plate and then receives 90 μl or parasites in media. The parasites
were in a concentration to have 50,000 parasites per well. After 72 hours 20 μl of MTS solution is added to each well in the 96 well plates. The plates were then incubated 37 °C for four hours to achieve optimal color development. After 4 h of incubation, the OD values were determined at 490 nm using a Spectra Max M2 (Molecular Devices, Sunnyvale, CA). The results were presented as the percentage of survivors (OD value with test compound divided by that of untreated control). Curve fitting using non-linear regression was done using trifox to obtain IC₅₀ values.

2. **Hsp 90 Assay**

The Hsp 90 fluorescence polarization assay was run by Hardik Patel in the laboratory of Prof. Gabriela Chiosis using the protocol described in their manuscript (Kim, J.; Felts, S.; Llauger, L.; He, H.; Huezo, H.; Rosen, N.; Chiosis, G. Development of a fluorescence polarization assay for the molecular chaperone Hsp 90. *J. Biomol. Screening* **2004**, *9*, 375-381) and using their standard compound PU-H71 as a control compound.

3. **p-Nitrophenyl phosphate phosphatase assay**

**SSH1-N461 Purification**

SSH1-N461 (truncated SSH1 with catalytic function) was subcloned into a pFastBac1 vector and transferred into a DH10Bac E. coli to generate a Bacmid, which was transfected with Sf9 insect cells and cultured in Sf900-SFM medium for 72 hours, to generate P1 virus. The P1 virus generated was further transfected with new Sf9 cells and cultured for 72 hours and the harvested cells were added in lysis buffer (Tris 20mM, NaCl 150 mM, Triton-X100 1%, 10 mM imidazole, with protease inhibitors) and centrifuged at 12,000 g for 15 minutes, following which the supernatant was collected. To the supernatant was added Ni sepharose high performance beads
and shaken for 1 hour at 4 °C, washed 3 times with lysis buffer and centrifuged for 2 minutes at 700 g. The SSH1-N461 was then eluted with the elution buffer – Tris 20 mM, NaCl 150 mM, 200 mM imidazole), and dialysated with dialysis buffer – Tris 20 mM, NaCl 150 mM, DTT 1 mM).

**pNPP Assay Procedure**

Each compound 10 mM 1µL (final 100 µM) was incubated with 10X reaction buffer 10 µL (Tris 500 mM pH 7.4, NaCl 1M, MgCl$_2$ 20 mM, DTT 10 mM) with SSH1-N461 (final 1 µM) at room temperature for 2 hours. pNPP 500 mM 20 µL (final 100 mM), was then added to the mixture (total volume 100 µL) and incubated at 37 °C and absorbances read at intervals of 1 hour.
Appendix II

Chapter 2 – NMR Spectra

![NMR Spectra Diagrams](image-url)
Appendix III

Chapter 3 – NMR Spectra

Chemical Shift (ppm)

Composition of the signal peaks:

- 7.35
- 7.37
- 6.57
- 6.86
- 4.19
Appendix IV

Chapter 4 – NMR Characterization

![NMR Characterization Diagram]
JWL-BE1-037_B_PROTON_01

H2N

H

Chemical Shift (ppm)

1.61
3.40
3.37
47.32
3.27
44.77
42.99
2.07
29.42
2.05
JWL-BE1-038_MONO_SUBS_BROMO_PROTON_01
1H
DMSO-d6
10 Hs

Chemical Shift (ppm)
Chemical Shift (ppm)

Normalized Intensity

JWL-BE1-061_2-RECRYS_PROTON_01
Chemical Shift (ppm)

Normalized Intensity

jwl-be1-060_2_P2_PROTON_01.esp

Chemical Shift (ppm)

Normalized Intensity
Appendix V

Chapter 7 – NMR Spectra

[Chemical structure image]

[Proton NMR spectrum image]

[Carbon NMR spectrum image]
Chemical Shift (ppm)

Normalized Intensity

Normalized Intensity
Chemical Shift (ppm)
Appendix VI

Chapter 6 – NMR Spectra
Appendix VII

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