Synthesis Of Pyridyl Ethyl Amides As Potential Antitrypanosomal Agents, And Synthesis Of Arylimidamide-Azole Hybrids As Potential Antileishmanial Agents

Jennifer Draper
SYNTHESIS OF PYRIDYL ETHYL AMIDES AS POTENTIAL ANTITRYPANOSOMAL AGENTS, AND SYNTHESIS OF ARYLIMIDEAMIDE-azoLE HYBRIDS AS POTENTIAL ANTILEISHMANIAL AGENTS

by

JENNIFER DRAPER

Under the Direction of David W. Boykin, Ph.D

ABSTRACT

Human African Trypanosomiasis (HAT) and Leishmaniasis are protozoal parasitic infections. Designated neglected tropical diseases and global in their impact, these diseases afflict the poorest people in the world. Treatments have remained essentially unchanged for decades and have poor efficacy and questionable safety profiles. In the first study, seventeen novel 2-[2-acylamidoethyl]-6(phenyl)pyridine analogues were designed using a ‘hit to lead’ strategy, synthesized and submitted for evaluation in vitro against Trypanosoma brucei rhodesiense (T. b. r.) for potential treatment against second stage HAT. Eight compounds gave T. b. r. IC50 values of 100nM or less; the best three compounds exhibited values of 64 nM, 12 nM, and 9 nM. In the second study, four novel N-(4-(5-(4-(5-(1H-azole-1-yl)pentyl)oxy)phenyl)furan-2-yl)phenyl) picolinimidamide hydrochloride hybrid analogues were designed using a molecular hybridization strategy for dual targeting, synthesized and submitted for evaluation in vitro against Leishmania. Four compounds exhibited IC50 values in the low micromolar range.

INDEX WORDS: Trypanosomiasis, Leishmaniasis, hit-to-lead, molecular hybridization
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DEDICATION

I dedicate my success in this work to my friends and family who have supported me with their sustaining love and confidence, never faltering through the many cycles of ups and downs, in my evolution through this process. Thank you, it is through your support and strength I was lifted to follow through.
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PART 1

1 Introduction

1.1 Disease Background

Human African trypanosomiasis (HAT), also known as sleeping sickness, is a disease caused by unicellular eukaryotic parasitic protozoa of the *Trypanosoma brucei* subgroup called trypanosomes\(^1\). Various species of the tsetse fly, genus Glossina, are the biological vector of trypanosomes\(^2\). Transmission of this disease occurs through the bite of the infected tsetse fly to susceptible host only in Sub-Saharan Africa, suitable regions to the tsetse fly habitat vector\(^2\). There are two forms of the disease, *T. brucei gambiense* and *T. brucei rhodesiense*, each caused by a different species of the *Trypanosoma brucei* subgroup, *gambiense* and *rhodesiense*, respectively\(^1\). This study will evaluate compounds against the *rhodesiense* form.

The incidence of the disease is variable but has shown a clear decrease in both forms over the past 10 years. In 2009, the number of new cases reported dropped below 10,000 for first time in 50 years and this trend was maintained in 2010. The estimated number of actual cases is currently 30,000.\(^3\)

Epidemiologically, incidence and prevalence of HAT, for both disease forms, are dispersed in geographically discrete foci of the tsetse fly which often are discontinuous. These foci can be defined under 4 different contexts divided into 2 areas. Gambiense form includes 3 areas: West and Central Africa show very low prevalence and the Democratic Republic of the Congo accounts for over 80% of total HAT cases. Rhodesiense areas include East and Southern Africa and report less than 200 cases per year accounting for less than 3% of total HAT cases reported. Approximately 70 million people are estimated to be at different levels of risk of contracting HAT
in Africa. Due to the discontinuous geographical distribution of the disease, spatially discrete estimates of population at risk have been calculated and classified in five categories of risk, ranging from “very high” to “very low” based on information including HAT reported cases and geographic distribution of human population.³

*T. brucei rhodesiense* is considered a zoonotic disease and is endemic to eastern sub-Saharan Africa. The disease develops rapidly upon transmission and can be detected early, but the condition is fatal if untreated causing death in a matter of weeks or months. The disease develops through two defined stages: stage one is defined as the haemolymphatic stage and involves trypanosomes in the haemolymphatic system while in stage two, defined as the meningoencephalitic stage (or late stage), the parasite has crossed the blood brain barrier to invade the CNS causing deterioration of neural tissue with subsequent symptoms including coma, disruption to sleep/wake patterns referred to as ‘sleeping sickness’, and death if untreated.¹ This study will focus on the design and synthesis of 2-[2-acylamidoethyl]-6-phenyl pyridines for the purpose of treating second stage HAT.

### 1.2 Current Drug Treatments

Currently four compounds are registered for use against HAT; two are used for first stage, haemolymphatic stage, disease (suramin and pentamidine, *Fig 1*) and two are used against second stage, meningoencephalitic stage, disease (melarsoprol and eflornithine, *Fig 2*).
Figure 1 Structure of Suramin and Pentamidine.

Figure 2 Structure of Melarsoprol and Eflornithine.

Pentamidine, first used in 1940, is the first-line treatment for early stage *T. b. gambiense* HAT. Although generally tolerated, undesirable side effects include hypoglycemia, prolongation of the QT interval on electrocardiogram, hypotension and gastrointestinal features.\(^1\) Suramin, discovered in 1921, is the first-line treatment for early stage *T. b. rhodesiense* HAT. Although effective, especially if administered early in the disease, undesirable side effects include renal failure, skin lesions, anaphylactic shock, bone marrow toxicity and neurological complications such as peripheral neuropathy.\(^1\)

Treatment for second stage HAT is more problematic as drugs must pass the blood-brain barrier to reach the parasite. Administration and toxicity of drugs for late stage HAT are also
among the problematic variables. Presently, melarsoprol, which acts on trypanothione, a parasite molecule that maintains an intracellular reducing environment, is the only effective drug available for treatment of late-stage *T. b. rhodesiense* HAT and is the most widely used treatment for *T. b. gambiense* HAT in resource poor countries where eflornithine has limited availability. However, melarsoprol is a toxic trivalent organoarsenic compound and undesirable side effects include encephalopathy, leading to a 5% fatality rate through treatment with this drug. Due to melarsoprol resistance in Central Africa, eflornithine, an ornithine decarboxylase inhibitor, has been designated as an effective treatment for late-stage *T. b. gambiense* HAT. All of these drugs are administered by injection and have undesirable side effects. Thus, the need for new drugs to treat diseases caused by this parasite is evident.¹

### 1.3 Drug Discovery Strategies – In the Absence of a Known Target

Current drug discovery strategies are generally based on identification and validation of biological targets which participate in the pathogenesis of the disease. Drug candidates can then be tested for activity on these targets and optimization of the candidate molecules typically follows.³ However, in this study, the target is unknown. In this case, an effective drug discovery strategy, called a structure modification ‘hit-to-lead’ strategy, involves defining a ‘hit’ for a putative target of unknown structure and allowing the ‘hit’ and optimized derivatives thereof, called a hit series, to define the active site of the putative unknown target.

This study focuses on the design and synthesis of analogues of *N*-((2-(6-phenylpyridin-2-yl)ethyl)benzamide which will be referred to as the hit compound *(Fig 3)*, a compound discovered by our laboratory to be active against *T. b. r.*⁴.
Figure 3 Structure of \( N-(2-\text{(6-phenylpyridin-2-yl)ethyl})\text{benzamide} \).

A small structure activity relationship (SAR) study was performed previous to this study, demonstrating important structural features of the hit molecule. Figure 4 illustrates various structural modifications of the pyridylethlamido analog hit series, establishing the necessary structural features for target compounds to provide activity against \( T. b. r. \). Allowing the hit molecule to serve as the reference, a significant loss in activity is observed by placing the 2-pyridylethlamido substituent at the 3-pyridyl position (DB 2141) as the \( T. b. r. \) IC\(_{50}\) value increases from 0.66 to 20.0 \( \mu \)M. An essentially equal loss in activity is observed when removing the nitrogen from the heterocycle (DB 2068) with the \( T. b. r. \) IC\(_{50}\) value increasing to 19.8 \( \mu \)M. Substituent modifications to the left terminus of the pyridyl nucleus demonstrate significant loss in activity as observed by altering the 6-phenylpyridyl substituent to the 5-pyridyl position (DB 2140) with \( T. b. r. \) IC\(_{50}\) increasing to 12.8 \( \mu \)M, however, the largest loss in activity is observed with the removal of the 6-phenylpyridyl substituent (DB 2139) with \( T. b. r. \) IC\(_{50}\) value increasing to above 90 \( \mu \)M. In evaluating structural modifications of this hit series, this SAR study assists in defining the size, shape, and functionality of the putative active site. The hit molecule was discovered to have good activity against \( T. b. r. \) with an IC\(_{50}\) value of 0.66 \( \mu \)M.
Figure 4 SAR previously establishing the hit molecule \( N-(2-(6\text{-}phenylpyridin-2\text{-}yl})\text{ethyl})\text{benzamide} \).  

1.4 Statement of Problem

Modifications of the hit compound are explored in a systematic fashion for the purpose of improving the activity against \( T. b. r. \) and ultimately treating late stage HAT. In general terms one varies the size, shape and functionality, which can participate in intermolecular interactions, of the hit molecule to learn what structural features are important for biological activity. In this study, we vary the structure of the pyridylethylamide analogs by altering the terminal positions while conserving the central pyridylethylamide nucleus-scaffold. It is desirable to focus on molecules with “drugable” physiochemical properties early in the hit-to-lead progress. To treat second stage HAT, it is important to design molecules with calculated polar surface areas approximately in the range of 50 to 90 and calculated log P values in the range of 2 - 3.5 to enhance the possibility of the compounds penetrating the blood-brain barrier.  

\begin{itemize}
  \item DB 2084 T.b.r. IC\textsubscript{50} = 0.66 \( \mu \text{M} \)
  \item DB 2068 T.b.r. IC\textsubscript{50} = 19.8 \( \mu \text{M} \)
  \item DB 2139 T.b.r. IC\textsubscript{50} \( \geq 90 \) \( \mu \text{M} \)
  \item DB 2140 T.b.r. IC\textsubscript{50} = 12.8 \( \mu \text{M} \)
  \item DB 2141 T.b.r. IC\textsubscript{50} = 20.0 \( \mu \text{M} \)
\end{itemize}
2 Results and Discussion

2.1 Results

Our study to vary the structure of the pyridylethlamide analogs by altering the terminal positions is summarized in Schemes 1 and 2. The approaches to the synthesis of these analogues are relatively short and readily performed. Schemes 1 and 2 outline the approach to the synthesis of 2-[2-acylamidoethyl] - 6(phenyl)pyridine analogues (7a-k & 10a-g). The first step in the synthesis of the various amide derivatives employs nucleophilic displacement of one bromide from 2,6 di-bromopyridine by the anion of tert-butyl cyanoacetate under moderate conditions, which we have previously extensively used. 2,6-Dibromopyridine (1) was stirred with 1.1 equivalents of tert-butyl cyanoacetate (2) and 2 equivalents of potassium carbonate in dimethylformamide under nitrogen atmosphere at 80 °C for 24 h to afford the resulting monosubstituted tert-butyl cyanoacetate analogue (3). This compound was then directly converted to the acetonitrile analogue (4) by heating under reflux in toluene in the presence of a catalytic amount of p-toluene sulfonic acid. Suzuki coupling between the nitrile and various boronic acids in the presence of 0.05 equivalents of Pd(PPh₃)₄ using a solvent mixture of toluene-ethanol afforded compound (5) in the second step of this synthesis. The resulting substituted pyridyl acetonitrile was then stirred in the presence of LiAlH₄ and AlCl₃ in ether and allowed to reflux 24 h providing the corresponding substituted pyridyl ethylamine (6). One equivalent of the primary amine was used directly in the last step in the presence of 1.13 equivalents of the appropriate carboxylic or carbonyl chlorides, a catalytic amount of triethylamine in anhydrous dichloromethane, and allowed to stir under nitrogen atmosphere at room temperature to afford the target compounds (7a-k & 10a-g).
Scheme 1 Synthesis of 2-[2-acylamidoethyl]-6(phenyl)pyridine analogues

Reagents and conditions (a) (i) K$_2$CO$_3$, DMF, 80°C (ii) HCl; (b) p-toluene sulphonic acid, Toluene, reflux (iii) Na$_2$CO$_3$; (c) Phenylboronic acid, Pd(PPh$_3$)$_4$, Na$_2$CO$_3$, Toluene, C$_2$H$_5$OH; (d) LiAlH$_4$, AlCl$_3$, Et$_2$O, reflux; (e) various carboxylic and carbonyl chlorides, Et$_3$N, CH$_2$Cl$_2$, anhydrous, rt.

Scheme 2 Synthesis of 2-[2-acylamidoethyl]-6(phenyl)pyridine analogues

Reagents and conditions (a) various boronic acids, Pd(PPh$_3$)$_4$, Na$_2$CO$_3$, dioxane, C$_2$H$_5$OH, 100°C; (b) LiAlH$_4$, AlCl$_3$, reflux; (c) carbonyl chloride, Et$_3$N, CH$_2$Cl$_2$, anhydrous, rt.
Table 1 Biological Activity of 2-[2-acylamidoethyl]-6(phenyl)pyridine analogues

![Chemical Structure]

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\(^a\) The in vitro activities were obtained using the trypomastigote bloodstream form of T. brucei rhodesiense strain. The IC\textsubscript{50} values are the mean of two independent assays. Coefficients of variation were less than 50\% \(^7\).

\(^b\) Cytotoxicity was evaluated using cultured L6 rat myoblast cells. were generated in an in-vitro assay.\(^7\)

\(^c\) ClogP and tPSA values were calculated using ChemBioDrawUltra 2010 software.
2.2 Discussion

Evaluation of biological activity for 2-[2-acylamidoethyl]-6(phenyl)pyridine analogues (7a-j & 10a-g) allows for interpretation of binding specificities for a putative active site of an unknown target. To investigate the tolerance of the active site for specificity of binding, various derivatives of \( N\)-(2-(6-phenylpyridin-2-yl)ethyl)benzamide (7a), the hit compound, were explored by varying the 6-phenylpyridyl and amide substituents. Table 1 contains the results for the biological evaluation for all these compounds.

Compounds 7a-7i explore the effectiveness of change at the amide phenyl site of 7a. Various halogen substituents on the phenyl ring were studied (7b-7h) and the activity was increased only with 7c and 7d with a fluorine atom in the \( m \) and \( p \) positions. The most active compound of this set, 7d, is about six fold more active than the parent 7a. Replacement of the phenyl with a cyclohexyl group, 7i, increased the activity by a factor of approximately two.

Replacement of the phenyl group of the amide unit with pyrrolidine or piperidine rings, thus converting the amide unit to a urea one, gives compounds 7j and 7k. The \( T. b. r. \) IC\(_{50}\) value for 7j is 100 nM and it shows low cytotoxicity, with a selectivity index of 2264. When compared to the hit 7a, this represents a six fold improvement in activity and ten-fold improvement in cytotoxicity. Compound 7k has a \( T. b. r. \) IC\(_{50}\) value of 50 nM and selectivity index of 3358 which represents a further increase in activity and lowering of cytotoxicity. It is possible that the amide carbonyl group of 7a serves as a hydrogen bond acceptor at the receptor binding site. Thus, the increase in activity of 7j and 7k may be due to the formation of a stronger hydrogen bond as a result of the more basic urea carbonyl groups. Due to the nanomolar \( T. b. r. \) activity and the excellent selectivity indices of 7j and 7k, the urea moiety was conserved for the remainder of the
study. From this point the pyrrolidine $7j$ and piperidine $7k$ with $T. b. r. IC_{50}$ values of 100 and 50 nm respectively will be referenced as the urea parent compounds.

On substituting fluorine for hydrogen in the ortho position of the 6-phenylpyridyl substituent ($10a$ & $10b$) of the urea parent compound, a modest decrease of $T. b. r. IC_{50}$ is observed with pyrrolidine substituted at the amide site ($10a$) and a larger decrease of $T. b. r. IC_{50}$ observed with piperidine substituted at the amide site ($10b$).

However, substitution of fluorine for hydrogen in the meta positions of the 6-phenylpyridyl substituent and pyrrolidine and piperidine at the amide site ($10c$ & $10d$) results in a decrease in $T. b. r. IC_{50}$, increasing activity to values of 41 nM and 12 nM respectively. Substitution of fluorine in the para positions also results in an increase of activity with piperidine as the amide substituent ($10f$) showing $T. b. r. IC_{50}$ of 24 nM. A more modest increase in $T. b. r. IC_{50}$ to 64 nM is observed with the pyrrolidine analogue ($10c$).

Substitution of hydrogen with a fluorine in both the m and p positions in the 6-phenylpyridyl substituent and with piperidine at the amide site ($10g$) results in the most active compound of the series showing a significant increase in activity giving a $T. b. r. IC_{50}$ value of 9 nM and giving an impressive selectivity index of greater than 16,000.

The ClogP values for all the F-substituted analogues are slightly higher than the parent urea compounds. This modest increase in hydrophobicity may contribute to the increased activity of these analogues, perhaps due to better uptake by the trypanosomes.

There is an apparent trend of increased activity of piperidine at the urea position. The three most active compounds from this study, $10g$, $10d$, $10f$, are substituted with piperidine at the amide site. The low nanomolar $T. b. r.$ activity and excellent selectivity indices of $10g$, $10d$ and $10f$ merit in vivo evaluation of these compounds to determine if further work on this series is warranted.
3 Experimental

All commercial reagents were used without purification. Melting points were determined on a Mel-Temp 3.0 apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F254 pre-coated aluminum sheets using UV light for detection. $^1$H, $^{13}$C, and $^{19}$F NMR were recorded on a Bruker 400 MHz spectrometer using the indicated solvents. $^1$H NMR were recorded using a frequency of 400 MHz, $^{13}$C NMR 100.6 MHz, and $^{19}$F NMR 376.5 MHz. Mass spectra were obtained from the Georgia State University Mass Spectrometry Laboratory, Atlanta, GA. Elemental analysis was performed by Atlantic Microlab Inc., Norcross, GA.

*tert–Butyl 2-(6-bromopyridin-2-yl)-2-cyanoacetate* (JD-1-7, 3). A mixture of the dibromo compound (1) (30 g, 126.64 mmol), *tert*-butyl cyanoacetate (2) (19.18 ml, 134.20 mmol) and K$_2$CO$_3$ (35 g, 253.28 mmol) in anhydrous DMF (75 ml) was stirred at 80 °C under nitrogen atmosphere for 24 h. The mixture was diluted with ice, acidified with conc. HCl to pH 1, and extracted with ethyl acetate. The organic layer was separated, washed with water, dried (MgSO$_4$), filtered and concentrated *in vacuo* to provide *tert–butyl 2-(6-bromopyridin-2-yl)-2-cyanoacetate* as a yellow solid (26.95g, 72%, 3). This compound was used directly in the next step.

*2-(6-Bromopyridin-2-yl)acetonitrile* (JD-1-10, 4). *Ter”-butyl 2-(6-bromopyridin-2-yl)-2-cyanoacetate* (3) (26.95g, 90.69 mmol) was dissolved in toluene (200 ml), and *p*-toluene sulphonic acid monohydrate (1.0 gm) was added. The mixture was heated under reflux for 12 h and then cooled to room temperature. The toluene was decanted, the black residue was extracted with ethyl acetate, and the two organic phases were combined, neutralized with NaHCO$_3$ solution (75 ml), dried (MgSO$_4$), filtered and concentrated *in vacuo*. The product was purified by column chromatography on silica gel using hexanes/ethyl acetate (70/30, v/v) as eluent to provide 2-(6-
bromopyridin-2-yl)acetonitrile as a yellow solid (5.7 gm, 36%, 4) mp = 46.5-48.5 °C (reported 46-46.5 °C). ⁶ ¹H NMR (CDCl₃): δ 7.64-7.6 (m, 1 H), 7.49-7.46 (m, 2 H), 3.95 (s, 2 H); ¹³C NMR (CDCl₃): δ 150.6, 141.1, 138.7, 126.8, 120.1, 115.3, 25.2.

2-(6-Phenylpyridin-2-yl)acetonitrile (JD-1-14 & 15, 5) Phenyl boronic acid (4.08g, 35.78 mmol) was added to a solution of 2-(6-bromopyridin-2-yl) acetonitrile (4.7g, 23.85 mmol) in 1,4-dioxane (35 ml) and de-airated under nitrogen atmosphere. A 2 M aqueous solution of Na₂CO₃ (35.8 ml, 71.55 mmol), ethanol (8 ml) and tetrakistriphenylphosphine palladium(0) (1.42 g, 1.19 mmol) were added to the de-airated reaction mixture and allowed to stir overnight at 100°C under nitrogen atmosphere. The reaction mixture was cooled to room temperature, separated with ethyl acetate, dried (MgSO₄), filtered and concentrated in vacuo. The product was purified by column chromatography on silica gel using hexanes/ethyl acetate (70/30 v/v) as eluent to provide 2-(6-phenylpyridin-2-yl)acetonitrile as a white solid (3.61g, 78%, 5) mp = 78-78.5°C (reported 80-80.5 °C). ⁶ ¹H NMR (CDCl₃): δ 8.16-8.14 (m, 2 H), 7.82-7.80 (m, 2 H ), 7.51-7.49 (m,2 H), 7.40 (s, 1H), 7.28 (s, 1 H 0, 4.03 (s, 2 H); ¹³C NMR (CDCl₃): δ 159.8, 156.3, 149.1, 136.9, 130.0, 127.3, 117.9, 116.3, 113.3, 113.1, 25.9; ESI-HRMS: m/z calculated for C₁₃H₁₀N₂: 195.0924, found: 195.0922.

2-(6-Phenylpyridin-2-yl)ethan-1-amine (JD-1-16, 6). Lithium aluminum hydride (190 mg, 4.99 mmol) suspended in ethyl ether (10 ml) was added to a slurry of aluminum chloride (670 mg, 4.99 mmol) in ethyl ether (15 ml). The reaction mixture was stirred for 15 min., after which 2-(6-phenylpyridin-2-yl)acetonitrile (970 mg, 4.99 mmol) dissolved in ethyl ether (15 ml) was added slowly, and the reaction mixture was refluxed under nitrogen atmosphere overnight. The reaction mixture was then cooled to room temperature and quenched slowly with water (9 ml), basified with 5 N sodium hydroxide, and extracted with ethyl ether (2 X 50 ml), dried (MgSO₄),
filtered and concentrated in vacuo to provide 2-(6-phenylpyridin-2-yl)ethan-1-amine (0.78g, 79%) as a yellow oil which was used directly in the next step.

2-Fluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide (DB2233, JD-1-26, 7b). 2-Fluorobenzoyl chloride (610 mg, 3.82 mmol) was added to a mixture of 2-(6-phenylpyridin-2-yl)ethan-1-amine (670 mg, 3.38 mmol), triethylamine (0.520 ml, 3.72 mmol) and anhydrous dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (9:1) as eluent providing 2-fluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide as a white solid (240 mg, 20%); mp = 114-116 °C. 1H NMR (DMSO-d$_6$/D$_2$O): δ 8.02 (d, J = 7.0 Hz, 2 H), 7.78-7.72 (m, 2 H), 7.56-7.40 (m, 5 H), 7.25-7.20 (m, 3 H), 3.69 (t, J = 6.5 Hz, 2 H), 3.05 (t, J = 6.5 Hz, 2 H); 19F NMR (DMSO-d$_6$ / Hexafluorobenzene): δ -116.70 (s), -164.90 (s); ESI: m/z calculated for C$_{20}$H$_{17}$FN$_2$O: 320.3602, found: 320.1403. Anal. Calcd for C$_{20}$H$_{17}$FN$_2$O + (0.15 OH$_2$): C, 74.98; H, 5.35; N, 8.74. Found: C, 74.53; H, 5.31; N, 8.59.

3-Fluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide (DB2256, JD-1-31, 7c). 3-Fluorobenzoyl chloride (776 mg, 4.90 mmol) was added to a mixture of 2-(6-phenylpyridin-2-yl)ethan-1-amine (860 mg, 4.34 mmol), triethylamine (0.670 ml, 4.77 mmol) and anhydrous dichloromethane (20 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (9:1) as eluent providing 3-fluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide as a white solid (440 mg, 28%); mp = 79.5-80.5 °C. 1H NMR (DMSO-d$_6$): δ 8.00 (d, J = 7.0 Hz, 2 H), 7.76-7.73 (m, 2 H), 7.62-7.51 (m, 2 H), 7.46-7.38 (m, 4 H), 7.32-7.21 (m, 2H), 3.66 (t, J = 6.5 Hz, 2 H), 3.04 (t, J = 6.5 Hz, 2 H); 19F NMR (DMSO-d$_6$ / Hexafluorobenzene): δ -115.22 (s), -164.90 (s); ESI: m/z calculated for C$_{20}$H$_{17}$FN$_2$O: 320.3602,
found: 320.1403. Anal. Calcd for C$_{20}$H$_{17}$FN$_2$O: C, 74.98; H, 5.35; N, 8.74. Found: C, 75.22; H, 5.15; N, 8.71.

4-Fluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide (DB2281, JD-1-70, 7d). 4-fluorobenzoyl chloride (0.256 g, 1.62 mmol) was added to a mixture of 2-(6-phenylpyridin-2-yl)ethan-1-amine (0.285 g, 1.44 mmol), triethylamine (220 ml, 1.58 mmol) and anhydrous dichloromethane (30 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1, 3:1, 2:1, 1:1) as eluent providing 4-fluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide as a white solid (279 mg, 60.5%); mp = 76 - 78 °C. $^1$H NMR (DMSO-d$_6$/D$_2$O): $\delta$ 8.01 (d, $J = 7.0$ Hz, 2H), 7.78-7.72 (m, 2H), 7.47-7.40 (m, 4H), 7.23 (d, $J = 7.5$ Hz, 1H), 7.08 (t, $J = 7.5$ Hz, 2H), 3.68 (t, $J = 6.5$ Hz, 2H), 3.02 (t, $J = 6.5$ Hz, 2H); $^{19}$F NMR (DMSO-d$_6$/Hexafluorobenzene): $\delta$ -112.08 (s), -164.90 (s); ESI: m/z calculated for C$_{20}$H$_{17}$FN$_2$O: 320.13, found: 320.14. Anal. Calcd for C$_{20}$H$_{17}$FN$_2$O: C, 74.98; H, 5.35; N, 8.74. Found: C, 74.95; H, 5.39; N, 8.72.

2,3-Difluoro-N-[2-(6-Phenylpyridine-2-yl)ethyl]benzamide (DB2257, JD-1-30, 7e). 2,3-Difluorobenzoyl chloride (1.04 g, 5.87 mmol) was added to a mixture of 2-(6-phenylpyridin-2-yl)ethan-1-amine (1.03 g, 5.20 mmol), triethylamine (0.800 ml, 5.72 mmol) and anhydrous dichloromethane (20 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (9:1) as eluent providing 2,3-difluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide as a white solid (640 mg, 36%); mp = 79-80 °C. $^1$H NMR (DMSO-d$_6$/D$_2$O): $\delta$ 8.01 (d, $J = 7.0$ Hz, 2H), 7.80-7.72 (m, 2H), 7.44–7.39 (m, 4H), 7.31–7.19 (m, 3 H), 3.68 (t, $J = 6.5$ Hz, 2 H), 3.04 (t, $J = 6.5$ Hz, 2 H); $^{19}$F NMR (DMSO-d$_6$/Hexafluorobenzene): $\delta$ -140.81,
-143.13 (dd, J = 8.3 Hz), -164.90 (s); ESI: m/z calculated for C$_{20}$H$_{16}$F$_2$N$_2$O: 338.3500, found: 338.1309. Anal. Calcd for C$_{20}$H$_{16}$F$_2$N$_2$O: C, 71.00; H, 4.77; N, 8.28. Found: C, 70.73; H, 4.97; N, 8.25.

2,6-Difluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide (DB2225, JD-1-20, 7f).

2,6-difluorobenzoyl chloride (580 mg, 3.71 mmol) was added to a mixture of 2-(6-phenylpyridin-2-yl)ethan-1-amine (651 mg, 3.28 mmol), triethylamine (0.500 ml, 3.61 mmol) and anhydrous dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1, 3:1, then 2:1) as eluent providing 2,6-difluoro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide as a light yellow solid (55 mg, 5%); mp = 106.5 - 108.5 °C. $^1$H NMR (DMSO-d$_6$ /D$_2$O): δ 8.01 (d, J = 7.0 Hz, 2H), 7.78-7.72 (m, 2H), 7.47-7.36 (m, 5H), 7.30-7.24 (m, 3H), 19F NMR (DMSO-d$_6$ / Hexafluorobenzene): δ -116.46 (s), -164.90 (s); ESI: m/z calculated for C$_{20}$H$_{16}$F$_2$N$_2$O: 338.3506, found: 338.1313. Anal. Calcd for C$_{20}$H$_{16}$F$_2$N$_2$O: C, 71.00; H, 4.77; N, 8.28. Found: C, 70.93; H, 5.07; N, 7.89.

2-Chloro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide (DB2245, JD-1-27, 7g).

2-Chlorobenzoyl chloride (628 mg, 3.59 mmol) was added to a mixture of 2-(6-phenylpyridin-2-yl)ethan-1-amine (630 mg, 3.18 mmol), triethylamine (0.490 ml, 3.50 mmol) and anhydrous dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1) as eluent providing 2-chloro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide as a white solid (370 mg, 35%); mp = 106.5-108.5 °C. $^1$H NMR (DMSO-d$_6$ /D$_2$O): δ 8.03 (d, J = 7.0 Hz, 2 H), 7.79-7.72 (m, 2 H), 7.47-7.36 (m, 5 H), 7.30-7.24 (m, 3 H),
3.67 (t, J = 6.5 Hz, 2 H), 3.04 (t, J = 6.5 Hz, 2 H); $^{13}$C NMR (CDCl$_3$): δ 165.6, 158.5, 155.7, 138.2, 136.5, 134.7, 129.9, 129.8, 129.2, 128.8, 128.0, 127.7, 125.9, 125.8, 121.0, 117.4, 38.1, 35.4; ESI: m/z calculated for C$_{20}$H$_{17}$ClN$_2$O: 336.8100, found: 336.1108. Anal. Calcd for C$_{20}$H$_{17}$FN$_2$O + (0.16 OH$_2$): C, 70.71; H, 5.14; N, 8.25. Found: C, 70.79; H, 5.06; N, 8.17.

**2,6-Dichloro-**N-**[2-(6-phenylpyridine-2-yl)ethyl]benzamide (DB2234, JD-1-28, 7h).**

2,6-Dichlorobenzoyl chloride (458 mg, 2.19 mmol) was added to a mixture of 2-(6-phenylpyridin-2-yl)ethan-1-amine (490 mg, 2.47 mmol), triethylamine (0.380 ml, 2.72 mmol) and anhydrous dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1) as eluent providing 2,6-dichloro-N-[2-(6-phenylpyridine-2-yl)ethyl]benzamide as a white solid (460 mg, 50%); mp = 101.5-103.5 °C. $^1$H NMR (DMSO-d$_6$): δ 8.02 (d, J = 7.0 Hz, 2H), 7.77-7.73 (m, 2 H), 7.54-7.38 (m, 6 H), 7.25 (d, J = 7.5 Hz, 1H), 3.72 (t, J = 6.5 Hz, 2 H), 3.05 (t, J = 6.5 Hz, 2 H); $^{13}$C NMR (DMSO-d$_6$): δ 163.0, 158.1, 154.9, 138.3, 137.0, 136.1, 130.6, 130.3, 128.4, 128.1, 127.5, 126.1, 121.5, 117.4, 38.0, 36.5; ESI: m/z calculated for C$_{20}$H$_{16}$Cl$_2$N$_2$O: 371.2598, found: 371.0718. Anal. Calcd for C$_{20}$H$_{16}$Cl$_2$N$_2$O: C, 64.70; H, 4.34; N, 7.55. Found: C, 64.58; H, 4.45; N, 7.48.

**N-[2-(6-Phenylpyridine-2-yl)ethyl]cyclohexanecarboxamide (DB2230, JD-1-21, 7i).**

Cyclohexanecarbonyl chloride (392 mg, 2.68 mmol) was added to a mixture of 2-(6-phenylpyridin-2-yl)ethan-1-amine (470 mg, 2.37 mmol), triethylamine (0.360 ml, 2.61 mmol) and dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (91:1, 4:1, then 3:1) as eluent providing N-[2-(6-phenylpyridine-2-yl)ethyl]cyclohexanecarboxamide as an off-white solid (200 mg, 27%); mp = 114-116 °C. $^1$H
NMR (DMSO-d$_6$): $\delta$ 7.96 (d, $J = 7.0$ Hz, 2 H), 7.74-7.67 (m, 2 H), 7.46-7.37 (m, 3 H), 7.16 (d, $J = 7.0$ Hz, 1H), 3.40 (t, $J = 6.5$ Hz, 2 H), 2.88 (t, $J = 6.5$ Hz, 2 H), 1.58-1.52 (m, 5 H); 13C NMR (DMSO-d$_6$): $\delta$ 174.5, 158.6, 154.9, 138.3, 136.9, 128.4, 128.1, 126.0, 121.3, 117.3, 43.5, 37.8, 37.0, 28.7, 24.9, 24.7; ESI: m/z calculated for C$_{20}$H$_{24}$N$_2$O: 308.4200, found: 308.1967 . Anal. Calcd for C$_{20}$H$_{24}$N$_2$O: C, 77.89; H, 7.84; N, 9.08. Found: C, 77.60; H, 7.83; N, 9.13.

$N$-[2-(6-Phenylpyridine-2-yl)ethyl]pyrrolidine-1-carboxamide (DB2222, JD-1-18, 7j).

Pyrrolidine carbonyl chloride (210 ml, 1.93 mmol) was added to a mixture of 2-(6-phenylpyridine-2-yl)ethan-1-amine (340 mg, 1.71 mmol), triethylamine (260 ml, 1.88 mmol) and dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate/ethyl acetate + 5% MeOH (4:1, 1:1, then 1:1 w/ 5% MeOH) as eluent providing $N$-[2-(6-phenylpyridine-2-yl)ethyl]pyrrolidine-1-carboxamide as a light yellow solid (300 mg, 59%); mp = 104-106 °C. $^1$H NMR (DMSO-d$_6$): $\delta$ 8.08 (d, $J = 7.2$ Hz, 2 H), 7.82-7.45 (m, 2 H), 7.53-7.40 (m, 2 H), 7.21 (dd, $J = 2.1$ Hz, $J = 4.1$ Hz, 1 H), 6.20 (br s, 1 H), 3.42 (q, $J = 6.6$ Hz, 2 H), 3.17 (t, $J = 6.5$, 4 H), 2.94 (t, $J = 7.3$, 2 H), 1.70-1.82 (m, 4 H); $^{13}$C NMR (DMSO-d$_6$): $\delta$ 159.0, 156.0, 154.8, 138.3, 137.0, 128.4, 128.1, 126.0, 121.3, 117.2, 44.7, 39.6, 38.0, 24.5; ESI: m/z calculated for C$_{18}$H$_{21}$N$_3$O: 296.1756, found: 296.1763 . Anal. Calcd for C$_{18}$H$_{21}$N$_3$O: C, 73.76; H, 7.49; N,13.58. Found: C, 73.56; H, 7.40; N,13.37.

$N$-[2-(6-Phenylpyridine-2-yl)ethyl]piperidine-1-carboxamide (DB2221, JD-1-17, 7k).

Piperidine carbonyl chloride (216 mg, 1.76 mmol) was added to a mixture of 2-(6-phenylpyridine-2-yl)ethan-1-amine (310 mg, 1.56 mmol), triethylamine (240 ml, 1.72 mmol) and dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen
atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1, then 1:1) as eluent providing N-[2-(6-phenylpyridine-2-yl)ethyl]piperidine-1-carboxamide as a light yellow solid (240 mg, 50%); mp = 103.5-104.5 °C. \(^1\)H NMR (DMSO-d\(_6\)):
\[ \delta \] 8.08 (d, \( J = 7.2 \) Hz, 2H), 7.79 (m, 2 H), 7.51-7.42 (m, 3 H), 7.20 (dd, \( J = 6.2 \) Hz, \( J = 6.2 \) Hz, 1 H), 6.54 (t, \( J = 5.1 \) Hz, 1 H), 3.43 (q, \( J = 6.6 \) Hz, 2 H), 3.23 (t, \( J = 5.2 \) Hz, 4 H), 2.95 (t, \( J = 7.3 \) Hz, 2 H), 1.49 (m, 2 H), 1.41-1.32 (d, \( J = 4.0 \) Hz, 4 H); \(^{13}\)C NMR (DMSO-d\(_6\)):
\[ \delta \] 159.0, 156.7, 154.8, 138.3, 136.9, 128.3, 128.1, 126.0, 121.3, 117.2, 43.7, 39.8, 37.8, 27.8, 23.6; ESI: m/z calculated for C\(_{19}\)H\(_{23}\)N\(_3\)O: 310.1922, found: 310.1919. Anal. Calcd for C\(_{19}\)H\(_{23}\)N\(_3\)O: C, 73.19; H, 7.17; N, 14.23. Found: C, 72.94; H, 7.16; N, 14.01.

2-(6-(2-fluorophenyl)pyridin-2-yl)acetonitrile (JD-1-61, 8a) (2-fluorophenyl)boronic acid (1.06 g, 7.60 mmol) was added to a solution of 2-(6-bromopyridin-2-yl) acetonitrile (1.00 g, 5.08 mmol) in 1,4-dioxane (15 ml) and de-airated with nitrogen. A 2 M aqueous solution of Na\(_2\)CO\(_3\) (7.62 mL, 15.24 mmol), ethanol (4 mL) and tetrakistriphenylphosphine palladium(0) (0.294 g, 0.254 mmol) were added to the de-airated reaction mixture and allowed to stir overnight at 100°C under nitrogen atmosphere. The reaction mixture was cooled to room temperature, separated with ethyl acetate, dried (MgSO\(_4\)), filtered, and concentrated in vacuo. The product was purified by column chromatography on silica gel using hexanes/ethyl acetate (9:1) as eluent to provide 2-(6-(2-fluorophenyl)pyridin-2-yl)acetonitrile as a white solid (0.770 g, 3.63 mmol, 71.3%, 5) mp = 55.5 – 56.5°C. \(^1\)H NMR (DMSO-d\(_6\)):
\[ \delta \] 7.97-7.92 (m, 2 H), 7.77, 7.74 (dd, \( J = 7.8 \) Hz, 1H), 7.53-7.48 (m, 1 H), 7.41 (d, \( J = 7.6 \) Hz, 1H), 7.38-7.32 (m, 2 H), 4.29 (s, 2 H); \(^{19}\)F NMR (DMSO-d\(_6\)/Hexafluorobenzene):
\[ \delta \] -119.40 (s), -164.90 (s); ESI-HRMS: m/z calculated for C\(_{13}\)H\(_9\)FN\(_2\): 212.22, found: 212.0832.
2-(6-(2-fluorophenyl)pyridin-2-yl)ethanamine (JD-1-62, 9a). Lithium aluminum hydride (0.131 g, 3.44 mmol) suspended in ethyl ether (10 mL) was added to a slurry of aluminum chloride (0.459 g, 3.44 mmol) in ethyl ether (15 mL). The reaction mixture was stirred for 15 minutes after which 2-(6-(2-fluorophenyl)pyridin-2-yl)acetonitrile (0.729 g, 3.44 mmol), dissolved in ethyl ether (25 mL), was added slowly and the reaction mixture was refluxed under nitrogen atmosphere overnight. The reaction mixture was then cooled to room temperature and quenched slowly with water dropwise (75 ml), basified with 5 N sodium hydroxide to pH 12, extracted with ethyl ether (2 X 50 ml), dried (MgSO₄), filtered, and concentrated in vacuo to provide 2-(6-(2-fluorophenyl)pyridin-2-yl)ethanamine (0.532 g, 2.46 mmol, 71.5%) as a yellow oil which was used directly in the next step.

N-(2-(6-(2-fluorophenyl)pyridin-2-yl)ethyl)pyrrolidine-1-carboxamide (DB2283, JD-1-67, 10a). Pyrrolidine carbonyl chloride (202 ml, 1.91 mmol) was added to a mixture of 2-(6-(2-fluorophenyl)pyridin-2-yl)ethanamine (0.365 g, 1.69 mmol), triethylamine (259 ml, 1.86 mmol) and dichloromethane (30 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1, 1:1) as eluent providing N-(2-(6-(2-fluorophenyl)pyridin-2-yl)ethyl)pyrrolidine-1-carboxamide as an off white solid (0.315 g, 59.5%).

1H NMR (DMSO-d₆): δ 7.96-7.92 (m, 1H), 7.81 (t, J = 7.8 Hz, 1H), 7.62-7.61 (m, 1H), 7.50-7.46 (m, 1H), 7.35-7.30 (m, 2H), 7.25 (d, J = 7.7 Hz, 1H), 6.21 (t, J = 5.2 Hz, 1H), 3.43-3.40 (m, 2H), 3.16 (t, J = 6.4 Hz, 4H), 2.95 (t, J = 7.2 Hz, 2H), 1.75 (s, 4H); 19F NMR (DMSO-d₆ / Hexafluorobenzene): δ -119.60 (s), -164.90 (s); ESI: m/z calculated for C₁₈H₂₀FN₃O: 313.37, found: 313.1681 Anal. Calcd for C₁₈H₂₀FN₃O: C, 68.99; H, 6.43; N, 13.41. Found: C, 69.28; H, 6.58; N, 13.41.
N-(2-(6-(2-fluorophenyl)pyridin-2-yl)ethyl)piperidine-1-carboxamide (DB2282, JD-1-68, 10b). Piperidine carbonyl chloride (0.334 g, 2.26 mmol) was added to a mixture of 2-(6-(2-fluorophenyl)pyridin-2-yl)ethanamine (0.433 g, 2.00 mmol), triethylamine (307 ml, 2.20 mmol) and dichloromethane (30 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1, 1:1) as eluent providing N-(2-(6-(2-fluorophenyl)pyridin-2-yl)ethyl)piperidine-1-carboxamide as an off white solid (0.360 g, 55%).

\[ \text{NMR (DMSO-d}_6\text{): } \delta \text{ 7.94 (t, } J = 7.8 \text{ Hz, 1H)}, 7.80 \text{ (t, } J = 7.7 \text{ Hz, 1 H}), 7.60 \text{ (d, } J = 7.5 \text{ Hz, 1 H)}, 7.52-7.45 \text{ (m, 1 H)}, 7.34-7.29 \text{ (m, 2 H)}, 7.24 \text{ (d, } J = 7.6 \text{ Hz, 1 H}), 6.54 \text{ (t, } J = 5.2 \text{ Hz, 1 H)}, 3.51-3.41 \text{ (m, 2 H)}, 3.21-3.20 \text{ (m, 4 H)}, 2.94 \text{ (t, } J = 7.1 \text{ Hz, 2 H}), 1.48-1.46 \text{ (m, 2 H)}, 1.22 \text{ (s, 4 H)}; \]

\[ \text{F NMR (DMSO-d}_6/\text{Hexafluorobenzene): } \delta \text{ -119.66 (s), -164.90 (s)}; \text{ ESI: m/z calculated for C}_{19}\text{H}_{22}\text{FN}_3\text{O: 327.40, found: 327.1825 Anal. Calcd for C}_{19}\text{H}_{22}\text{FN}_3\text{O: C, 69.70; H, 6.77; N, 12.83. Found: C, 69.844; H, 6.75; N, 12.90.} \]

2-(6-(3-fluorophenyl)pyridin-2-yl)acetonitrile (JD-1-36, 8b). (3-fluorophenyl)boronic acid (1.59 g, 11.42 mmol) was added to a solution of 2-(6-bromopyridin-2-yl) acetonitrile (1.50 g, 7.61 mmol) in 1,4-dioxane (15 ml) and de-aired with nitrogen. A 2 M aqueous solution of \( \text{Na}_2\text{CO}_3 \) (11.41 ml, 22.83 mmol), ethanol (4 mL) and tetrakistriphenylphosphine palladium(0) (0.440 g, 0.381 mmol) were added to the de-aired reaction mixture and allowed to stir overnight at 100°C under nitrogen atmosphere. The reaction mixture was cooled to room temperature, separated with ethyl acetate, dried (MgSO\(_4\)), filtered, and concentrated \textit{in vacuo}. The product was purified by column chromatography on silica gel using hexanes/ethyl acetate (19:1) as eluent to provide 2-(6-(3-fluorophenyl)pyridin-2-yl)acetonitrile as a white solid (0.880 g, 4.15 mmol, 54.5%, 5) \text{mp} = 38.0 – 39.0 °C. \[ \text{NMR (DMSO-d}_6\text{): } \delta \text{ 7.94-7.87 (m, 4 H), 7.53-7.48 (m, 1 H)}, \]

...
7.37 (d, J = 7.5 Hz, 1 H), 7.25 (t, J = 8.0 Hz, 1 H), 4.25 (s, 2 H); $^{19}$F NMR (DMSO-d$_6$/Hexafluorobenzene): δ -115.11 (s), -164.90 (s); ESI-HRMS: m/z calculated for C$_{13}$H$_9$FN$_2$: 212.22, found: 212.0828.

2-(6-(3-fluorophenyl)pyridin-2-yl)ethanamine (JD-1-40, 9b). Lithium aluminum hydride (0.150 g, 3.93 mmol) suspended in ethyl ether (10 mL) was added to a slurry of aluminum chloride (0.524 g, 3.93 mmol) in ethyl ether (15 mL). The reaction mixture was stirred for 15 minutes after which 2-(6-(3-fluorophenyl)pyridin-2-yl)acetonitrile (0.834 g, 3.93 mmol), dissolved in ethyl ether (25 mL), was added slowly and the reaction mixture was refluxed under nitrogen atmosphere overnight. The reaction mixture was then cooled to room temperature and quenched slowly with water dropwise (75 ml), basified with 5 N sodium hydroxide to pH 12, extracted with ethyl ether (2 X 50 ml), dried (MgSO$_4$), filtered, and concentrated in vacuo to provide 2-(6-(3-fluorophenyl)pyridin-2-yl)ethanamine (0.682 g, 3.15 mmol, 80.2 %) as a yellow oil which was used directly in the next step.

$N$-(2-(6-(3-fluorophenyl)pyridin-2-yl)ethyl)pyrrolidine-1-carboxamide (DB2271, JD-1-41, 10c). Pyrrolidine carbonyl chloride (214 mL, 2.02 mmol) was added to a mixture of 2-(6-(3-fluorophenyl)pyridin-2-yl)ethanamine (0.387 mg, 1.79 mmol), triethylamine (275 mL, 1.97 mmol) and dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (9:1, 4:1, 1:1, 1:1 + 1% MeOH) as eluent providing $N$-(2-(6-(3-fluorophenyl)pyridin-2-yl)ethyl)pyrrolidine-1-carboxamide as an off white solid (0.307 g, 0.980 mmol, 54.7%) mp = 112-113 °C. $^1$H NMR (DMSO-d$_6$): δ 7.93-7.87 (m, 2 H), 7.84-7.79 (m, 2 H), 7.23-7.22 (q, J = 14.2 Hz, 1 H), 7.27-7.22 (m, 2 H), 6.18 (t, J = 5.2 Hz, 1 H), 3.44-3.41 (m, 2 H), 3.17-3.16 (m, 4 H) 2.94 (t, J = 7.15 Hz, 2H), 1.74 (s, 4 H); $^{19}$F NMR (DMSO-d$_6$/Hexafluorobenzene): δ -115.11 (s), -164.90 (s); ESI-HRMS: m/z calculated for C$_{13}$H$_9$FN$_2$: 212.22, found: 212.0828.
Hexafluorobenzene): δ -115.37 (s), -164.90 (s); ESI: m/z calculated for C\textsubscript{18}H\textsubscript{20}FN\textsubscript{3}O: 313.37, found: 313.1669 Anal. Calcd for C\textsubscript{18}H\textsubscript{20}FN\textsubscript{3}O: C, 68.99; H, 6.43; N, 13.41. Found: C, 68.96; H, 6.35; N, 13.37.

\textit{N-}(2-\textit{6}-(3-fluorophenyl)pyridin-2-\textit{y}l)ethyl)piperidine-1-carboxamide (DB2270, JD-1-42, 10d). Piperidine carbonyl chloride (0.215 g, 1.46 mmol) was added to a mixture of 2-\textit{6}-(3-fluorophenyl)pyridin-2-\textit{y}l)ethanamine (0.280 g, 1.29 mmol), triethylamine (200 mL, 1.42 mmol) and dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1, 2:3) as eluent providing \textit{N-}(2-\textit{6}-(3-fluorophenyl)pyridin-2-\textit{y}l)ethyl)piperidine-1-carboxamide as an off white solid (0.155 g, 0.473 mmol, 36.7%) mp = 115-116 °C. \textsuperscript{1}H NMR (DMSO-d\textsubscript{6}): δ 7.93-7.87 (m, 2H), 7.83-7.77 (m, 2 H), 7.51 (q, J = 14.3 Hz, 1 H), 7.27-7.17 (m, 2 H), 6.52 (t, J = 5.2 Hz, 1 H), 3.42-3.40 (m, 2 H), 3.21 (m, 4 H), 2.93 (t, J = 7.2 Hz, 2 H), 1.47-1.46 (m, 2 H), 1.36-1.35 (m, 4 H); \textsuperscript{19}F NMR (DMSO-d\textsubscript{6} / Hexafluorobenzene): δ -115.37 (s), -164.90 (s); ESI: m/z calculated for C\textsubscript{19}H\textsubscript{22}FN\textsubscript{3}O: 327.40, found: 327.1645 Anal. Calcd for C\textsubscript{19}H\textsubscript{22}FN\textsubscript{3}O: C, 69.70 H, 6.77; N, 12.83. Found: C, 69.58; H, 6.75; N, 12.76.

2-\textit{6}-(4-fluorophenyl)pyridin-2-\textit{y}l)acetonitrile (JD-1-49, 8c). (4-fluorophenyl)boronic acid (1.94 g, 13.95 mmol) was added to a solution of 2-(6-bromopyridin-2-\textit{y}l) acetonitrile (1.832 g, 9.30 mmol) in 1,4-dioxane (15 mL) and de-airated with nitrogen. A 2 M aqueous solution of Na\textsubscript{2}CO\textsubscript{3} (14.0 mL, 27.9 mmol), ethanol (4 mL) and tetrakistriphenylphosphine palladium(0) (0.537 g, 0.465 mmol) were added to the de-airated reaction mixture and allowed to stir overnight at 100°C under nitrogen atmosphere. The reaction mixture was cooled to room temperature, separated with ethyl acetate, dried (MgSO\textsubscript{4}), filtered, and concentrated in vacuo. The product was
purified by column chromatography on silica gel using hexanes/ethyl acetate (19:1) as eluent to provide 2-(6-(3-fluorophenyl)pyridin-2-yl)acetonitrile as a white solid (1.51 g, 7.12 mmol, 76.5 %) mp = 62.0 – 64.0 °C. 1H NMR (DMSO-d6): δ 8.17-8.13 (m, 2 H), 8.05-8.00 (m, 2 H), 7.36-7.30 (m, 3 H), 4.26 (s, 2 H); 19F NMR (DMSO-d6 / Hexafluorobenzene): δ -115.00 (s), -164.90 (s); ESI-HRMS: m/z calculated for C13H9FN2: 212.22, found: 212.0828.

2-(6-(4-fluorophenyl)pyridin-2-yl)ethanamine (JD-1-51, 9c). Lithium aluminum hydride (0.252 g, 6.644 mmol) suspended in ethyl ether (10 mL) was added to a slurry of aluminum chloride (0.886 g, 6.644 mmol) in ethyl ether (15 mL). The reaction mixture was stirred for 15 minutes after which 2-(6-(4-fluorophenyl)pyridin-2-yl)acetonitrile (1.41 g, 6.644 mmol), dissolved in ethyl ether (25 mL), was added slowly and the reaction mixture was refluxed under nitrogen atmosphere overnight. The reaction mixture was then cooled to room temperature and quenched slowly with water dropwise (75 mL), basified with 5 N sodium hydroxide to pH 12, extracted with ethyl ether (2 X 50 ml), dried (MgSO4), filtered, and concentrated in vacuo to provide 2-(6-(4-fluorophenyl)pyridin-2-yl)ethanamine (1.2 g, 5.55 mmol, 83.5 %) as a yellow oil which was used directly in the next step.

N-(2-(6-(4-fluorophenyl)pyridin-2-yl)ethyl)pyrolidine-1-carboxamide (DB2273, JD-1-52, 10e). Pyrolidine carbonyl chloride (330 mL, 3.07 mmol) was added to a mixture of 2-(6-(4-fluorophenyl)pyridin-2-yl)ethanamine (0.587 mg, 2.71 mmol), triethylamine (420 mL, 2.98 mmol) and dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (4:1, 1:1, 1:1 + 3% MeOH) as eluent providing N-(2-(6-(4-fluorophenyl)pyridin-2-yl)ethyl)pyrolidine-1-carboxamide as an off white solid ( 0.420 g, 1.34 mmol, 49.5%) mp = 121.0-121.8 °C. 1H NMR (DMSO-d6): δ 8.14-8.11 (m, 2 H), 7.80-7.75 (m,
2 H), 7.30 (t, J = 8.9 Hz, 2 H), 7.19, 7.18 (dd, J = 6.0 Hz, 1 H), 6.18 (t, J = 5.1 Hz, 1 H), 3.43 (q, J = 13.00 Hz, 2 H), 3.16 (t, J = 6.4 Hz, 4 H), 2.94 (t, J = 7.3 Hz, 2 H), 1.76-1.73 (m, 4 H); 19F NMR (DMSO-\textit{d}6 / Hexafluorobenzene): δ -115.69 (s), -164.90 (s); ESI: m/z calculated for C_{18}H_{20}F_{3}N_{3}O: 313.37, found: 313.1669

Anal. Calcd for C_{18}H_{20}F_{3}N_{3}O: C, 68.99; H, 6.43; N, 13.41. Found: C, 69.12; H, 6.61; N, 13.37.

\textit{N-}(2-(6-(4-fluorophenyl)pyridin-2-yl)ethyl)piperidine-1-carboxamide (DB2274, JD-1-53, 10f). Piperidine carbonyl chloride (0.472 g, 3.20 mmol) was added to a mixture of 2-(6-(4-fluorophenyl)pyridin-2-yl)ethanamine (0.613 g, 2.83 mmol), triethylamine (434 mL, 3.11 mmol) and dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on silica gel with hexanes/ethyl acetate (1:5, 7:3, 1:1) as eluent providing \textit{N-}(2-(6-(4-fluorophenyl)pyridin-2-yl)ethyl)piperidine-1-carboxamide as an off white solid (0.593 g, 1.81 mmol, 64.0%) mp = 96.4 – 97.4 °C. 1H NMR (DMSO-\textit{d}6): δ 8.19-8.10 (m, 2H), 7.83-7.71 (m, 2 H), 7.30 (t, J = 8.8 Hz, 2 H), 7.18, 7.17 (dd, J = 6.0 Hz, 1 H), 6.52 (t, J = 5.0 Hz, 1 H), 3.41 (q, J = 12.75 Hz, 2 H), 3.21 (t, J = 5.2 Hz, 4 H), 2.92 (t, J = 7.2 Hz, 2 H), 1.47 (m, 2 H), 1.36-1.35 (m, 4 H); 19F NMR (DMSO-\textit{d}6 / Hexafluorobenzene): δ -115.73 (s), -164.90 (s); ESI: m/z calculated for C_{19}H_{22}F_{3}N_{3}O: 327.40, found: 327.1825 Anal. Calcd for C_{19}H_{22}F_{3}N_{3}O: C, 69.70 H, 6.77; N, 12.83. Found: C, 69.91; H, 6.91; N, 12.78.

\textit{2-}(6-(3,4-fluorophenyl)pyridin-2-yl)acetonitrile (JD-1-55, 8d). (3,4-fluorophenyl)boronic acid (1.20 g, 7.61 mmol) was added to a solution of 2-(6-bromopyridin-2-yl) acetonitrile (1.00 g, 5.08 mmol) in 1,4-dioxane (15 mL) and de-airated with nitrogen. A 2 M aqueous solution of Na$_2$CO$_3$ (7.60 mL, 15.23 mmol), ethanol (4 mL) and tetrakistriphenylphosphine palladium(0) (0.294 g, 0.254 mmol) were added to the de-airated
reaction mixture and allowed to stir overnight at 100°C under nitrogen atmosphere. The reaction mixture was cooled to room temperature, separated with ethyl acetate, dried (MgSO₄), filtered, and concentrated in vacuo. The product was purified by column chromatography on silica gel using hexanes/ethyl acetate (19:1) as eluent to provide 2-(6-(3,4-fluorophenyl)pyridin-2-yl)acetonitrile as a white solid (0.932 g, 4.05 mmol, 79.8%, 5) mp = 53.9 – 54.9 °C. ¹H NMR (DMSO-d₆): δ 8.15-8.10 (m, 1 H), 7.97-7.89 (m, 3 H), 7.58-7.52 (m, 1 H), 7.38-7.37 (m, 1 H), 4.26 (s, 2 H); ¹⁹F NMR (DMSO-d₆ / Hexafluorobenzene): δ -140.63, -140.80 (dd, J = 8.3 Hz), -164.90 (s); ESI-HRMS: m/z calculated for C₁₃H₈F₂N₂: 230.21, found: 231.0739.

2-(6-(3,4-fluorophenyl)pyridin-2-yl)ethanamine (JD-1-59, 9d). Lithium aluminum hydride (0.182 g, 4.80 mmol) suspended in ethyl ether (10 mL) was added to a slurry of aluminum chloride (0.640 g, 4.80 mmol) in ethyl ether (15 mL). The reaction mixture was stirred for 15 minutes after which 2-(6-(3,4-fluorophenyl)pyridin-2-yl)acetonitrile (0.932 g, 4.05 mmol), dissolved in ethyl ether (25 mL), was added slowly and the reaction mixture was refluxed under nitrogen atmosphere overnight. The reaction mixture was then cooled to room temperature and quenched slowly with water dropwise (75 mL), basified with 5 N sodium hydroxide to pH 12, extracted with ethyl ether (2 X 50 ml), dried (MgSO₄), filtered, and concentrated in vacuo to provide 2-(6-(3,4-fluorophenyl)pyridin-2-yl)ethanamine (0.470 g, 2.01 mmol, 49.5%) as a yellow oil which was used directly in the next step.

N-(2-(6-(3,4-fluorophenyl)pyridin-2-yl)ethyl)piperidine-1-carboxamide (DB2280, JD-1-62, 10g). Piperidine carbonyl chloride (0.444 g, 3.01 mmol) was added to a mixture of 2-(6-(3,4-fluorophenyl)pyridin-2-yl)ethanamine (0.470 g, 2.01 mmol), triethylamine (310 ml, 2.21 mmol) and dichloromethane (15 ml). The reaction mixture was allowed to stir at room temperature overnight under nitrogen atmosphere. The residue was purified by column chromatography on
silica gel with hexanes/ethyl acetate (4:1, 1:1) as eluent providing N-(2-(6-(3,4-fluorophenyl)pyridin-2-yl)ethyl)pyridine-1-carboxamide as an off white solid (0.273 g, 0.790 mmol, 39.3%) mp = 118 - 120 °C. \(^1\)H NMR (DMSO-d$_6$): \(\delta\) 8.17-8.09 (m, 1 H), 8.00-7.94 (m, 1 H), 7.80-7.72 (m, 2 H), 7.56-7.50 (m, 1 H), 7.20 (d, \(J = 6.2\) Hz, 1 H), 6.55-6.50 (m, 1 H), 3.55-3.50 (m, 2 H), 3.20 (d, \(J = 4.2\) Hz, 4 H), 2.92-2.90 (m, 2 H), 1.46 (br s, 2 H), 1.34 (br s, 4H); \(^19\)F NMR (DMSO-d$_6$ / Hexafluorobenzene): \(\delta\) -140.65, -141.00 (dd, \(J = 8.3\) Hz), -164.90 (s); ESI: m/z calculated for C$_{19}$H$_{21}$F$_2$N$_3$O: 345.39, found: 345.1582 Anal. Calcd for C$_{19}$H$_{21}$F$_2$N$_3$O: C, 66.07; H, 6.13; N, 12.17. Found: C, 66.27; H, 6.16; N,12.20.

PART 2

1 Introduction

1.1 Disease Background

Leishmaniasis is a disease caused by unicellular eukaryotic parasitic protozoa called *Leishmania*, a genus under the Trypanosomitida order (class Kinetoplastida). There are over 20 *Leishmania* species which cause the disease, most of which are zoonotic. Species are categorized as “New World” species (Western Hemisphere) and “Old World” species (Eastern Hemisphere). These include the *L. mexicana* complex with 3 main species (*L. amazonensis*, *L. venezuelensis*, and *L. mexicana*); the *L. donovani* complex with 2 species (*L. infantum* (known as *L. chagasi* in the New World), and *L. donovani*); *L. major*; *L. tropica*; *L. aethiopica*; and the subgenus *Viannia* with 4 main species (*L. (V.) peruviana*, *L. (V.) braziliensis*, *L. (V.) panamensis*, and *L. (V.) guyanensis*). The biological vectors of these parasites are various species of the sandfly,
genera *Phlebotomus* and *Lutzomyia*. This disease is transmitted through the bite of an infected female sandfly, genus *Phlebotomus* in the Old World, genus *Lutzomyia* in the New World, to susceptible host. \(^8,9,10\)

Geographic distribution of the disease is worldwide, found in parts of more than 90 countries. With the exception of Antarctica, *Leishmania* species have been reported on every continent. This organism is primarily endemic to tropical and subtropical regions. Old World leishmaniasis is found in regions of Africa (predominately Northern Africa tropical regions), Southern Europe, Asia, and the Middle East; however, it is not found in the Pacific Islands or Australia. New World leishmaniasis is found in Central America, South America, and regions of Mexico; however, it is not found in Uruguay or Chile. Human disease mainly occurs in the Mediterranean Basin, South-East Asia, East Africa, Afro-Eurasia, and Latin America. While 20,000 to 30,000 deaths occur annually, disease incidence is estimated to be 1.3 million new cases per year. The disease is considered a neglected tropical disease and affects the poorest people on the planet. \(^10,11\)

The disease presents in three main forms: cutaneous, mucocutaneous, and visceral. Symptoms of the disease vary with the *Leishmania* species responsible for the infection. Certain infections remain asymptomatic.

Visceral leishmaniasis (VL), also known as kala-azar, is caused by *L. donovani* and fatal if untreated. Characterized by weight loss, irregular bouts of fever, anemia, and enlargement of the spleen and liver, it is highly endemic in East Africa and the Indian subcontinent. With an estimated 200,000 to 400,000 new cases of VL occurring worldwide annually, over 90% of these cases occur in six countries: India, Sudan, South Sudan, Brazil, Ethiopia, and Bangladesh. \(^8,12\)
Cutaneous leishmaniasis (CL), caused by *L. major*, is the most common form of leishmaniasis causing serious disability and ulcers on exposed parts of the body which can leave life-long scars. Disease incidence is estimated to be 0.7 million to 1.3 million new cases per year with approximately 95% of CL cases occurring in the regions of the Mediterranean basin, the Middle East, Central Asia, and the Americas; however, over two-third of CL new cases occur in six countries: Colombia, Brazil, Algeria, Afghanistan, Syria, and Iran. 8,13

Mucocutaneous Leishmaniasis, caused by *L. braziliensis*, is characterized by partial or total mucous membrane destruction of the mouth, nose, and throat. Approximately 90% of mucocutaneous leishmaniasis cases occur in Brazil, Peru and Bolivia. 8,14

1.2 Current Drug Treatments

The current basis of treatment for leishmaniasis is chemotherapy, relying on a limited selection of drugs with extreme limitations including excessive treatment cost, toxicity, problems with routes of administration, and questionable efficacy in endemic populations. Antileishmanial therapy has predominantly depended upon pentavalent antimonials including sodium stibogluconate (*Fig 5*) and meglumine antimonite (*Fig 6*) for more than 70 years. 15

![Figure 5 Structure of sodium stibogluconate.](image)

![Figure 6 Structure of Meglumine antimoniate.](image)
However, in parts of the world, resistance has limited the utility of antimonials\textsuperscript{16}, in which case Amphotericin B (\textit{Fig 7}) and Pentamidine (\textit{Fig 8}) are utilized as second line medications in the event that antimonial drugs fail.\textsuperscript{15}

![Figure 7 Structure of Amphotericin B.](image)

Amphotericin B is a polyene antibiotic which has been found to be highly effective for the treatment of antimonial resistant \textit{L. donovani} and for cases of mucocutaneous leishmaniasis that have not responded to antimonials\textsuperscript{17}. The drug is given by \textit{i.v.} infusion and has many side effects including nephrotoxicity, anemia and hypokalemia. The high cost of the drug is another limiting factor for use in developing countries.

![Figure 8 Structure of Pentamidine.](image)

In 1952, pentamidine was shown to be a relatively safe and not only an effective first-line treatment for cutaneous leishmaniasis, but also, as a second-line treatment for visceral leishmaniasis.\textsuperscript{18} Since the introduction of miltefosine (\textit{Fig 9}) at the start of this century, there have been no novel antileishmanial compounds approved for treatment in human beings.\textsuperscript{15} A high cure
rate was reported in patients with visceral leishmaniasis by administrating the drug orally for 28 days. Although it is the only drug which is given orally, it has a major limitation of being teratogenic and this excludes its use in women of childbearing age.

![Figure 9 Structure of Miltefosine.](image)

Current treatment for this disease remains unsatisfactory as most of the drugs were developed decades ago and do not meet current standards. Thus, the need for new drugs to treat diseases caused by this parasite is evident.

1.3 Drug Discovery Strategies – Dual Targeting

In recent years, there has been growing use of the dual targeting approach to develop new drugs. Due to problems with the use of conventional combination therapy (cocktail of drugs), a strategy employed by clinicians to treat unresponsive patients, researchers have been motivated to investigate molecular hybridization (MH) techniques. MH techniques employ the design of hybrid drugs, essentially two drug pharmacophores that have been combined into one single molecule to interact with multiple targets, or amplify activity on the bio target in a synergistic fashion, or counterbalance known side-effects associated with one hybrid part. Pharmacophore hybridization provides an attractive alternative to conventional therapy with the distinction that the two drugs are covalently linked, yet administered as a single unit.

The MH design strategy essentially involves two or more known ligand sub-units (bio-active units) which are linked together either directly or with spacer units which may be rigid or
flexible (Fig 10). The fusion of two or more recognized bioactive subunits leads to new hybrid structural design, maintaining and even amplifying characteristics of the original prototypes. Dual targeting and the development of hybrid molecules, with improved activity when compared to the parent drugs, is a concept experiencing expanding usage as a drug design strategy.\textsuperscript{21}

![Diagram showing molecular hybridization]

Figure 10 Molecular hybridization.\textsuperscript{21}

The MH design strategy has been successfully employed in a number of cancer chemotherapy models. Tubulin inhibitors as single entities have proven successful as anticancer agents and have provided researchers motivation to design hybrid structures. Tubulin inhibitor based hybrids demonstrate significant anticancer potential.\textsuperscript{21}

Microtubules are constructed from repeating $\alpha/\beta$ subunits forming the spindle fibers. Microtubules direct chromosomes during mitosis. Previous studies demonstrate that microtubule assembly and disassembly can be blocked by a range of agents which bind to specific sites of the $\beta$-tubulin subunit thereby disrupting the spindle fibers, arresting cells in mitosis and eventually leading to apoptotic cell death. Presently, three binding sites have been defined: a) Taxane-
binding pocket, b) Colchicine site close to the α/β interface, c) Area where the vinca alkaloids bind. The established literature shows within the class of Tubulin inhibitors, chalcones, colchicines, vinca alkaloids, combrestatins and paclitaxel based hybrids have been designed and present promising potential as anticancer agents.21

One such hybridization design for anticancer agents employs the linking of chalcones to imidazolones, thus creating chalcone-imidazolones hybrids. Chalcones are known to interact with tubulin at its colchicines binding site and exhibit potent toxicity to numerous cancer cell lines. Imidazolones are recognized for their apoptosis inducing and Hdm 2 E3 ligase inhibiting ability. The documented medicinal properties of chalcones and imidazolones led to the design of chalcone linked imidazolones. The hybridized derivatives were evaluated for their anti-cancer activity against 53 human tumor cell lines derived from nine different cancer types: lung, CNS, colon, ovarian, renal, breast, leukemia, prostate, and melanoma. Many compounds presented good anti-cancer activity.21

Molecular hybridization techniques have also been used successfully to deliver new antiprotozoal chemical agents. The clinical lifespan of drugs used as antiprotozoal agents, especially monotherapeutic drugs, is almost invariably reduced by emerging drug resistance. Therefore, the concept of combining two or more drugs with differing modes of action is an attractive approach to provide hybrid molecules as the next generation antiprotozoal agents.22

Pentamidine and benzimidazole hybrid molecules have recently been reported to demonstrate antileishmanial activity. As previously mentioned, pentamidine, an aromatic diamidine, is an effective first-line treatment for cutaneous leishmaniasis, but also, as a second-line treatment for visceral leishmaniasis. The unique structural architecture of benzimidazole is recognized as an important scaffold in medicinal chemistry due to its many biological activities.
The rational hybrid design strategy in this study conserved the central pentyldioxyphenyl spacer in pentamidine while the terminal diamidine groups were replaced with a 5-substituted benzimidazole sub-unit. The hybrids were tested against five species of parasites: *Trichomonas vaginalis, Leishmania mexicana, Giardia lamblia, Entamoeba histolytica, and Plasmodium berghei*. The bioactivity observed indicate enhancement of antiprotozoal activity with inclusion of the benzimidazole into the pentamidine moiety, potentially due to the electron-donating capacity of the benzimidazole sub-units.22

Current research reports that azole-arylimidamide (AIA) hybrids demonstrate antileishmanial activity.23 In the past our laboratory focused on developing novel AIAs as potential anti-parasitic drugs.23,24 This class of molecules, with DB766 (*Fig 11*) being the most active, has had success as a treatment for Leishmaniasis in animals.25 A recent study suggests CYP5122A1, an identified cytochrome P450 essential for ergosterol biosynthesis, virulence, drug response, and survival in *Leishmania*, to be the target for AIA antileishmanial activity.26 Azole antifungal agents are known to act as competitive inhibitors of CYP51, lanosterol 14α-demethylase, also an identified cytochrome P450, and also active against Leishmaniasis.27 Azoles including posaconazole 28 and ketoconazole29 (*Fig 11*) have been successfully used to treat Leishmaniasis. Both of these classes of compounds inhibit different pathways in the cytochrome P450 system and therefore have different modes of action, hence hybrid molecules of AIA and azole units are of potential interest.26 This study will focus on the design and synthesis of AIA-azole hybrids such as *N*-((4-((5-(1H-azole-1-yl)pentyl)oxy)phenyl)furan-2-yl)phenyl)picolinimidamide hydrochloride analogues for the purpose of treating Leishmaniasis.
1.4 Statement of Problem

In an effort to demonstrate proof of concept of antileishmanial activity of AIA-azole hybrids, we decided to tether azole units from posaconazole and ketoconazole to the diphenylfuran frame work of DB766. The overall shapes of AIAs and azoles are similar with nitrogen-containing heterocycles bound to a linear or curved linker in each. It is hypothesized that a hybrid molecule containing one arylimidamide terminal unit suitably linked to an azole unit may combine the antileishmanial properties of individual azoles and AIAs. Thus, such molecules could interact with azole target (CYP51) and the suggested AIA target (CYP5122A1)\textsuperscript{26}. Initially, flexible linkers of variable length will be used to attach the two pharmacophores to identify an effective geometry.
2 Results and Discussion

2.1 Results

The synthesis of these prototype molecules employs a strategy of first attaching the linked azole unit to the diphenylfuran frame work and then generating the arylimidamide unit which is outlined in Scheme 3.

The first step employs a Stille coupling reaction between 4-bromonitrobenzene and 2-(tri-n-butylstannyl)furan in the presence of 0.05 equivalents of Pd(PPh₃)₃ using dioxane as the solvent to afford compound (3). This product was then brominated utilizing 1.2 equivalents of NBS in DMF at room temperature in the second step of this synthesis providing compound (4). Next, dibromoalkanes (6) were added to a solution of the p-hydroxyphenylboronic pinacol ester (5) in the presence of dry K₂CO₃, in acetone and vigorously stirred overnight to provide the 2-(4-(bromoalkoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolanes (7). Then, Suzuki coupling between the 2-bromofuran (4) and 2-(4-(bromoalkoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (7) in the presence of 0.05 equivalents of Pd(PPh₃)₄ using a solvent mixture of toluene-ethanol afforded compounds (8). Compounds (8) were then directly converted to the azole analogues (9) in an arylation reaction with imidazole or triazole in the presence of NaH in DMF at room temperature. The 1-((4-(5-(4-nitrophenyl)furan-2-yl)phenoxy)alkyl)-1H-azole (9) was reduced to yield the corresponding arylamine (10) in a hydrogenation reaction facilitated by 10% PdC under 50 PSI of hydrogen gas. One equivalent of the aryl amine (10) was used directly in the last step, cooled in an ice bath, to which 1.1 equivalents of S-(2-naphthylmethyl)-2-pyridyl thioimidate hydrobromide was added and allowed to stir and slowly come to RT overnight. The resulting product was then converted to the free base and then the corresponding HCl salt using
freshly prepared ethanolic HCl solution, affording the target compounds (11) as hydrochloride salts.

**Scheme 3 Synthesis of N-(4-(5-(4-((1H-azol-1-yl)alkoxy)phenyl)furan-2-yl)phenyl) picolinimidamide hydrochloride analogues**

Reagents and conditions: (a) Pd(PPh$_3$)$_4$, dioxane, 90°C (b) NBS, DMF, rt (c) K$_2$CO$_3$, acetone, reflux (d) Pd(PPh$_3$)$_4$, K$_2$CO$_3$, MeOH, toluene, 80°C (e) azole, NaH, DMF, rt (f) H$_2$, PdC, EtOH-ETOAc (g) (i) S-(2-naphthylmethyl-2-pyridylthiomidate hydeobromide, EtOH (ii) NaOH (iii) Ethanolic HCl

2.2 Discussion

Preliminary data for the four new hybrid compounds may be found in **Table 2**. It is encouraging that the IC$_{50}$ values are in the low micromolar range as this suggests that these prototype molecules can be optimized to enhance activity. It is not prudent to engage in significant
SAR analysis with only four compounds. Nonetheless, it appears that in this set that imidazole is superior to triazole for the azole portion of the hybrid. Clearly, a number of modifications including the two pharmacophores and the linker are required in order to determine if this approach will be fruitful.

Table 2 Biological Activity of $N$-(4-(5-(4-((1H-azol-1-yl)alkoxy)phenyl)furan-2-yl)phenyl) picolinimidamide hydrochloride analogues against \textit{L. amazonensis}$^{30}$

<table>
<thead>
<tr>
<th>Code 1</th>
<th>Code 2</th>
<th>n</th>
<th>X, Y</th>
<th>IC$_{50}$ (µM) versus intracellular \textit{L. amazonensis}</th>
</tr>
</thead>
<tbody>
<tr>
<td>11a</td>
<td>DB2336</td>
<td>5</td>
<td>$\text{N}$</td>
<td>$1.53 \pm 0.56$</td>
</tr>
<tr>
<td>11b</td>
<td>DB2337</td>
<td>5</td>
<td>$\text{N}$</td>
<td>$3.56 \pm 1.40$</td>
</tr>
<tr>
<td>11c</td>
<td>DB2342</td>
<td>6</td>
<td>$\text{N}$</td>
<td>$1.82 \pm 0.58$</td>
</tr>
<tr>
<td>11d</td>
<td>DB2343</td>
<td>6</td>
<td>$\text{N}$</td>
<td>$5.22 \pm 1.94$</td>
</tr>
</tbody>
</table>

The IC$_{50}$ values were generated in an \textit{in-vitro} assay using the hybrids for \textit{L. amazonensis} intracellular amastigotes $^{30}$

3 Experimental

All commercial reagents were used without purification. Melting points were determined on a Mel-Temp 3.0 apparatus and are uncorrected. TLC analysis was carried out on silica gel 60 F254 pre-coated aluminum sheets using UV light for detection. $^1$H and $^{13}$C NMR were recorded on a Bruker 400 MHz spectrometer using the indicated solvents. Mass spectra were obtained from the Georgia State University Mass Spectrometry Laboratory, Atlanta, GA. Elemental analysis was performed by Atlantic Microlab Inc., Norcross, GA.
2-(4-nitrophenyl)furan (3) Tetrakistriphenylphosphine palladium (0.5 mmol) was added to a stirred mixture of the 2-(tributylstannyl) furan (11 mmol) and 1-bromo-4-nitrobenzene (10 mmol) in de-airedated dioxane (25 mL) under a nitrogen atmosphere. The vigorously stirred mixture was heated at 90-100 °C for 24 h. The solvent was evaporated under reduced pressure, the resulting solid was partitioned between ethyl acetate (200 mL) and 5 mL of concentrated ammonia, washed with water, passed through celite, dried over sodium sulfate and evaporated. Purification was by column chromatography on silica gel, using hexanes/ethyl acetate (93/7, v/v). The solid attained was then recrystallized from hexanes/ethyl acetate. Yellow solid, yield (68 %); mp 134-135 °C; 1HNMR (DMSO-d6) δ 6.71 (d, J = 3.6 Hz, 1H), 7.02 (br s, 1H), 7.23 (d, J = 3.6 Hz, 1H), 7.90 (d, J = 8.8 Hz, 2H), 7.98 (d, J = 8.8 Hz, 2H).

2-bromo-5-(4-nitrophenyl)furan (4) N-bromosuccinimide (2.13 gm, 12 mmol) was added portion-wise to a stirred solution of the previous nitro comp (10 mmol) in dimethylformamide (20 ml). The reaction mixture was stirred at room temperature for 12 h then poured onto cold water, the precipitate was collected and dried. Purification by column chromatography on silica gel, using hexanes/ethyl acetate (95/5, v/v). Yellow solid, yield (96 %); mp 141-143 °C; 1HNMR (DMSO-d6) δ 6.83 (d, J = 3.6 Hz, 1H), 7.35 (d, J = 3.6 Hz, 1H), 7.90 (d, J = 8.8 Hz, 2H), 8.26 (d, J = 8.8 Hz, 2H); HRMS: m/z calculated for C10H7BrNO3: 267.9609, found: 267.9602 (M+ +1).

General Procedure: 2-(4-(bromoalkoxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (7) Dibromoalkanes (36 mmol) were added to a solution of the p-hydroxyphenylboronic acid ester (6 mmol) and dry K2CO3 (12 mmol) in anhydrous acetone (30 ml) under a nitrogen atmosphere. The vigorously stirred mixture was allowed to reflux for 48 h,
K₂CO₃ was filtered and washed with acetone (5 ml) then the combined filtrate was evaporated under reduced pressure. Purification was by column chromatography on silica gel, using hexanes/ethyl acetate (93/7, v/v).

2-(4-((5-bromopentyl)oxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (7a) White solid (66 %) mp 99-101 °C; ¹H NMR (CDCl₃) δ 1.35 (s, 12 H), 1.62-1.65 (m, 2 H), 1.82-1.85 (m, 2 H), 1.94-1.97 (m, 2 H), 3.45 (t, J =6 Hz, 2 H), 4.03 (t, J = 6 Hz, 2 H), 6.91 (d, J = 8.4 Hz, 2H), 7.76 (d, J =8.4 Hz, 2 H); HRMS: m/z calculated for C₁₇H₂₇BBBrO₃: 369.1237, found: 369.1264 (M⁺ +1);

2-(4-((6-bromohexyl)oxy)phenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (7b) White solid (75 %) mp 98-99 °C; ¹H NMR (CDCl₃, ppm): δ 7.77 (d, J = 8.4 Hz, 2H), 6.90 (d, 2H), 4.02-4.00 (t, J = 6.6 Hz, 2H), 3.49-3.44 (t, J = 6.9 Hz, 2H), 1.91-1.82 (m, 4H), 1.54-1.50 (m, 4H), 1.38 (s, 12 H).

General Procedure: 2-(4-(bromoalkoxy)phenyl)-5-(4-nitrophenyl)furan (8) 2.3 ml deaerated 2 M aqueous solution of K₂CO₃ and p-hydroxyphenyl boronic acid ester (7) (2.73 mmol) in 5 ml deaerated methanol were added to a stirred solution of 2-bromo-5-(4-nitrophenyl)furan (2.28 mmol), and tetrakistriphenylphosphine palladium (0.114 mmol) in deaerated toluene (25 mL) under a nitrogen atmosphere. The vigorously stirred mixture was warmed to 80 °C for 24 h. The solvent was evaporated under reduced pressure. Purification was by column chromatography on silica gel, using hexanes/ethyl acetate (90/10, v/v).

2-(4-((5-bromopentyl)oxy)phenyl)-5-(4-nitrophenyl)furan (8a) Orange solid, yield (62 %); mp 70-71 °C; ¹H NMR (DMSO-d₆) δ 1.67-1.68 ( m, 2 H), 1.85 (m, 2 H), 1.96-2.00 (m, 2 H), 3.47 (br s, 2 H), 4.02 (br s, 2 H), 6.66 (br s, 1 H), 6.94-6.96 (m, 3 H), 7.68 (d, J =8.4 Hz, 2H), 7.78 (d, J =8 Hz, 2H), 8.22 (d, J = 8 Hz, 2H).
2-(4-((6-bromohexyl)oxy)phenyl)-5-(4-nitrophenyl)furan (8b) Yellow solid, yield (59 %); mp 76-77 °C; $^1$HNMR (DMSO-$d_6$) δ 1.46 (m, 4 H), 1.82 (br s, 2 H), 1.84 (br s, 2 H), 3.55 (t, $J$ =6.4 Hz, 2H), 4.03 (t, $J$ =6.4 Hz, 2H), 7.03-7.05 (m, 3 H), 7.40 (d, $J$ = 3.6 Hz, 1 H), 7.80 (d, $J$ = 8.8 Hz, 2H), 8.03 (d, $J$ = 8.8 Hz, 2H); HRMS: m/z calculated for C$_{22}$H$_{23}$BrNO$_4$: 444.0810, found: 444.0799 (M$^+$ +1).

General Procedure: 1-((4-(5-(4-nitrophenyl)furan-2-yl)phenoxy)alkyl)-1H-azole (9)
The previous bromoalkoxy (8) compounds (2 mmol) were added to a solution of the azole (2 mmol) and NaH (2.5 mmol) in dry DMF (10 ml) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 12 h, then poured on ice-water (50 ml), filtered and dried. Purification by recrystallization from ethyl acetate/hexanes.

1-((5-(4-(5-(4-nitrophenyl)furan-2-yl)phenoxy)pentyl)-1H-imidazole (9a) Orange solid, yield (73 %); mp 61-62 °C; $^1$HNMR (CDCl$_3$) δ 1.28 (br s, 2H), 1.60-1.80 (m, 4H), 4.01-4.03 (m, 2 H), 4.23-4.24 (m, 2 H), 6.81 (d, $J$ = 3.6 Hz, 1 H), 7.94-6.97 (m, 3 H), 7.11 (br s, 1H), 7.61 (br s, 1H), 7.70 (d, $J$ = 8.8 Hz, 2 H), 7.84 (d, $J$ = 8.8 Hz, 2H), 8.03 (br s, 1H), 8.27 (d, $J$ = 8.8 Hz, 2H); HRMS: m/z calculated for C$_{24}$H$_{24}$N$_3$O$_4$: 418.1785, found: 418.1767 (M$^+$ +1).

1-((5-(4-(5-(4-nitrophenyl)furan-2-yl)phenoxy)pentyl)-1H-1,2,4-triazole (9b) Orange solid, yield (67 %); mp 69-70 °C; $^1$HNMR (CDCl$_3$) δ 1.27 (br s, 2H), 1.86-1.87 (m, 2H), 2.00-2.02 (m, 2 H), 4.01-4.03 (m, 2 H), 4.23-4.24 (m, 2 H), 6.49 (br s, 1H), 6.95-6.97 (m, 3 H), 7.71 (d, $J$ = 8 Hz, 2H), 7.84 (d, $J$ = 8.8 Hz, 2H), 7.98 (br s, 1H), 8.09 (br s, 1H), 8.28 (d, $J$ = 8.8 Hz, 2H); HRMS: m/z calculated for C$_{23}$H$_{23}$N$_4$O$_4$: 419.1719, found: 419.1706 (M$^+$ +1).

1-((6-(4-(5-(4-nitrophenyl)furan-2-yl)phenoxy)hexyl)-1H-imidazole (9c) Orange solid, yield (63 %); mp 63-64 °C; $^1$HNMR (DMSO-$d_6$) δ 1.25-127 (m, 2H), 1.42-1.44 (m, 2H), 1.69-1.72 (m, 4 H), 3.94-3.97 (m, 4 H), 6.88 (br s, 1H), 7.01-7.04 (m, 3 H), 7.16 (br s, 1H), 7.41 (d, $J$ = 3.6
Hz, 1H), 7.62 (br s, 1H), 7.79 (d, J = 8.8 Hz, 2H), 8.02 (d, J = 8.8 Hz, 2H), 8.27 (d, J = 8.8 Hz, 2H); HRMS: m/z calculated for C_{25}H_{26}N_{3}O_{4}: 432.1923, found: 432.1909 (M^+ +1).

1-(6-(4-(5-(4-nitrophenyl)furan-2-yl)phenoxy)hexyl)-1H,1,2,4-triazole (9d) Orange solid, yield (71 %); mp 68-69 °C; ^1HNMR (DMSO-d_{6}) δ 1.17-1.23 (m, 2H), 1.28-1.30 (m, 2H), 1.70-1.73 (m, 4 H), 3.99 (t, J = 6.4 Hz, 2H), 4.19 (t, J = 6.4 Hz, 2H), 7.02-7.04 (m, 3 H), 7.41 (d, J = 3.6 Hz, 1H), 7.80 (d, J = 8.8 Hz, 2H), 7.95 (br s, 1 H), 8.03 (d, J = 8.8 Hz, 2H), 8.28 (d, J = 8.8 Hz, 2H), 8.52 (br s, 1 H); HRMS: m/z calculated for C_{24}H_{25}N_{4}O_{4}: 388.2025, found: 388.2029 (M^+ +1).

General Procedure: 4-(5-(4-(1H-azol-1-yl)alkoxy)phenyl)furan-2-yl)aniline (10) To a de-aereated solution of the previous nitrocompound (5 mmol) in ethyl acetate/ethanol (60ml: 20ml) mixture was added Pd/C (10 %) (0.2 gm). Stirring in a parr hydrogenator under 50 PSI until the uptake of hydrogen ceased, the consumption of hydrogen gave a clear solution. The solution was filtered through celite, and the solvent was removed under reduced pressure, the residue formed was used directly in the next step without further purification. Since the amine is easily oxidized, it is imperative to proceed with the next step immediately.

4-(5-(4-(5-(1H-imidazol-1-yl)pentyl)oxy)phenyl)furan-2-yl)aniline (10a) White solid, yield (89 %); mp 59-61 °C; ^1HNMR (DMSO-d_{6}) δ 1.37 (br s, 2 H), 1.75 (br s, 4 H), 3.98 (br s, 4 H), 5.34 (s, 2 H), 6.62 (m, 3 H), 6.79 (br s, 1 H), 6.88 (br s, 1 H), 6.96 (d, J = 7.6 Hz, 2 H), 7.17 (br s, 1 H), 7.44 (d, J = 7.6 Hz, 2 H), 7.63-7.65 (m, 3 H); HRMS: m/z calculated for C_{24}H_{25}N_{3}O_{2}: 388.2025, found: 388.2029 (M^+ +1).

4-(5-(4-(5-(1H-1,2,4-triazol-1-yl)pentyl)oxy)phenyl)furan-2-yl)aniline (10b) White solid, yield (92 %); mp 61-62 °C; ^1HNMR (DMSO-d_{6}) δ 1.37 (br s, 2 H), 1.74 (br s, 2 H), 1.85 (br s, 2 H), 3.98 (br s, 2 H), 4.21 (br s, 2 H), 5.32 (s, 2 H), 6.62 (m, 3 H), 6.79 (br s, 1 H), 6.96 (d, J =
7.2 Hz, 2 H), 7.43 (d, J = 7.2 Hz, 2 H), 7.64 (d, J = 7.6 Hz, 2 H), 7.96 (br s, 1 H), 8.53 (br s, 1 H);
HRMS: m/z calculated for C_{23}H_{25}N_{4}O_{2}: 389.1978, found: 389.1964 (M^+ +1).

4-(5-(4-((6-(1H-imidazol-1-yl)hexyl)oxy)phenyl)furan-2-yl)aniline (10c) White solid, yield (97 %); mp 51-52 °C; 1^1HNMR (DMSO-d6) δ 1.40 (br s, 4 H), 1.51 (br s, 4 H), 3.97-4.00 (m, 4 H), 4.79 (s, 2 H), 6.52 (d, J = 3.2 Hz, 1 H), 6.57 (br s, 1 H), 6.73 (d, J = 8.4 Hz, 2 H), 6.91 (d, J = 8.4 Hz, 2 H), 7.28 (br s, 2 H), 7.54-7.56 (m, 3 H), 7.66 (d, J = 8.4 Hz, 2 H); HRMS: m/z calculated for C_{25}H_{28}N_{3}O_{2}: 402.2182, found: 402.2185 (M^+ +1).

4-(5-(4-((6-(1H,1,2,4-triazol-1-yl)hexyl)oxy)phenyl)furan-2-yl)aniline (10d) White solid, yield (97 %); mp 51-52 °C; 1^1HNMR (DMSO-d6) δ 1.28 (br s, 2 H), 1.44 (br s, 2 H), 1.70-1.80 (m, 4 H), 3.97 (br s, 2 H), 4.18 (br s, 2 H), 5.33 (s, 2 H), 6.60-6.62 (m, 3 H), 6.79 (br s, 1 H), 6.96 (d, J = 8 Hz, 2 H), 7.45 (d, J = 7.6 Hz, 2 H), 7.66 (d, J = 8 Hz, 2 H), 7.95 (br s, 1 H), 8.52 (s, 1 H); HRMS: m/z calculated for C_{24}H_{27}N_{4}O_{2}: 403.2129, found: 403.2122 (M^+ +1).

**General Procedure:** \(N-(4-(4-((1H-azol-1-yl)alkoxy)phenyl)furan-2-yl)phenyl) picolinimidamide hydrochloride (11) S-(2-Naphthylmethyl)-2-pyidyl thioimidate hydrobromide (1.87 mmol) was added to a cooled solution of the amino azoles (10) (1.7 mmol) in dry ethanol (30 mL) in an ice bath. The mixture was stirred at room temperature for overnight. The organic solvent was evaporated under reduced pressure to yield an oil product. Dry ether (100 mL) was added to the oil and the mixture was stirred at room temperature for 1 h. A precipitate formed and was filtered and washed with dry ether. The solid was dissolved in ethanol (2 ml); the solution was cooled to 0 °C in an ice bath and 10% NaOH was added until pH reached approximately 10. The free base was extracted with ethyl acetate (3 × 50 mL). The organic layer was washed with distilled water, dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure. The resulting solid freebase was treated with dry hexanes and then filtered. The free base was
suspended in dry ethanol (20 mL) and cooled to 0 °C in an ice bath. Freshly prepared ethanolic HCl solution (2 ml) was added to the suspension and the mixture was stirred at room temperature for overnight. The resulting red solution was concentrated under reduced pressure to yield a. The solid was recrystallized twice from dry ethanol and dry ether and filtered.

\[ N-(4-(5-(4-(1H-imidazol-1-yl)pentyl)oxy)phenyl)furan-2-yl)phenyl) \]

picolinimidamide hydrochloride (11a) Yellow solid, yield (55 %); mp 80-82 °C dec.; \(^1\)HNMR (DMSO-\(d_6\)) \(\delta\) 1.39-1.40 (m, 2 H), 1.76-1.77 (m, 2 H), 1.88-1.92 (m, 2 H), 4.01-4.04 (m, 4 H), 6.97 (d, \(J = 3.2\) Hz, 1 H), 7.02 (d, \(J = 8.4\) Hz, 2 H), 7.18 (d, \(J = 3.2\) Hz, 1 H), 7.22 (br s, 1 H), 7.38 (s, 1 H), 7.48 (s, 1 H), 7.55 (d, \(J = 8.4\) Hz, 2 H), 7.71 (br s, 2 H), 7.77 (d, \(J = 8.4\) Hz, 2 H), 7.84 (d, \(J = 8.4\) Hz, 2 H), 7.97 (d, \(J = 8\) Hz, 2 H), 8.21-8.25 (m, 1 H), 8.52 (d, \(J = 8\) Hz, 1 H), 11.88 (s, 1 H); \(^1\)CNMR (DMSO-\(d_6\)) \(\delta\) 24.4, 27.9, 28.0, 55.4, 66.9, 106.1, 108.8, 114.4, 122.2, 123.8, 124, 124.5, 124.5, 124.6, 125.9, 128, 129.6, 132.6, 132.6, 137.8, 143.9, 148.5, 149.2, 150.5, 152.8, 157.9, 158.8; HRMS: m/z calculated for C\(_{30}\)H\(_{30}\)N\(_5\)O\(_2\): 492.2400, found: 492.2390 (base M\(^+\) +1); Anal. Calcd. For C\(_{30}\)H\(_{30}\)N\(_5\)O\(_2\). 2 HCl. 1.75 H\(_2\)O: C, 60.45; H, 5.83; N, 11.75; Found: C, 60.14; H, 5.61; N, 11.39.

\[ N-(4-(5-(4-(1H,1,2,4-triazol-1-yl)pentyl)oxy)phenyl)furan-2-yl)phenyl) \]

picolinimidamide hydrochloride (11b) Yellow solid, yield (49 %); mp 89-91 °C dec.; \(^1\)HNMR (DMSO-\(d_6\)) \(\delta\) 1.38-1.40 (m, 2 H), 1.76-1.79 (m, 2 H), 1.88-1.89 (m, 2 H), 4.00 (d, \(J = 6\) Hz, 2 H), 4.28 (d, \(J = 6\) Hz, 2 H), 6.97 (d, \(J = 3.2\) Hz, 1 H), 7.01 (d, \(J = 8.8\) Hz, 2 H), 7.18 (d, \(J = 3.2\) Hz, 1 H), 7.55 (d, \(J = 8.4\) Hz, 2 H), 7.76 (d, \(J = 8.4\) Hz, 2 H), 7.87 (br s, 1 H), 7.97 (d, \(J = 8\) Hz, 2 H), 8.23 (br s, 2 H), 8.50 (d, \(J = 7.6\) Hz, 1 H), 8.90 (m, 2 H), 9.37 (s, 1 H), 10.14 (s, 1 H), 11.86 (s, 1 H); \(^1\)CNMR (DMSO-\(d_6\)) \(\delta\) 24.4, 27.8, 29, 53.2, 66.1, 106.1, 108.9, 114.3, 122.9, 123.8, 124, 124.5, 124.6, 126.7, 128.3, 128.7, 129.6, 132.6, 138, 143.9, 148.9, 149.2, 150.5, 152.8, 157.9, 159;
HRMS: m/z calculated for C_{29}H_{29}N_{6}O_{2}: 493.2352, found: 493.2338 (base M\(^+\) +1); Anal. Calcd. For C_{29}H_{28}N_{6}O_{2}. 2 HCl. 1.25 H_{2}O: C, 59.24; H, 5.57; N, 14.29; Found: C, 59.02; H, 5.31; N, 13.98.  

\(-N-(4-(5-(4-(6-(1H-imidazol-1-yl)hexyl)oxy)phenyl)furan-2-yl)phenyl)\) picolinimidamide hydrochloride (11c) Yellow solid, yield (47%); mp 78-80 °C dec.; \(^1\)HNMR (DMSO-\(d_6\)) \(\delta\) 1.29-1.40 (m, 4 H), 1.71-1.81 (m, 4 H), 3.99 (br s, 2 H), 4.23-4.30 (m, 2 H), 6.97-7.00 (m, 3 H), 7.17 (br s, 1 H), 7.55 (br s, 2 H), 7.70-7.76 (m, 4 H), 7.86 (br s, 2 H), 7.95 (br s, 2 H), 8.21 (br s, 1 H), 8.59 (br s, 1 H), 8.88 (br s, 1 H), 9.32 (s, 1 H), 10.19 (s, 1 H), 11.96 (s, 1 H); \(^{13}\)CNMR (DMSO-\(d_6\)) \(\delta\) 24.1, 25.1, 27.9, 28.1, 54, 65.3, 106.1, 108.8, 113.6, 122.7, 123.8, 124, 124.4, 124.6, 125.6, 128.7, 129, 129.6, 132.6, 138.6, 143.9, 148.6, 149.7, 150.5, 152.1, 157.9, 159.3; HRMS: m/z calculated for C_{31}H_{32}N_{5}O_{2}: 506.2556, found: 506.2549 (base M\(^+\) +1); Anal. Calcd. For C_{31}H_{31}N_{5}O_{2}. 2 HCl. 2 H_{2}O: C, 60.59; H, 6.07; N, 11.40; Found: C, 60.34; H, 6.02; N, 11.13.  

\(-N-(4-(5-(4-(6-(1H,1,2,4-triazol-1-yl)hexyl)oxy)phenyl)furan-2-yl)phenyl)\) picolinimidamide hydrochloride (11d) Yellow solid, yield (44%); mp 81-83 °C dec.; \(^1\)HNMR (DMSO-\(d_6\)) \(\delta\) 1.29-1.40 (m, 4 H), 1.70-1.80 (m, 4 H), 3.99 (br s, 2 H), 4.25 (br s, 2 H), 6.97 (s, 1 H), 7.02 (d, \(J = 7.2\) Hz, 2 H), 7.18 (br s, 1 H), 7.56 (d, \(J = 7.2\) Hz, 2 H), 7.76 (d, \(J = 7.2\) Hz, 2 H), 7.86, (br s, 1 H), 7.97 (d, \(J = 7.2\) Hz, 2 H), 8.22 (br s, 1 H), 8.32 (s, 1 H), 8.59 (d, \(J = 7.2\) Hz, 1 H), 8.89 (s, 1 H), 9.07 (s, 1 H), 9.40 (s, 1 H), 10.19 (s, 1 H), 11.96 (s, 1 H); \(^{13}\)CNMR (DMSO-\(d_6\)) \(\delta\) 24.1, 25.2, 27.8, 28.3, 55.8, 66.9, 106, 108.1, 114.4, 122.3, 123.6, 123.9, 124.5, 124.6, 126.7, 128.5, 128.7, 129.6, 132.6, 138.4, 143.9, 146.1, 149.2, 150.5, 152.9, 157.2, 158.8; HRMS: m/z calculated for C_{30}H_{31}N_{6}O_{2}: 507.2503, found: 507.2492 (base M\(^+\) +1); Anal. Calcd. For C_{30}H_{30}N_{6}O_{2}. 2 HCl. 2.5 H_{2}O: C, 57.69; H, 5.97; N, 13.46; Found: C, 57.36; H, 5.88; N, 13.29.
REFERENCES


5. Paohesh H., Lenz G. B. **Medicinal Chemical Properties of Successful Central Nervous System Drugs.** *NeuroRx.* 2005, 2, 541-553.


10. Iowa State University College of Veterinary Medicine. **Leishmaniasis (Cutaneous and Visceral).** Retrieved October 29, 2014, from
http://www.cfsph.iastate.edu/Factsheets/pdfs/leishmaniasis.pdf


Appendix A - Part 1

Appendix A.1 - $^1H$ NMR

(7b)
(7h)
Appendix A.2 - $^{13}$C NMR

(7g)
Appendix B - Part 2

Appendix A.1 – $^1H$ NMR

(11a)
Appendix A.2 - $^{13}$C NMR

(11d)