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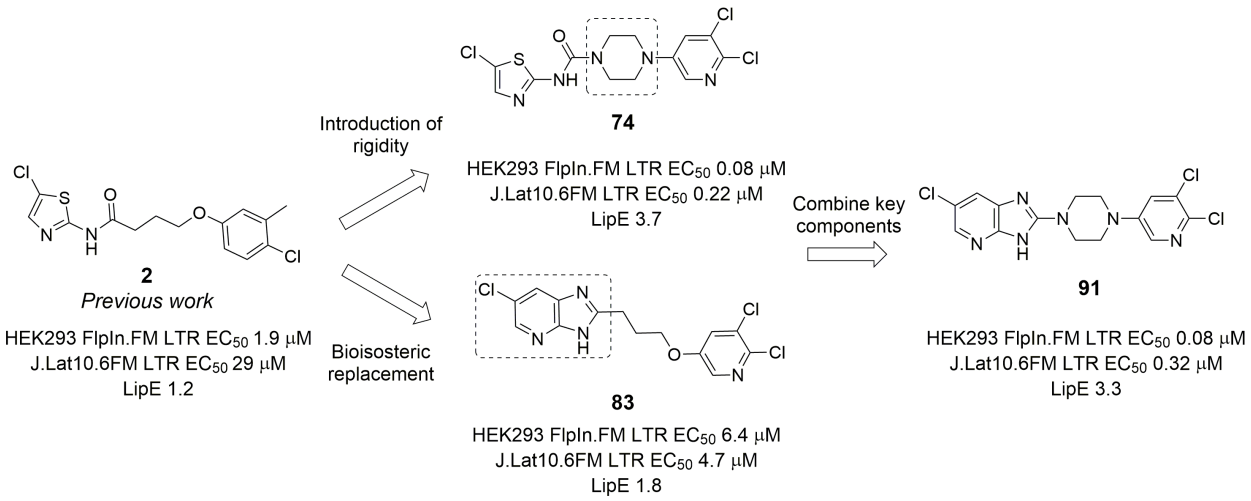

Optimization of 5-Substituted Thiazolyl Ureas and 6-Substituted Imidazopyridines as Potential HIV-1 Latency Reversing Agents

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KEYWORDS

HIV-1, AIDS, latency, thiazole, imidazopyridine, transcription.

ABSTRACT

A persistent latent reservoir of virus in CD4+ T cells is a major barrier to cure HIV. Activating viral transcription in latently infected cells using small molecules is one strategy being explored to eliminate latency. We previously described the use of a FlpIn.FM HEK293 cellular assay to identify and then optimize the 2-acylaminothiazole class to exhibit modest activation of HIV gene expression. Here, we implement two strategies to further improve the activation of viral gene expression and physicochemical properties of this class. Firstly, we explored rigidification of the central oxy-carbon linker with a variety of saturated heterocycles, and secondly, investigated bioisosteric replacement of the 2-acylaminothiazole moiety. The optimization process afforded lead compounds (**74** and **91**) from the 2-piperazinyl thiazolyl urea and the imidazopyridine class. The lead compounds from each class demonstrate potent activation of HIV gene expression in the FlpIn.FM HEK293 cellular assay (both with LTR EC_{50}s of 80 nM) and in the Jurkat Latency 10.6 cell model (LTR EC_{50} 220 and 320 nM respectively), but consequently activate gene expression non-specifically in the FlpIn.FM HEK293 cellular assay (CMV EC_{50} 70 and 270 nM respectively) manifesting in cellular cytotoxicity. The lead compounds have potential for further development as novel latency reversing agents.

INTRODUCTION

The emergence of antiretroviral therapies (ART) 25 years ago has allowed for the control of HIV-1 by suppressing plasma viral loads to undetectable levels.[1, 2] However, discontinuation of treatment results in rebound of the virus, constraining patients to a lifelong drug treatment in order to subdue HIV replication and consequently decrease the severity of opportunistic pathogenic and viral infections.[3, 4] This in itself poses many challenges including the need for long term compliance, the emergence of multidrug resistant viruses and drug toxicities resulting from life-long therapy.[5] The virus can rebound after cessation of ART due to long lived and proliferating latently infected CD4+ T cells that harbour replication-competent virus.[6, 7] These latently infected cells are established early after infection, prior to the detection of viremia,[8, 9] and persist on ART, presenting a major barrier to the eradication of the virus.[10, 11]

One strategy being investigated to eliminate latently infected CD4+ T cells is known as “shock and kill”. The aim of this approach is the pharmacological activation of proviral transcription and subsequent virion production. Infected cells would then be susceptible to immune clearance and viral cytopathic effects, allowing for their elimination. Used in combination with ART, this approach could potentially eradicate or reduce the number of latently infected cells allowing for enhanced virological control when ART is stopped.[12, 13]

A diverse range of epigenetic proteins have been investigated as latency reversing agents (LRAs) for the “shock and kill” approach and include histone deacetylase (HDACs),

bromodomain and extra-terminal motif (BET) proteins, histone methyltransferases (HMT) and protein kinase C (PKC) activators.[14, 15] Several inhibitors of these proteins have progressed to human clinical trials. One example is the HDAC inhibitor, Vorinostat. In this clinical trial, people living with HIV (PLWH) on suppressive ART were treated with Vorinostat (Figure 1) for 14 days and showed increased levels of CD4+ T cell associated unspliced (CA-US) HIV RNA,[16] however, plasma HIV RNA, concentration of HIV DNA, integrated DNA and inducible virus in CD4+ T-cells remained unchanged. Significant changes in host gene expression were also observed, questioning the safety and long-term use of Vorinostat. Similar findings with preclinical and clinical trials on other HDAC inhibitors, including panobinostat[17, 18] and romidepsin[19, 20] and the BET inhibitor, JQ1[21,22] (Figure 1). In general, epigenetic modulators demonstrate poor efficacy in ex vivo models and do not induce substantial increases in intracellular HIV-1 mRNA or free virions in patient cells.[22] Therefore, new LRAs are needed with a novel mechanism of action.

We recently reported on the design of a FlpIn.FM luciferase reporter cellular assay capable of detecting small molecules that activate HIV-1 gene expression.[23] The FlpIn.FM reporter cell line will detect both transcriptional and post-transcriptional steps of gene expression, including effects on RNA capping, RNA splicing, RNA transport, RNA-modifications, RNA stability and translation of protein. The HEK293 derived reporter cell line expresses a HIV-1 Nef – click beetle red (CBR) fusion protein from a spliced mRNA expressed under direction from the long terminal repeat (LTR) HIV promoter, allowing for detection of early viral gene expression. The cell line also contains a click beetle green (CBG) luciferase reporter driven from a cytomegalovirus (CMV) promoter allowing for the detection of non-specific host cell protein expression.

We used the HEK293 FlpIn.FM cell line in a high throughput screen and the identified the 2-acylaminothiazole class (Figure 2).[23] Our initial optimization efforts concentrated on

defining structure activity relationship (SAR) of the 2-acylaminothiazole class and resulted in compounds with enhanced HIV gene expression (LTR) in both HEK293 FlpIn.FM and the Jurkat 10.6 latent HIV-GFP reporter cell line. The optimized compounds also increased CA-US HIV RNA in resting CD4+ T cells isolated from PLWH on ART. While a modest enhancement in HIV gene expression was demonstrated with optimized analogues, the selectivity window between activation of HIV and non-specific (CMV) gene expression was limited. This is comparable to the level of selectivity observed with epigenetic modulators used as LRAs in clinical trials.[23]

In this report we describe further optimization of the 5-substituted 2-acylaminothiazole class focused on rigidifying the central oxy-carbon chain and bioisosteric substitution of the 2-acylaminothiazole moiety to improve LTR specific activity and physicochemical properties. We show the 5-substituted thiazolyl urea and 6-substituted imidazopyridine analogues derived from this study have enhanced HIV-1 LTR activity in both the FlpIn.FM cell line and J.Lat10.6 cell lines and improved *in vitro* metabolic stability.

RESULTS AND DISCUSSION

The initial focus of the research reported here was to rigidify the central aliphatic chain, linking the thiazole and the pendant aryl group (Figure 2), by replacement with a variety of 4-, 5-, 6- and 7- membered unsaturated heterocyclic systems. It was proposed that this change could improve HIV-1 LTR activity by reducing conformational energy required for the substituted thiazolyl and aryl motifs to engage with pockets of the unknown cellular target. In addition, it was hypothesized, the incorporation of heterocyclic systems may improve aqueous solubility and *in vitro* metabolism. We previously reported the 2-acylaminothiazole class possessed poor aqueous solubility (<1.6 μM at pH 6.5) and was rapidly degraded by both human and mouse liver microsomes.[23] In addition, the thiazole moiety is a known

toxicophore because of cytochrome P450-mediated oxidative conversion of the 4,5-unsaturated bond to an epoxide which can act as a nucleophile trap.[24] While 4- or 5-substitution of the thiazole ring is known to suppress this metabolic event, we endeavoured to eliminate this liability by introducing a 5,6-bicyclic heterocycle that is a bioisoteric replacement for the 2-acylaminothiazole moiety. Herein, we describe efforts to improve HIV-1 LTR specific potency and physiochemical properties by concentrating on substituting the central aliphatic linker and the 2-acylaminothiazole moiety with 4-, 5-, 6- and 7- membered heterocyclic and 5,6-bicyclic systems respectively.

Chemistry

The synthesis of 2-thiazoloyl urea analogues (Tables 1-5) started with the preparation of phenyl 2-thiazolecarbamates (**4**) by reaction of 5-substituted 2-aminothiazoles (**3**) with phenyl chloroformate under basic conditions (Scheme 1). The heterocyclic substituted aryl building block (**6**) was generated employing a Buchwald–Hartwig amination between an aryl halide (**5**) and a N-Boc protected heterocycle (L in Tables 1-5) followed by N-Boc deprotection. The phenyl 2-thiazolecarbamate (**4**) and the heterocyclic substituted aryl building block (**6**) were then used to afford the 2-thiazoloyl urea analogues (**7**) in Tables 1-5.

The synthesis of the imidazopyridine analogues (Table 6) began with the alkylation of a substituted phenol or pyridinol (**8**) with a protected halogenated butanoate (Scheme 2). Deprotection of the ester followed and the carboxylic acid (**9**) was then transformed into a acid chloride using $POCl_3$. The acid chloride was on-reacted with a substituted pyridine-diamine yielding the imidazopyridine analogues (**10**) in Table 6.

Access to the 2-piperazine substituted imidazopyridines (Table 7) started with the condensation of a substituted pyridine-diamine (**11**) with disuccinimidyl carbonate under

aprotic conditions to yield the imidazopyridinone (**12**, Scheme 3). The imidazopyridinone was then reacted with $POCl_3$ to yield the 2-chloroimidazopyridine (**13**). Finally, the nucleophilic substitution of a substituted aryl piperazine (**14**) with the 2-chloroimidazopyridine (**13**) afforded the 2-piperazine substituted imidazopyridines (**15**) in Table 7.

Structure and Activity Relationship

Our previous SAR studies established the 5-position of the thiazole ring and 3,4-disubstitution on the aryl ring of the 2-acylaminothiazole class were important for HIV-1 LTR activity.[23] In particular, combinations of 5-methyl or 5-chloro substitution on the thiazole ring and 3-methyl and 4-chloro disubstitution on the phenoxy ring (Figure 2), were optimal for HIV-1 LTR activity and selectivity against the CMV non-specific reporter, and thus this substitution pattern would be used as a benchmark and remain consistent for the analogues produced in the present study.

We first investigated the suitability of 5- and 6- membered saturated heterocycles as surrogates for an oxy-carbon linker between the carbonyl and the aryl ring. The 5- and 6-membered heterocycles were proposed because they have similar steric spacing compared to the 4-atom oxy-carbon linker. The thiazolyl urea analogues generated were evaluated against the HEK293 FlpIn.FM cell line and their LTR and CMV activities are shown in Tables 1 and 2. The results show that introduction of the piperazine, seen in compounds **16** and **17**, gave a 4- and 20- fold improvement in LTR activity (EC_{50} 0.6 and 0.4 μM) compared to **1** and **2** (Figure 2), respectively. It was also noted that the 3-chloro and 4-methyl substitution on the aryl ring seen in **34** and **35** (LTR EC_{50} of 0.47 and 0.25 μM) (Table 2) did not affect activity compared to **16** and **17**, indicating that the configuration of the 3- and 4- chloro and methyl

substitution was interchangeable with the thiazolyl urea and consistent with previous SAR with the 2-acylaminothiazole class.[23]

The selectivity window between the non-specific CMV reporter and the LTR reporter for all analogues shown in Tables 1 and 2, was approximately 2- to 3- fold, except for analogues, **36** and **37**. The observed selectivity window was generally consistent for all analogues produced throughout this report, and therefore our efforts herein concentrated on improving the LTR activity. While CMV activity was monitored, achieving selectivity between the LTR and CMV reporter was not the ongoing primary focus.

Analogues with heterocycles replacing the oxy-carbon linker such as 4-hydroxy, 4-amino and 4-methylamino piperidine (**18-22**) and 3-hydroxy and 3-amino piperidine (**23-27**) were all inactive (LTR EC_{50} >20 μM) (Table 1). The corresponding 5-membered systems, the 3-amino and 3-hydroxy pyrrolidine analogues, **28-31**, displayed LTR activity (EC_{50} 0.79 – 6.2 μM) comparable to **1** and **2**, but *N*-methylation of the 3-amino pyrrolidine functionality (**32** and **33**) was detrimental to LTR activity. Analogues **36** and **37** with the 4-pipecolic amide scaffold retained LTR activity (EC_{50} 2.5 and 2.0 μM) (Table 2) compared to 2-acylaminothiazoles **1** and **2**.

Smaller 4-membered heterocycles were all investigated as replacements for the oxy-carbon linker (Table 2). The analogues, **38** and **39**, with 3-aminoazetidine functionality maintained comparable LTR activity (EC_{50} 0.67 and 0.23 μM) to the piperazine analogues **34** and **35**. *N*-Methylation of the 3-amino group of the azetidine moiety (**40** and **41**) was detrimental to LTR potency, mirroring the SAR observed with the *N*-methylation of the 3-amino pyrrolidine analogues, **32** and **33**. The 3-oxy azetidine analogues, **42** and **43**, exhibited comparable LTR activity (EC_{50} 0.22 and 0.53 μM) to **38** and **39**, suggesting that the nitrogen and oxygen atoms are interchangeable at this position, consistent with the SAR previously reported with the 2-acylaminothiazoles series.[23]

In addition to the 3-amino (**38** and **39**) and 3-oxy azetidine (**42** and **43**), the piperazine heterocycle (**34** and **35**) was the most suitable replacement for the oxy-carbon linker, therefore we further explored iterations on the piperazine (Table 2). Expanding the piperazine ring to homo-piperazine (**44** and **45**) resulted in 20- and 100-fold losses in activity when compared to the corresponding piperazine compounds (**34** and **35**). 2-Methyl substituted piperazine analogues (**46-49**) were consistently less potent (EC_{50} 1.3 to 8.9 μM) than the unsubstituted piperazine analogues (**34** and **35**). The oxo-piperazine analogue, **50** (EC_{50} 0.6 μM), was 2-fold less active than **35**, although it had enhanced LipE (3.0). The 2,6-diazaspiro[3.3]heptane heterocycle was also trialled because Reilly *et al*. demonstrated it was a suitable piperazine isostere with reduced cytotoxicity.[25] It was found that 2,6-diazaspiro[3.3]heptane scaffold was not a suitable replacement for piperazine in this instance, with the analogues **51** and **52** displaying modest LTR activity (EC_{50} 40 and 16 μM). Overall, the unsubstituted piperazine was established as the most appropriate group for replacement of the oxy-carbon linker.

Another goal of this work was to improve the *in vitro* metabolism and solubility, as the 2-acylaminothiazole analogues (Figure 2) were shown to possess poor aqueous solubility and modest *in vitro* metabolic stability (Table 8).[23] Although the LipE was increased with the piperazine analogue **35** compared to **1**, the aqueous solubility was still limited (<1.6 μM at pH 2.0 and pH 6.5), however, the metabolic stability of **35** in mouse liver microsomes (Cl_{int} 53 μL/min/mg protein) was enhanced compared to the 2-acylaminothiazole **1** (Table 8). A similar outcome was also observed for the 3-amino azetidine analogue **39** with modest metabolic stability in mouse liver microsomes (Cl_{int} 49 μL/min/mg protein) and low aqueous solubility. Analogue **29** with 3-amino pyrrolidine functionality possessed poor metabolic stability in mouse microsomes (Cl_{int} 151 μL/min/mg protein), while the inclusion of the oxo-piperazine group (**50**) enhanced stability in mouse liver microsomes (Cl_{int} 29 μL/min/mg

protein) and improved LipE, but did not improve aqueous solubility. Overall, this data demonstrated further improvements in aqueous solubility and metabolism were required.

To further enhance LTR mediated gene-expression and potentially improve physicochemical properties, a variety of substitutions on the aryl group were explored while retaining the 5-methyl thiazolyl piperazine pharmacophore (Table 3). Consistent with previous observations (Figure 2) that the 3-mono and 3,4-disubstitution are important for LTR potency,[23] the thiazolyl piperazine analogues with either no substitution, 4-chloro or 3-chloro substitution (**53** and **54**) were significantly less potent than the disubstituted 3-, 4-chloro methyl analogues, **16** and **34**. The 3,4-dichloro substituted analogue (**55**) was equipotent to the two chloro-methyl substituted analogues (**16** and **34**) again suggesting that the chloro and methyl groups are interchangeable in the 3- and 4- positions. Introduction of a nitrile substituent on the 3-position of the aryl group (**56**) modestly improved LTR activity (EC_{50} LTR of 0.23 μM) and LipE (3.7).

We previously determined a chloro, nitrile or trifluoromethyl substituent in the 5-position of the thiazole was important for LTR activity and improving metabolic stability. In particular, the nitrile substitution was shown to decrease thiazole oxidation.[23] In an effort to further improve LTR activity and metabolic stability, we next explored the impact of substitution in the 5-position of the thiazole ring on LTR activity while maintaining either a 4-substitution or a 3,4-disubstition on the aryl ring (surveyed in Table 3). The results in Table 4 show that 5-chloro and 5-nitrile thiazole substitution with a 4-chloro aryl substitution (**57** and **58**) gives a 2- and 7-fold improvement in LTR activity (EC_{50} 0.35 and 1.5 μM) compared to the 5-methyl substituted analogue (**54**). Similarly, 5-chloro and 5-nitrile thiazole substitution with a 3-chloro-4-methyl aryl group (**35** and **60)** resulted in a 2-fold improvement in LTR activity (EC_{50} 0.24 and 0.24 μM) compared to the 5-methyl substituted comparator analogue **34**, while the 5-trifluoromethyl substitution (**61**) was 2-fold less active

(EC_{50} 1.3 μM). The 5-chloro substitution on the thiazole with a 4-nitrile aryl substitution (**65**) was less active than its 5-methyl substituted counterpart (**56**). An undecorated thiazole analogue (**59**) possessed moderate LTR activity (EC_{50} 3.4 μM), but in contrast, we previously showed that the unsubstituted 2-acylaminothiazole was inactive,[23] highlighting the influence of the piperazinyl scaffold on LTR potency. Overall, analogues with 5-chloro and 5-nitrile thiazole substitution and a 3,4-disubstituted aryl group, **17** and **62**-**64**, consistently displayed improved LTR activity compared to its 5-methyl thiazole counterpart (Table 3). In addition, the inclusion of 5-nitrile substitution on the thiazole enhanced LipE values (>2.6).

The changes made to the 5-thiazole and aryl substituents enhanced the metabolic stability of **63** in the presence of mouse liver microsomes (Cl_{int} 15 μL/min/mg protein) when compared to **35** (Cl_{int} 53 μL/min/mg protein) (Table 8). Previous analysis of our 2-acylaminothiazoles, identified several potential metabolites including those predicted to originate from the mono-oxidation of both the phenyl ring and the 3- or 4- methyl substituent on the aryl ring.[23] Mass spectrometry analysis of **63** only detected a single mono-oxidation site (Table S1) demonstrating that installation of 3,4-dichloro substitution on the aryl ring significant improves intrinsic clearance. The in vitro metabolism data on **65** (Cl_{int} 38 μL/min/mg protein) (Table 8) provides further evidence of improved metabolic stability when a methyl substituent is not present on the thiazole or the pendant aryl ring.

To further improve metabolic stability and enhance aqueous solubility of the thiazolyl urea series, we explored whether differentially substituted pyridines would be a suitable replacement for the 3,4-disubstituted aryl ring. Analogues were designed to incorporate an endocyclic nitrogen in the 5- or 6- position while maintaining 3,4-disubstitution on the aryl ring in combination with a 5-substituted thiazole (Table 5). The results of the analogues evaluated in FlpIn.FM HEK293 assay show the 5-methyl thiazole analogue **66** with 6-(3-cyano-4-methylpyridine) substitution exhibited modest LTR activity (EC_{50} 4.2 μM).

Consistent with previous trends, transition from the 5-methyl to 5-chloro substituent on the thiazole (**67**) resulted in a significant increase in potency (EC_{50} 0.46 μM). This trend was also observed for the analogues **68** and **69** comprising a 5-(2-cyano-3-methylpyridine) system (EC_{50} of 10 μM vs. 0.21 μM). The similar LTR activity observed between **67** and **69** suggests the location of the endocyclic nitrogen appears to be unimportant for LTR activity. Substitution of a trifluoromethyl group into the 4-pyridyl position (**70** and **71**) was also tolerated (LTR EC_{50} 0.73 and 0.92 μM), but the 5-chloro analogue **71** was 2-fold less active than nitrile counterpart **67**. Furthermore, the inclusion of the nitrile substituent resulted in increased LipE values for analogues **67** (3.4) and **69** (4.0). Analogues **72**-**75** comprised of 3,4-dichloropyridin-5-yl substitution in combination with substitution of methyl, nitrile, chloro and trifluoromethyl in the 5-position of the thiazole, respectively, showed a 2- to 10-fold increase in LTR activity (EC_{50} <1.0 μM) and improved LipE compared to their phenyl counterparts (**55**, **62** and **63**). Compound **74** exhibited the greatest LTR potency (EC_{50} 80 nM) of the thiazolyl urea series. To evaluate whether a pyrimidine system could replace the substituted pyridinyl system, the analogues **76** and **77** were generated, but pyrimidine substitution was not tolerated (EC_{50} >40 and 8 μM).

The LipE values were improved for the pyridinyl analogues in Table 5 compared to the aryl analogues, but the aqueous solubility was not improved (Table 8). The metabolic stability of the pyridinyl analogue **74** in mouse liver microsomes (Cl_{int} 32 μL/min/mg protein) was comparable to the corresponding aryl analogues **35** and **50**. Similarly, the pyridinyl analogue **73** possessed similar metabolic stability (Cl_{int} 22 μL/min/mg protein) than the comparator phenyl analogue **63** (Cl_{int} 15 μL/min/mg protein). Overall, incorporating a heterocyclic nitrogen in the aryl ring was beneficial for LTR activity and LipE, maintained in vitro metabolism, but did not improve aqueous solubility.

Unsubstituted thiazoles are notorious toxicophores due to their susceptibility for cytochrome P450 mediated oxidative metabolism of the 4,5-double bond producing an epoxide, which is a nucleophilic trap. In vitro metabolism studies, including our own, have shown that 5-substitution of the thiazole ring can mitigate this oxidative event.[23, 26] To overcome the oxidative metabolism, we investigated the bioisoteric replacement of the 2-aminoacylthiazole system. We hypothesized a low-lying σ* orbital of the sulfur atom in the thiazole was acting as an electron acceptor for an electron rich oxygen atom on the amide/urea carbonyl. This interaction is likely to result in a non-covalent intramolecular interaction capable of fixing the rotational confirmation of carboxamide in relation to the thiazole. Non-covalent sulfur interactions are a common phenomenon and have been recently reviewed.[27] We proposed the imidazopyridine scaffold could act as a suitable bioisosteric replacement for the 2-acylaminothiazole moiety, in which the pyridine nitrogen would mimic the thiazole nitrogen and the imidazole NH would emulate the 2-acylaminothiazole NH.

To determine if the imidazopyridine system was an appropriate replacement for the 2-acylaminothiazole structure, a set of analogues were generated and evaluated in the FlpIn.FM HEK293 cellular assay (Table 6). The results show that the 6-methyl and 6-chloro imidazopyridine analogues **78** and **79** exhibited modest LTR activity (EC_{50} 12 and 9.4 μM) comparable to the LTR activity of the corresponding 2-acylaminothiazoles **1** and **2** (Figure 2). The imidazopyridine analogues **78**, **80** and **82** possessed similar LTR activity suggesting 3,4-disubstitution pattern on the phenoxy aryl ring was not as sensitive to change as the 2-acylaminothiazole scaffold. The imidazopyridine analogues with the 5-chloro substituent, **79**, **81** and **83** were consistently more active than the 6-methyl substituted imidazopyridine analogues **78**, **80** and **82**, demonstrating the same trend seen with the 2-acylaminothiazole class. The thiazopyridine analogues, **84** and **85** were inactive (LTR EC_{50} >40 μM), demonstrating the requirement for an NH in the 3-position of the imidazopyridine. The in

vitro metabolism of **79** and **83** in the presence of mouse liver microsomes was high (Cl_{int} >866 and 122 μL/min/mg protein) (Table 8). It was discovered the high metabolism was a result of a result of mono and bis-oxygenation of the aryl phenoxy ring (Table S1). The introduction of the imidazopyridine system did not improve aqueous solubility, for example **79** and **83** has comparable solubility to the 2-acylaminothiazole analogues in Table 8. Although no improvement in in vitro physiochemical properties was observed, the LTR activity of the imidazopyridine analogues was comparable to the corresponding 2-acylaminothiazole derivatives, reinforcing imidazopyridine as a suitable bioisosteric replacement.

To improve LTR mediated gene-expression and physicochemical properties of the imidazopyridine scaffold, we next replaced the oxy-carbon chain with piperazine; an approach previously undertaken with the thiazolyl urea class (Tables 1-5). A focus set of 2-piperazinyl imidazopyridine analogues was synthesized and evaluated in the FlpIn.FM HEK293 cell assay (Table 7). The results show the imidazopyridine analogues **86**, **88** and **90** (EC_{50} 0.75 - 3.2 μM) are moderately less potent than the corresponding thiazolyl urea analogues, **34**, **62** and **72**. The 6-halo imidazopyridine analogues **87** and **91** are 10-fold more potent (EC_{50} 0.32 and 0.08 μM) than the 6-methyl derivatives **86** and **90** (EC_{50} 3.2 and 1.3 μM), consistent with the trend observed for the 5-chloro thiazolyl urea class. In addition, imidazopyridine analogue **91** was equipotent (EC_{50} of 0.08 μM) to **74**, suggesting the 2-piperazinyl imidazopyridine derivatives were closely aligned with the trends in SAR and LTR activity of the 2-piperazinyl thiazolyl urea series, further supporting the application of the imidazopyridine bioisostere. The imidazopyridine analogues **86** and **91** exhibited low metabolic stability in the presence of mouse liver microsomes (Cl_{int} 224 and 132 μL/min/mg protein) (Table 8) compared to the corresponding thiazolyl urea derivatives **35** and **74**. The aqueous solubility of **86** and **91** at pH 6.5 was also low, but the solubility at pH 2 was

enhanced (25 - 100 μM), because of the presence of an ionizable nitrogen on the imidazopyridine system.

In summary of the SAR (Figure 3), it was initially determined that the piperazine, 3-aminoazetidine and 3-aminopyrrolidine were suitable replacements for the oxy-carbon chain substituted producing the thiazolyl urea scaffold with improved LTR activity (Tables 1-5) and physicochemical properties (Table 8) compared to the 2-acylaminothiazole class. The SAR of the thiazolyl urea series was also closely aligned with the 2-aminoacylthiazole class.[23] In general, either 3,4-dichloro or 3,4-chloro methyl substitution on the aryl ring and either 5-chloro or 5-nitrile substitution on the thiazole were optimal for LTR directed gene expression. This activity and SAR trend was also observed for the imidazopyridine bioisosteric replacements of the 2-acylaminothiazole and the thiazolyl urea series (Table 6 and 7). 5-Chloro and 3,4-dichloro substitution on both the 2-acylaminothiazole and the thiazolyl urea series displayed the most robust in vitro metabolic stability in comparison to other substitution patterns (Table 8). The imidazopyridine system exhibited slightly improved aqueous solubility but possessed significantly lower metabolic stability compared to 2-acylaminothiazole and the thiazolyl urea series suggesting an intrinsic metabolic liability associated with the bicyclic scaffold.

In general, improvements in LTR activated reporter gene expression also resulted in proportionate increases in CMV activity. In cases where selectivity was observed, LTR activity reached a maximum before decreasing or plateauing with increasing compound concentration (Figure S1), suggesting that either off-target cellular toxicity circumvents transcriptional or modulates post-transcriptional gene expression, or an increase in transcriptional activation and gene expression perturbs cellular homeostasis leading to cell death.

Evaluation of Cellular Growth Inhibition

The CMV reporter was built into the FlpIn.FM HEK239 cell line to detect fluctuations in non-specific Luc reporter protein expression. To determine whether increased LTR and CMV reporter activity are related to host cytotoxicity, a selection of compounds was evaluated in a Cell Titre Glo growth inhibition assay using the HepG2 cell line (Table 9). In this assay, all the analogues tested inhibited cell growth more potently than the 2-acylamidothiazole analogue **2**. The HepG2 cell growth inhibition data robustly correlated with the LTR activity, suggesting transcriptional activation was related to cell growth inhibition. This observation provides a reason why the LTR activity reaches a maximum before gradually decreasing with higher concentrations of compound (Figure S1). This is consistent with clinically used LRA's such as Romidepsin and Panobinostat, whereby the cell growth inhibition is linked to their mechanism of action.[28] Compounds with increasing LTR activity were also associated with potent CMV activity, however, the window of selectivity between the CMV and LTR reporter was not consistent for each analogue, and therefore it is difficult to ascertain whether cell growth inhibition is directly associated with LTR or CMV activity. It is unknown or this class of HIV LRAs whether it will be possible to improve cell cytotoxicity while maintaining LTR potency, but the strong correlation with both the LTR and CMV activity suggests gene expression from these viral promoters and cellular toxicity are intrinsically linked. The association between activation of non-specific gene expression and cell toxicity is a common trait in other established LRAs.[28] Ongoing efforts to uncover the mechanism of action of the 2-acylaminothiazole and thiazolyl urea series may assist in understanding this relationship and improving the selectivity between cell growth inhibition and activation of LTR gene expression.

Evaluation of Thiazolyl Ureas Against Targets Previously Linked to HIV Latency

Our efforts to establish the mechanism by which the thiazolyl urea analogues enhance cellular LTR activity began with profiling a representative compound against a panel of epigenetic enzymes known to be associated with HIV gene expression[29] (Table S2 to S4). **74** displayed no inhibitory activity when profiled against all histone deacetylase (HDAC) isoforms and SIRT1 (Table S2). Compound **74** also exhibited no detectable inhibition of nine methyltransferases and four acetyltransferases previously linked to HIV-1 transcriptional modulation in patients with chronic HIV-1 infection and latency[29, 30] (Tables S3 and S4). The thiazolyl urea scaffold possess a kinase hinge-binding motif and is closely related to reported cyclin dependent kinase inhibitors,[31,32] and therefore we screened a representative analogue (**35**) against 369 kinases at a concentration of 0.5 μM (Table 5). The results show analogue **35** did not show significant inhibition of any of the kinases in the panel, except for the modest inhibition (40-50%) of three cyclin-dependent kinase 9 (CDK9) proteins, cyclin K, T1 and T2. Subsequently, **74** was tested against 22 kinases of the CDK family (Table S6) at concentration of 0.5 μM. Consistent with the kinase inhibition data on **35**, **74** exhibited approximately 60% inhibition the CDK9 proteins, cyclin K, T1 and T2. CDK9 proteins are known to be recruited by Tat to initiate HIV-1 transcription by phosphorylating the COOH-terminus of RNA-pol II and increasing processivity.[33] While this is within the realm of pharmacological relevance as a potential mechanism, direct inhibition of CDK-9 in HIV-1 replication assays has been shown to potently block HIV-1 transcription.[34] In addition, the cell growth inhibition and LTR activity for both **35** and **74** (EC_{50} <0.1 μM) is significantly lower than the measured CDK9 inhibition values. Therefore, it is likely that the CDK9 kinase activity is unrelated to observed cellular and LTR activity. In summary, the screening of analogues against a panel of epigenetic proteins and kinases suggests the thiazolyl urea series has a mechanism of action not previously associated with HIV gene expression. Target identification of the thiazolyl urea class will be key aspect of future studies.

Evaluation of Analogues in the Jurkat Latency 10.6 Cell Line

The J.Lat10.6 clone is a latently infected T-cell line commonly employed to evaluate the activity of LRAs to activate the HIV LTR directed reporter gene expression.[35, 36] This cell line contains a full-length integrated HIV-1 genome with green fluorescent protein (GFP) in place of the Nef gene. Treatment of J.Lat10.6 with TNF-α or compound activates viral gene expression which is quantified by flow cytometry.

We evaluated a selection of the analogues using the J.Lat10.6 cell line and compared their LTR activity to selection of known LRAs (Table 9). The results show compounds **29**, **35**, **39**, **50**, **63** and **73** enhanced the LTR GFP reporter with an EC_{50} range of 1 - 5 µM, comparable to the activity of known LRAs, Vorinostat and JQ1 (EC_{50} 2.3 and 8.1 µM). The LTR activity displayed by the thiazolyl urea analogues was a significant improvement on the LTR activity of the 2-amainoacylthiazole **2** reported previously (EC_{50} 29 µM).[23] The analogues, **65**, **74** and **91**, potently enhanced the LTR GFP reporter (EC_{50} <0.5 µM) comparable to the activity of the known LRAs, Romidepsin (EC_{50} 0.22 µM) and Panobinostat (EC_{50} 0.13 µM). There is a robust correlation between the FlpIn.FM HEK293 LTR activity and the J.Lat10.6 LTR reporter, and this is most evident with the analogues **74** and **91,** which display potent activity in both cell lines. Overall, the LTR activated Luc-reporter gene expression from the FlpIn.FM HEK293 cell assay robustly correlates with the LTR activated GFP expression in the J.Lat10.6 cellular model, demonstrating the utility of the FlpIn.FM HEK293 in detecting LRAs.

CONCLUSIONS

The FlpIn.FM HEK293 cellular assay was previously utilized to identify the 2-acylaminothiazole hit class. Exploration of the structure activity relationship afforded compounds with modest activity in HIV cellular models (Figure 2). Building on our previous work, we applied two design strategies to further enhance physicochemical properties and improve activity in HIV gene expression models. The first approach explored rigidification of the central oxy-carbon linker with a variety of 4-, 5-, 6- and 7- membered saturated heterocycles. Piperazine, 3-aminopyrrolidine and 3-azetidine were the most suitable surrogates for the central oxy-carbon linker (thiazolyl urea analogues **74** and **91**) which exhibited a 20-fold enhancement in LTR activity (EC_{50} 80 and 80 nM) compared to the 2-acylaminothiazole **2** (EC_{50} 1.9 μM). The thiazolyl urea analogues also possessed enhanced metabolic stability in the presence of both human and mouse liver microsomes (for example **63**, Cl_{int} 11 and 15 μL/min/mg protein), however aqueous solubility remains low and will require further optimization.

The second approach investigated the bioisosteric replacement of the 2-acylaminothiazole moiety with imidazopyridine framework. We determined the imidazopyridine scaffold was a reasonable bioisostere and mirrored the structure activity relationship and the LTR activity observed with the 2-acylaminothiazole and the thiazolyl urea scaffolds. The imidazopyridine analogues possessed low metabolic stability in the presence of both human and mouse liver microsomes (for example, **86** Cl_{int} 115 and 224 μL/min/mg protein), suggesting an intrinsic liability with the scaffold that could not be determined through metabolism identification. Future work is required to address both metabolism and aqueous solubility of the imidazopyridine class to permit its utility as an LRA in in vivo studies.

The thiazolyl urea and imidazopyridine series potently activated LTR activity in the FlpIN.FM HEK293 cellular assay, but also variably activated the non-specific transcription via the CMV reporter. Only a select few analogues displayed a significant window of

selectivity (>10-fold for **36-37**, **63**, **66** and **70**) and in general, most analogues displayed a 2- to 3- fold difference between LTR and CMV activity (Tables 1-7). It is unknown whether the same mechanism of action is responsible for modulating both the LTR and CMV promoter directed gene expression, but the robust correlation in the HIV LTR directed reporter activity and cellular toxicity (Table 9) would suggest the mechanisms are intrinsically linked. Therefore, it is proposed the cellular toxicity of the analogues is the result of a significant increase in HIV-promoter directed protein expression that disrupts cellular homeostasis and consequently manifests in cellular toxicity. The non-selective transcriptional and/or post-transcriptional cytotoxicity observed is a reoccurring trait in epigenetic modulators, such as Romidepsin and Panobinostat (Table 9), commonly utilized in the shock and kill approach.[28] It is likely that identifying the cellular target(s) of the thiazolyl urea and imidazopyridine series will help unravel the mechanisms imparting the cytotoxicity observed. Our efforts to elucidate the cellular target involved screening thiazolyl urea compounds against a panel of epigenetic proteins and kinases but did not reveal the genuine target (Tables S2-S6). The discovery of the mechanism remains a key aspect of our future work.

In summary, we have used a 2-aminoacylthiazole scaffold as a template to prepare a set of thiazolyl ureas and imidazopyridine analogues that potently activate HIV transcription in FlpIn.FM HEK293 and J.Lat10.6 latency models. Furthermore, the activity observed with lead analogues (**74** and **91**) is comparable to the clinical evaluated LRAs, Romidepsin and Panobinostat, and highlights the potential of this class for application to modify HIV gene expression as a strategy to eliminate latent provirus.

EXPERIMENTAL SECTION

Biology Experimental.

FlpIn.FM HEK293 Cellular Assay. The FlpIn.FM HEK293 cellular assay was performed according to previously described protocol.[23] Briefly, FlpIn.FM HEK293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FCS. A total of 1500 cells in 25 μL of DME media with 5% FCS were transferred to each well of the assay plate using a Multidrop reagent dispenser. Plates were left at room temperature in a single layer for 1 h to allow adhesion to commence and were then incubated at 37 °C and 5% CO_2 for 48 h. Assay plates were cooled to 20 °C prior to addition of 10 μL of Chroma-Glo reagent to each assay plate well. The plates were incubated at 20 °C for 2 min, and plates were read on a plate reader (Em: 613 and 537 nm). Percent activity of each compound was calculated for the ClickBeetle Red (CBR) luminescence and ClickBeetle Green luminescence (CBG) according to the following equation: % activity = 100 – (100 × (luminescence$_{compound}$ – mean luminescence$_{negative\ control}$/mean luminescence$_{positive\ control}$ – mean luminescence$_{negative\ control}$)). For calculating the EC_{50} values of LTR- and CMV-driven gene expression, the raw data was first normalized between the DMSO-treated negative control (set to 0%) and the PMA-treated positive control (set to 100%). Data points associated with cytotoxicity were excluded where LTR (ClickBeetle Red) activity begins to decrease with increasing compound concentration. EC_{50} values were then obtained using XY analysis, nonlinear regression (curve fit), and [agonist] versus normalized response–variable slope analysis using Domatics Software.

Jurkat Latency 10.6 Clone (J.Lat10.6) Cellular Assay. The J.Lat10.6 cellular assay was performed according to previously described protocol.[35] Briefly, cells were maintained in 90% RPMI 1640 and 10% FBS supplemented with penicillin G (100 U/mL), streptomycin (100 μg/mL), and l-glutamine (2 mM, 0.3 mg/mL). J.Lat10.6 cells (4×10^4) were seeded into a 96-well format in 50 μL of complete RPMI (supplemented with 10% FCS and 1% Pen/Strep). Compounds were then added in 50 μL of complete RPMI and plates incubated at

37°C for 48 h. Bioluminescence was measured using a FACSCalibur. Positive population data was normalized using the positive control (TNF-α) and negative control (DMSO) using GraphPad Prism. The EC_{50} was extrapolated in the same manner as reported above for the HEK293 FlpIn.FM dual reporter cellular assay.

HepG2 Cell Growth Inhibition Assay. The HepG2 cellular assay was performed according to previously described protocol.[23] Briefly, HepG2 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal calf serum (FCS) in a humidified incubator at 37 °C and 5% CO2. Assay plates were created by seeding 1000 cells in 50 μL of DMEM with 10% FCS into each well of 384-well tissue culture treated plates. Ten-point dilution series of compounds were prepared in DMSO and transferred to the assay plate. Plates were then incubated at 37 °C and 5% CO_2 for 48 h. Cell Titer-Glo reagent was added and bioluminescence measured using an Envision plate reader. Cytotoxicity was calculated as a percentage using DMSO as the positive growth control and 10 μM bortezomib as a negative growth control. EC_{50} values were calculated using a four-parameter log dose, nonlinear regression analysis, with sigmoidal dose−response (variable slope) curve fit and constraint parameters of 0 and 100% using GraphPad Prism (v6.05).

Aqueous Solubility Determination Using Nephelometry. The compound in DMSO was spiked into either pH 6.5 phosphate buffer or 0.01 M HCl (pH 2.0) with the final DMSO concentration being 1%. Samples were then analysed via nephelometry to determine a solubility range.

In Vitro Metabolism Using Mouse and Human Liver Microsomes. In vitro metabolism and metabolism identification studies were performed following the previously described procedure.[23]

Chemistry Experimental.

General Chemistry Procedures. Solvents were obtained commercially and used without further purification. Chromatography was performed with silica gel 60 (particle size 0.040-0.063 µm) using an automated purification system. NMR spectra were recorded on a Bruker Avance DRX 300. Chemical shifts are reported in ppm on the δ scale and referenced to the appropriate solvent peak. MeOD, DMSO-d_6, Acetone-D_6 and $CDCl_3$ contain H_2O.

LCMS were recorded on either a Waters LCMS system composed of a Waters 3100 Mass Detector, Waters 2996 Diode Array Detector, Waters 2545 Binary Pump, Waters SFO System Fluidics Organizer and a Waters 2767 Sample Manager (Method A), or an Agilent LCMS system composed of an Agilent G6120B Mass Detector, 1260 Infinity G1312B Binary pump, 1260 Infinity G1367E HiPALS autosampler and 1260 Infinity G4212B Diode Array Detector (Method B). Conditions for LCMS Method A were as follows, column: Kinetex TM XB-C18 5µm 4.6 × 50mm, injection volume 10 µL, 5-100% B over 3 min (solvent A: water 0.1% formic acid; solvent B: AcCN 0.1% formic acid), flow rate: 1.5 mL/min, detection: 100-600 nm, acquisition time: 6 min. Conditions for LCMS Method B were as follows, column: Poroshell 120 EC-C18, 2.1 × 50 mm 2.7 Micron at 20 ºC, injection volume 2 µL, gradient: 5-100% B over 3 min (solvent A: water 0.1% formic acid; solvent B: AcCN 0.1% formic acid), flow rate: 0.8 mL/min, detection: 254 nm, acquisition time: 5 min. LCMS conditions used to assess purity of final compounds were as follows, column: Phenomenex Gemini C18, 2.0 × 50 mm; injection volume 20 µL; gradient: 0-100% Buffer B over 6 min (buffer A: 0.1% formic acid in autoclaved MilliQ water; buffer B: 0.1% formic acid in 100% acetonitrile), flow rate: 1.0 mL/min, detection: 214 or 224 nm. Unless otherwise noted, all compounds were found to be >95% pure by this method. HRMS were acquired through The Bio21 Mass Spectrometry and Proteomics Facility using a Thermo Scientific™ nano-LC Q Exactive™ Plus Mass spectrometer.

General Procedure A: 4-(4-Chloro-3-methylphenyl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**16**). **92** (30 mg, 0.14 mmol), **93** (37 mg, 0.16 mmol) and caesium carbonate (93 mg, 0.29 mmol) were combined in 1,4-dioxane (1 mL) and stirred at reflux for 5 h under N_2. The reaction was then cooled to room temperature and the reaction mixture diluted with EtOAc (20 mL), and then washed with water (10 mL) and brine (10 mL). The organic layer was then dried with Na_2SO_4 and concentrated *in vacuo*. The crude residue was then purified by column chromatography (100% CyHex to 50% EtOAc/CyHex) to obtain **16** as a solid (19 mg, 38%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (d, *J* 8.6 Hz, 1H), 6.94 (d, *J* 1.3 Hz, 1H), 6.80 (d, *J* 2.9 Hz, 1H), 6.71 (dd, *J* 8.7, 3.0 Hz, 1H), 3.78 - 3.64 (m, 4H), 3.24 - 3.11 (m, 4H), 2.41 - 2.33 (m, 6H). MS, m/z = 351 (100) $[M+H]^+$, 353 (30). HRMS found: (M + H) 351.1038; $C_{16}H_{19}ClN_4OS$ requires (M +H), 351.1041.

4-(4-Chloro-3-methylphenyl)-N-(5-chlorothiazol-2-yl)piperazine-1-carboxamide (**17**). General Procedure A was followed using **94** (40 mg, 0.16 mmol) and **92** (30 mg, 0.14 mmol) to obtain **17** as a solid (41 mg, 78%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.23 (d, *J* 8.8 Hz, 1H), 7.15 (s, 1H), 6.80 (d, *J* 2.9 Hz, 1H), 6.70 (dd, *J* 8.7, 3.0 Hz, 1H), 3.75 - 3.63 (m, 4H), 3.23 - 3.12 (m, 4H), 2.35 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.9, 152.8, 148.9, 136.3, 132.3, 129.1, 125.8, 120.0, 118.8, 115.1, 48.9, 43.6, 20.0. MS, m/z = 371 (100) $[M+H]^+$, 373 (60). HRMS found: (M + H) 371.0493; $C_{15}H_{16}Cl_2N_4OS$ requires (M + H), 371.0495.

4-(4-Chloro-3-methylphenoxy)-N-(5-methylthiazol-2-yl)piperidine-1-carboxamide (**18**). General Procedure A was followed using **93** (30 mg, 0.13 mmol) and **95** (26 mg, 0.12 mmol) to obtain **18** as a solid (20 mg, 47%). ^{1}H-NMR (300 MHz; CDCl3): δ 7.23 (d, *J* 8.6 Hz, 1H), 6.92 (s, 1H), 6.80 (d, *J* 2.9 Hz, 1H), 6.69 (dd, *J* 3.0, 8.7 Hz, 1H), 4.49 (tt, *J* 3.4, 6.4 Hz, 1H), 3.81 - 3.63 (m, 2H), 3.61 - 3.44 (m, 2H), 2.40 - 2.29 (m, 6H), 2.04 – 1.74 (m, 4H). MS, m/z = 365 (100) $[M+H]^+$, 367 (30). HRMS found: (M + H) 366.1037; $C_{17}H_{20}ClN_3O_2S$ requires (M +H), 366.1038.

4-(4-Chloro-3-methylphenoxy)-N-(5-chlorothiazol-2-yl)piperidine-1-carboxamide (**19**). General Procedure A was followed using **94** (32 mg, 0.13 mmol) and **95** (26 mg, 0.12 mmol) to obtain **19** as a solid (38 mg, 47%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.24 (d, *J* 8.6 Hz, 1H), 7.13 (s, 1H), 6.81 (d, *J* 2.9 Hz, 1H), 6.70 (dd, *J* 2.9, 8.8 Hz, 1H), 4.57 - 4.47 (m, 1H), 3.76 - 3.51 (m, 4H), 2.35 (s, 3H), 2.02 – 1.81 (m, 4H). MS, m/z = 386 (100) $[M+H]^+$. HRMS found: (M + H) 386.0490; $C_{16}H_{17}Cl_2N_3O_2S$ requires (M +H), 386.0492.

4-((4-Chloro-3-methylphenyl)amino)-N-(5-methylthiazol-2-yl)piperidine-1-carboxamide (**20**). General Procedure A was followed using **93** (16 mg, 0.069 mmol) and **96** (13 mg, 0.058 mmol) to obtain **20** as a solid (10 mg, 47%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.12 (d, *J* 8.4 Hz, 1H), 6.93 (br. s., 1H), 6.48 (d, *J* 2.4 Hz, 1H), 6.39 (dd, *J* 2.9, 8.6 Hz, 1H), 4.16 - 4.04 (m, 2H), 3.56 - 3.37 (m, 2H), 3.17 - 3.04 (m, 2H), 2.36 (s, 3H), 2.30 (s, 3H), 2.16 - 2.06 (m, 2H), 1.43 - 1.31 (m, 2H). MS, m/z = 365 (100), 367 (30) $[M+H]^+$. HRMS found: (M + H) 365.1197; $C_{17}H_{21}ClN_4OS$ requires (M +H), 365.1198.

4-((4-Chloro-3-methylphenyl)amino)-N-(5-chlorothiazol-2-yl)piperidine-1-carboxamide (**21**). General Procedure A was followed using **94** (18 mg, 0.069 mmol) and **96** (13 mg, 0.058 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **21** as a solid (2.5 mg, 11%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.24 - 7.12 (m, 2H), 6.61 - 6.45 (m, 2H), 4.22 - 4.03 (m, 2H), 3.56 - 3.43 (m, 1H), 3.14 (ddd, *J* 3.3, 11.3, 14.3 Hz, 2H), 2.38 - 2.27 (m, 3H), 2.25 - 2.11 (m, 2H), 1.61 - 1.39 (m, 2H). MS, m/z = 385 (100), 387 (60) [M+H]+. HRMS found: (M + H) 385.0650; $C_{16}H_{18}Cl_2N_4OS$ requires (M +H), 385.0651.

4-((4-Chloro-3-methylphenyl)(methyl)amino)-N-(5-chlorothiazol-2-yl)piperidine-1-carboxamide (**22**). General Procedure A was followed using **94** (17 mg, 0.065 mmol) and **98** (13 mg, 0.055 mmol) to obtain **22** as a solid (10 mg, 46%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ

7.22 - 7.11 (m, 2H), 6.69 (d, *J* 2.9 Hz, 1H), 6.62 (dd, *J* 3.0, 8.7 Hz, 1H), 4.30 - 4.16 (m, 2H), 3.81 - 3.66 (m, 1H), 3.09 – 2.91 (m, 2H), 2.74 (s, 3H), 2.35 (s, 3H), 1.90 - 1.62 (m, 4H). MS, m/z = 399 (100), 401 (60) $[M+H]^+$. HRMS found: (M + H) 399.0808; $C_{17}H_{20}Cl_2N_4OS$ requires (M +H), 399.0808.

3-(4-Chloro-3-methylphenoxy)-N-(5-chlorothiazol-2-yl)piperidine-1-carboxamide (**23**). General Procedure A was followed using **94** (15 mg, 0.059 mmol) and **99** (12 mg, 0.053 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **23** as a solid (3.4 mg, 17%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.22 (d, *J* 8.8 Hz, 1H), 7.16 (s, 1H), 6.78 (d, *J* 3.3 Hz, 1H), 6.68 (dd, *J* 2.9, 8.6 Hz, 1H), 4.34 (td, *J* 3.4, 6.5 Hz, 1H), 3.87 - 3.79 (m, 1H), 3.66 - 3.42 (m, 3H), 2.32 (s, 3H), 2.04 – 1.86 (m, 3H), 1.67 - 1.55 (m, 1H). MS, m/z = 386 (100) $[M+H]^+$, 388 (60). HRMS found: (M + H) 386.0490; $C_{16}H_{17}Cl_2N_3O_2S$ requires (M +H), 386.0492.

(S)-3-((4-Chloro-3-methylphenyl)amino)-N-(5-methylthiazol-2-yl)piperidine-1-carboxamide (**24**). General Procedure A was followed using **93** (16 mg, 0.067 mmol) and **100** (15 mg, 0.067 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **24** as a solid (5.0 mg, 21%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.11 (d, *J* 8.58 Hz, 1H), 6.94 (s, 1H), 6.50 (d, *J* 2.86 Hz, 1H), 6.42 (dd, *J* 3.0, 8.7 Hz, 1H), 4.12 - 4.02 (m, 1H), 3.82 - 3.62 (m, 1H), 3.52 - 3.41 (m, 1H), 3.40 - 3.29 (m, 1H), 3.14 - 3.01 (m, 1H), 2.36 (s, 3H), 2.28 (s, 3H), 2.10 – 1.97 (m, 1H), 1.92 – 1.79 (m, 1H), 1.70 – 1.53 (m, 2H). MS, m/z = 365 (100) $[M+H]^+$. HRMS found: (M + H) 365.1197; $C_{17}H_{21}ClN_4OS$ requires (M +H), 365.1198.

(S)-3-((4-Chloro-3-methylphenyl)amino)-N-(5-chlorothiazol-2-yl)piperidine-1-carboxamide (**25**). General Procedure A was followed using **94** (17 mg, 0.067 mmol) and **100** (15 mg, 0.067 mmol). The crude residue was then purified by reverse phase preparatory HPLC using

a gradient of 95% water/ACN to 100% ACN to obtain **25** as a solid (5.3 mg, 21%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.16 - 7.10 (m, 2H), 6.51 (d, *J* 2.4 Hz, 1H), 6.43 (dd, *J* 2.5, 8.7 Hz, 1H), 4.05 – 3.98 (m, 1H), 3.73 - 3.63 (m, 1H), 3.53 - 3.44 (m, 1H), 3.39 - 3.29 (m, 1H), 3.10 (dd, *J* 7.9, 13.4 Hz, 1H), 2.29 (s, 3H), 2.09 - 2.01 (m, 1H), 1.91 - 1.82 (m, 1H), 1.72 - 1.57 (m, 2H). MS, m/z = 385 (100) $[M+H]^+$, 387 (60). HRMS found: (M + H) 385.0649; $C_{16}H_{18}Cl_2N_4OS$ requires (M +H), 385.0651.

(R)-3-((4-Chloro-3-methylphenyl)amino)-N-(5-methylthiazol-2-yl)piperidine-1-carboxamide (**26**). General Procedure A was followed using **93** (31 mg, 0.13 mmol) and **101** (30 mg, 0.13 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **26** as a solid (2.8 mg, 6%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.11 (d, *J* 8.6 Hz, 1H), 6.94 (s, 1H), 6.50 (d, *J* 2.4 Hz, 1H), 6.42 (dd, *J* 2.9, 8.4 Hz, 1H), 4.14 - 4.05 (m, 1H), 3.82 - 3.62 (m, 1H), 3.52 - 3.41 (m, 1H), 3.41 - 3.30 (m, 1H), 3.10 (dd, *J* 7.4, 13.3 Hz, 1H), 2.36 (s, 3H), 2.29 (s, 3H), 2.10 – 1.98 (m, 1H), 1.93 - 1.81 (m, 1H), 1.76 - 1.52 (m, 2H). MS, m/z = 365 (100) $[M+H]^+$. HRMS found: (M + H) 365.1195; $C_{17}H_{21}ClN_4OS$ requires (M +H), 365.1198.

(R)-3-((4-Chloro-3-methylphenyl)amino)-N-(5-chlorothiazol-2-yl)piperidine-1-carboxamide (**27**). General Procedure A was followed using **94** (34 mg, 0.13 mmol) and **101** (30 mg, 0.13 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **27** as a solid (3.1 mg, 6%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.16 - 7.10 (m, 2H), 6.70 – 6.48 (m, 2H), 4.25 – 4.12 (m, 1H), 3.90 - 3.80 (m, 1H), 3.53 - 3.17 (m, 3H), 2.31 (s, 3H), 1.90 - 2.05 (m, 1H), 1.75 – 1.54 (m, 3H). MS, m/z = 385 (100) $[M+H]^+$, 387 (60). HRMS found: (M + H) 385.0648; $C_{16}H_{18}Cl_2N_4OS$ requires (M +H), 385.0651.

3-((4-Chloro-3-methylphenyl)amino)-N-(5-methylthiazol-2-yl)pyrrolidine-1-carboxamide (**28**). General Procedure A was followed using **93** (18 mg, 0.076 mmol) and **103** (16 mg, 0.076 mmol) to obtain **28** as a solid (13 mg, 49%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.14 (d, *J* 8.6 Hz, 1H), 6.92 (s, 1H), 6.47 (d, *J* 2.6 Hz, 1H), 6.38 (dd, *J* 8.6, 2.9 Hz, 1H), 4.12 - 4.05 (m, 1H), 3.82 – 3.74 (m, 1H), 3.66 - 3.52 (m, 2H), 3.44 - 3.34 (m, 1H), 2.39 - 2.18 (m, 7H), 2.05 – 1.95 (m, 1H). MS, m/z = 351 (100) $[M+H]^+$, 353 (30). HRMS found: (M + H) 351.1041; $C_{16}H_{19}ClN_4OS$ requires (M +H), 351.1041.

3-((4-Chloro-3-methylphenyl)amino)-N-(5-chlorothiazol-2-yl)pyrrolidine-1-carboxamide (**29**). General Procedure A was followed using **94** (20 mg, 0.076 mmol) and **103** (16 mg, 0.076 mmol) to obtain **29** as an oil (12 mg, 43%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.14 (d, *J* 8.6 Hz, 2H), 6.46 (d, *J* 2.6 Hz, 1H), 6.37 (dd, *J* 8.5, 3.0 Hz, 1H), 4.14 - 4.06 (m, 1H), 3.83 - 3.69 (m, 1H), 3.65 - 3.50 (m, 2H), 3.44 – 3.34 (m, 1H), 2.39 - 2.21 (m, 4H), 2.09 – 1.93 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.1, 151.8, 144.7, 136.9, 132.6, 129.7, 123.8, 115.8, 112.2, 52.8, 51.9, 44.2, 31.4, 20.3 (1 quaternary C not observed). MS, m/z = 371 (100) $[M+H]^+$, 373 (30). HRMS found: (M + H) 371.0492; $C_{15}H_{16}Cl_2N_4OS$ requires (M +H), 371.0495.

3-(4-Chloro-3-methylphenoxy)-N-(5-methylthiazol-2-yl)pyrrolidine-1-carboxamide (**30**). General Procedure A was followed using **93** (19 mg, 0.080 mmol) and **104** (17 mg, 0.080 mmol) to obtain **30** as a solid (9.3 mg, 33%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.23 (d, *J* 8.6 Hz, 1H), 6.92 (d, *J* 1.1 Hz, 1H), 6.74 (d, *J* 2.9 Hz, 1H), 6.63 (dd, *J* 2.9, 8.6 Hz, 1H), 4.99 - 4.81 (m, 1H), 3.86 - 3.49 (m, 4H), 2.45 - 2.09 (m, 8H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.5, 155.2, 152.5, 137.4, 133.2, 129.8, 126.7, 126.6, 118.2, 114.1, 75.9, 51.6, 44.0, 31.2, 20.3, 11.5. MS, m/z = 352 (100) $[M+H]^+$, 354 (30). HRMS found: (M + H) 352.0881; $C_{16}H_{18}ClN_3O_2S$ requires (M +H), 352.0881.

3-(4-Chloro-3-methylphenoxy)-N-(5-chlorothiazol-2-yl)pyrrolidine-1-carboxamide (**31**). General Procedure A was followed using **94** (21 mg, 0.080 mmol) and **104** (17 mg, 0.080 mmol) to obtain **31** as a solid (11 mg, 64%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.25 (d, *J* 8.6 Hz, 1H), 7.15 (s, 1H), 6.75 (d, *J* 3.1 Hz, 1H), 6.64 (dd, *J* 2.97, 8.69 Hz, 1H), 5.00 - 4.93 (m, 1H), 3.86 - 3.59 (m, 4H), 2.35 (s, 4H), 2.30 - 2.09 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.5, 155.1, 151.9, 137.5, 134.0, 129.9, 126.9, 118.2, 114.0, 75.8, 51.6, 44.1, 31.2, 20.3 (1 quaternary C not observed). MS, m/z = 372 (100). $[M+H]^+$, 374 (60). HRMS found: (M + H) 372.0331; $C_{15}H_{15}Cl_2N_3O_2S$ requires (M +H), 372.0335.

3-((4-Chloro-3-methylphenyl)(methyl)amino)-N-(5-methylthiazol-2-yl)pyrrolidine-1-carboxamide (**32**). General Procedure A was followed using **93** (13 mg, 0.053 mmol) and **105** (10 mg, 0.045 mmol) to obtain **32** as an oil (8 mg, 49%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.19 (d, *J* 8.6 Hz, 1H), 6.92 (s, 1H), 6.74 - 6.60 (m, 2H), 4.33 (quin, *J* 7.3 Hz, 1H), 3.76 - 3.63 (m, 2H), 3.54 - 3.32 (m, 2H), 2.79 (s, 3H), 2.33 (d, *J* 4.4 Hz, 6H), 2.22 - 2.08 (m, 2H). MS, m/z = 365 (100) $[M+H]^+$, HRMS found: (M + H) 365.1196; $C_{17}H_{21}ClN_4OS$ requires (M +H), 365.1198.

3-((4-Chloro-3-methylphenyl)(methyl)amino)-N-(5-chlorothiazol-2-yl)pyrrolidine-1-carboxamide (**33**). General Procedure A was followed using **94** (14 mg, 0.053 mmol) and **105** (10 mg, 0.045 mmol) to obtain **33** as an oil (8 mg, 49%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.23 - 7.18 (m, 1H), 7.14 (br s, 1H), 6.73 (d, *J* 2.9 Hz, 1H), 6.65 (dd, *J* 3.1, 8.8 Hz, 1H), 4.33 (quin, *J* 7.4 Hz, 1H), 3.75 - 3.62 (m, 2H), 3.55 - 3.33 (m, 2H), 2.80 (s, 3H), 2.34 (s, 3H), 2.22 - 2.10 (m, 2H). MS, m/z = 212 (100) $[M+H]^+$, 214 (30). HRMS found: (M + H) 385.0648; $C_{16}H_{18}Cl_2N_4OS$ requires (M +H), 385.0651.

4-(3-Chloro-4-methylphenyl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**34**). General Procedure A was followed using **93** (31 mg, 0.14 mmol) and **106** (26 mg, 0.12

mmol) to obtain **34** as a solid (13 mg, 30%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.12 (dd, *J* 8.4, 0.7 Hz, 1H), 6.97 - 6.90 (m, 2H), 6.77 - 6.70 (m, 1H), 3.77 - 3.63 (m, 4H), 3.27 - 3.11 (m, 4H), 2.37 (d, *J* 1.1 Hz, 3H), 2.29 (s, 3H). MS, m/z = 351 (100) $[M+H]^+$, 353 (30). HRMS found: (M + H) 351.1037; $C_{16}H_{19}ClN_4OS$ requires (M +H), 351.1041.

4-(3-Chloro-4-methylphenyl)-N-(5-chlorothiazol-2-yl)piperazine-1-carboxamide (**35**). General Procedure A was followed using **94** (35 mg, 0.14 mmol) and **106** (26 mg, 0.12 mmol) to obtain **35** as a solid (32 mg, 69%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.17 - 7.08 (m, 2H), 6.98 - 6.89 (m, 1H), 6.75 (dd, *J* 8.4, 2.6 Hz, 1H), 3.79 - 3.59 (m, 4H), 3.31 - 3.06 (m, 4H), 2.30 (s, 3H). MS, m/z = 371 (100) $[M+H]^+$, 373 (60). HRMS found: (M + H) 371.0493; $C_{15}H_{16}C_{l2}N_4OS$ requires (M +H), 371.0495.

1-(3-Chloro-4-methylphenyl)-N-(5-methylthiazol-2-yl)piperidine-4-carboxamide (**36**). **108** (33 mg, 0.13 mmol), 5-methyl-2-aminothiazole (18 mg, 0.16 mmol), EDCI (30 mg, 0.16 mmol), and DMAP (1.9 mg, 0.013 mmol) in DCE (5 mL) were stirred at 45 ^{o}C for 16 h. To the reaction was added 10% citric acid solution (10 mL) and the mixture extracted with DCM (2 × 10 mL). The organic layer was then washed with 10% $NaHCO_3$ solution (1 × 15 mL), dried with $MgSO_4$ and concentrated in vacuo. The crude residue was then purified by column chromatography gradient eluting with 100% CyHex to 60% EtOAc/CyHex to obtain **36** as a solid (24 mg, 53%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.04 - 7.17 (m, 2H), 6.96 (d, *J* 2.6 Hz, 1H), 6.80 (dd, *J* 2.6, 8.4 Hz, 1H), 3.73 (td, *J* 3.1, 12.4 Hz, 2H), 2.90 - 2.72 (m, 2H), 2.68 - 2.51 (m, 1H), 2.42 (ad, J 2.0 Hz, 3H), 2.31 (s, 3H), 2.10 - 2.00 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 172.8, 156.2, 150.1, 134.6, 133.7, 131.3, 126.0, 124.9, 115.8, 114.9, 48.2, 41.0, 27.4, 18.5, 11.0. MS, m/z = 350 (100) $[M+H]^+$, 352 (30). HRMS found: (M + H) 350.1090; $C_{17}H_{20}ClN_3OS$ requires (M +H), 350.1089.

1-(3-Chloro-4-methylphenyl)-N-(5-methylthiazol-2-yl)piperidine-4-carboxamide (**37**). The procedure used for **36** was followed using **108** (33 mg, 0.13 mmol) and 5-chlorothiazol-2-amine.HCl (27 mg, 0.16 mmol) to obtain **37** as a solid (29 mg, 60%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 10.45 (s, 1H), 7.12 (d, *J* 8.8 Hz, 1H), 6.96 (s, 1H), 6.85 - 6.76 (m, 1H), 3.78 - 3.64 (m, 2H), 2.88 - 2.72 (m, 2H), 2.63 - 2.55 (m, 1H), 2.30 (s, 3H), 2.12 – 1.98 (m, 4H). MS, m/z = 370 (100) $[M+H]^+$, 372 (60). HRMS found: (M + H) 370.0539; $C_{16}H_{17}Cl_2N_3OS$ requires (M +H), 370.0542.

3-((3-Chloro-4-methylphenyl)amino)-N-(5-methylthiazol-2-yl)azetidine-1-carboxamide (**38**). General Procedure A was followed using **93** (22 mg, 0.094 mmol) and **110** (20 mg, 0.10 mmol) to obtain **38** as a solid (12 mg, 38%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.03 (d, *J* 8.1 Hz, 1H), 6.93 (d, *J* 1.1 Hz, 1H), 6.51 (d, *J* 2.4 Hz, 1H), 6.35 (dd, *J* 2.4, 8.1 Hz, 1H), 4.46 - 4.34 (m, 2H), 4.32 - 4.17 (m, 1H), 3.84 (dd, *J* 4.6, 8.8 Hz, 2H), 2.34 (d, *J* 1.1 Hz, 3H), 2.26 (s, 3H). MS, m/z = 337 (100) $[M+H]^+$, 339 (60). HRMS found: (M + H) 337.0882; $C_{15}H_{17}ClN_4OS$ requires (M +H), 337.0885.

3-((3-Chloro-4-methylphenyl)amino)-N-(5-chlorothiazol-2-yl)azetidine-1-carboxamide (**39**). General Procedure A was followed using **94** (23 mg, 0.094 mmol) and **110** (20 mg, 0.10 mmol) to obtain **39** as a solid (10 mg, 31%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.19 (s, 1H), 7.05 (d, *J* 7.9 Hz, 1H), 6.54 (d, *J* 2.2 Hz, 1H), 6.37 (dd, *J* 2.5, 8.5 Hz, 1H), 4.56 - 4.48 (m, 2H), 4.40 - 4.27 (m, 1H), 3.94 (dd, *J* 5.0, 8.9 Hz, 2H), 2.27 (s, 3H). ^{13}C NMR (75 MHz; $CDCl_3$) δ 158.3, 153.6, 144.5, 135.2, 133.4, 131.6, 126.3, 113.6, 112.0, 56.9, 43.5, 18.9 (1 quaternary C not observed). MS, m/z = 357 (100) $[M+H]^+$, 359 (60). HRMS found: (M + H) 357.0333 $C_{14}H_{14}Cl_2N_4OS$ requires (M +H), 357.0338.

3-((3-Chloro-4-methylphenyl)(methyl)amino)-N-(5-methylthiazol-2-yl)azetidine-1-carboxamide (**40**). General Procedure A was followed using **93** (24 mg, 0.10 mmol) and **111**

(24 mg, 0.11 mmol) to obtain **40** as a solid (12 mg, 33%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.09 (d, *J* 8.4 Hz, 1H), 6.94 (s, 1H), 6.73 (d, *J* 2.6 Hz, 1H), 6.55 (dd, *J* 2.6, 8.4 Hz, 1H), 4.37 - 4.24 (m, 3H), 4.09 – 4.03 (m, 2H), 2.87 (s, 3H), 2.38 - 2.32 (m, 3H), 2.29 (s, 3H). MS, m/z = 351 (100) [M+H]+, 353 (30). HRMS found: (M + H) 351.1038; $C_{16}H_{19}ClN_4OS$ requires (M +H), 351.1041.

3-((3-Chloro-4-methylphenyl)(methyl)amino)-N-(5-chlorothiazol-2-yl)azetidine-1-carboxamide (**41**). General Procedure A was followed using **94** (26 mg, 0.10 mmol) and **111** (24 mg, 0.11 mmol) to obtain **41** as a solid (15 mg, 40%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.17 - 7.08 (m, 2H), 6.75 (d, *J* 2.4 Hz, 1H), 6.6 (dd, *J* 2.6, 8.4 Hz, 1H), 4.44 - 4.25 (m, 3H), 4.14 - 4.02 (m, 2H), 2.88 (s, 3H), 2.30 (s, 3H). MS, m/z = 371 (100) $[M+H]^+$, 373 (60). HRMS found: (M + H) 371.0493; $C_{15}H_{16}Cl_2N_4OS$ requires (M +H), 371.0495.

3-(3-Chloro-4-methylphenoxy)-N-(5-methylthiazol-2-yl)azetidine-1-carboxamide (**42**). General Procedure A was followed using **93** (26 mg, 0.11 mmol) and **113** (22 mg, 0.11 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **42** as a solid (4.9 mg, 13%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.14 (d, *J* 8.4 Hz, 1H), 6.95 (d, *J* 1.3 Hz, 1H), 6.75 (d, *J* 2.6 Hz, 1H), 6.57 (dd, *J* 2.6, 8.4 Hz, 1H), 5.00 – 4.83 (m, 1H), 4.48 - 4.31 (m, 2H), 4.20 – 3.99 (m, 2H), 2.47 – 2.18 (m, 6H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.9, 154.7, 135.0, 133.0, 131.6, 129.4, 126.8, 115.3, 113.2, 65.8, 56.5, 19.1, 11.6 (1 quaternary C not observed). MS, m/z = 338 (100) $[M+H]^+$. HRMS found: (M + H) 338.0725; $C_{15}H_{16}ClN_3O_2S$ requires (M +H), 338.0725.

3-(3-Chloro-4-methylphenoxy)-N-(5-chlorothiazol-2-yl)azetidine-1-carboxamide (**43**). General Procedure A was followed using **94** (28 mg, 0.11 mmol) and **113** (22 mg, 0.11 mmol) to obtain **43** as a solid (4.9 mg, 13%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.19 - 7.10 (m, 2H), 6.76 (d, *J* 2.6 Hz, 1H), 6.57 (dd, *J* 2.6, 8.4 Hz, 1H), 5.03 – 4.90 (m, 1H), 4.52 - 4.37 (m,

2H), 4.14 (dd, *J* 4.4, 9.9 Hz, 2H), 2.31 (s, 3H). MS, m/z = 358 (100) $[M+H]^+$, 360 (60). HRMS found: (M + H) 358.0179; $C_{14}H_{13}Cl_2N_3O_2S$ requires (M +H), 358.0179.

4-(3-Chloro-4-methylphenyl)-N-(5-methylthiazol-2-yl)-1,4-diazepane-1-carboxamide (**44**). General Procedure A was followed using **93** (25 mg, 0.11 mmol) and **115** (24 mg, 0.11 mmol) to obtain **44** as a solid (16 mg, 41%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.05 (dd, *J* 0.7, 8.6 Hz, 1H), 6.95 (s, 1H), 6.70 (d, *J* 2.6 Hz, 1H), 6.52 (dd, *J* 2.6, 8.6 Hz, 1H), 3.78 - 3.67 (m, 2H), 3.67 - 3.51 (m, 4H), 3.41 (t, *J* 6.3 Hz, 2H), 2.36 (d, *J* 1.3 Hz, 3H), 2.25 (s, 3H), 2.14 – 1.99 (m, 2H). MS, m/z = 365 (100) $[M+H]^+$, 367 (30). HRMS found: (M + H) 365.1196; $C_{17}H_{21}ClN_4OS$ requires (M +H), 365.1198.

4-(3-Chloro-4-methylphenyl)-N-(5-chlorothiazol-2-yl)-1,4-diazepane-1-carboxamide (**45**). General Procedure A was followed using **94** (28 mg, 0.11 mmol) and **115** (24 mg, 0.11 mmol) to obtain **45** as a solid (15 mg, 37%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.23 (d, J = 9.0 Hz, 1H), 6.94 (s, 1H), 6.75 (d, J = 2.9 Hz, 1H), 6.53 (dd, J = 2.9, 9.0 Hz, 1H), 3.81 - 3.69 (m, 2H), 3.67 - 3.47 (m, 4H), 3.42 (t, J = 6.2 Hz, 2H), 2.36 (s, 3H), 2.13 – 1.89 (m, 2H). MS, m/z = 385 (100) $[M+H]^+$, 387 (60). HRMS found: (M + H) 385.0654; $C_{16}H_{18}Cl_2N_4OS$ requires (M +H), 385.0651.

4-(3-Chloro-4-methylphenyl)-2-methyl-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**46**). General Procedure A was followed using **93** (25 mg, 0.11 mmol) and **118** (24 mg, 0.11 mmol) to obtain **46** as a solid (15 mg, 39%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.11 (d, *J* 8.4 Hz, 1H), 6.94 (d, *J* 1.1 Hz, 1H), 6.89 (d, *J* 2.4 Hz, 1H), 6.71 (dd, *J* 2.6, 8.4 Hz, 1H), 4.53 – 4.41 (m, 1H), 4.01 (d, *J* 13.2 Hz, 1H), 3.55 - 3.32 (m, 3H), 2.97 (dd, *J* 3.6, 11.9 Hz, 1H), 2.78 (dt, *J* 3.6, 11.6 Hz, 1H), 2.37 (d, *J* 1.1 Hz, 3H), 2.29 (s, 3H), 1.38 (d, *J* 6.8 Hz, 3H). MS, m/z = 365 (100), 367 (30) $[M+H]^+$. HRMS found: (M + H) 365.1196; $C_{17}H_{21}ClN_4OS$ requires (M +H), 365.1198.

4-(3-Chloro-4-methylphenyl)-N-(5-chlorothiazol-2-yl)-2-methylpiperazine-1-carboxamide (**47**). General Procedure A was followed using **94** (25 mg, 0.098 mmol) and **118** (22 mg, 0.098 mmol) to obtain **47** as a solid (14 mg, 37%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.17 (s, 1H), 7.15 - 7.09 (m, 1H), 6.90 (d, *J* 2.6 Hz, 1H), 6.72 (dd, *J* 2.4, 8.4 Hz, 1H), 4.43 - 4.29 (m, 1H), 4.01 – 3.89 (m, 1H), 3.59 - 3.34 (m, 3H), 2.99 (dd, *J* 3.4, 12.2 Hz, 1H), 2.81 (dt, *J* 3.5, 11.7 Hz, 1H), 2.30 (s, 3H), 1.42 (d, *J* 6.6 Hz, 3H). MS, m/z = 385 (100), 387 (60) $[M+H]^+$. HRMS found: (M + H) 385.0647; $C_{16}H_{18}Cl_2N_4OS$ requires (M +H), 385.0651.

4-(3-Chloro-4-methylphenyl)-3-methyl-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**48**). General Procedure A was followed using **93** (14 mg, 0.060 mmol) and **120** (13 mg, 0.060 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **48** as a solid (4 mg, 18%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.12 (d, *J* 8.8 Hz, 1H), 7.00 – 6.86 (m, 2H), 6.73 (dd, *J* 2.5, 8.3 Hz, 1H), 4.08 – 3.96 (m, 1H), 3.86 – 3.73 (m, 2H), 3.64 - 3.52 (m, 1H), 3.48 - 3.35 (m, 1H), 3.25 - 3.07 (m, 2H), 2.38 (s, 3H), 2.30 (s, 3H), 1.04 (d, *J* 6.2 Hz, 3H). MS, m/z = 365 (100), 367 (30) $[M+H]^+$. HRMS found: (M + H) 365.1197; $C_{17}H_{21}ClN_4OS$ requires (M +H), 365.1198.

4-(3-Chloro-4-methylphenyl)-N-(5-chlorothiazol-2-yl)-3-methylpiperazine-1-carboxamide (**49**). General Procedure A was followed using **94** (14 mg, 0.055 mmol) and **120** (12 mg, 0.055 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **49** as a solid (5 mg, 24%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.22 - 7.09 (m, 2H), 6.92 (d, *J* 2.4 Hz, 1H), 6.75 (dd, *J* 2.5, 8.3 Hz, 1H), 4.01 – 3.88 (m, 1H), 3.83 - 3.55 (m, 3H), 3.50 - 3.37 (m, 1H), 3.25 - 3.10 (m, 2H), 2.30 (s, 3H), 1.04 (d, *J* 6.4 Hz, 3H). MS, m/z = 385 (100), 387 (60) $[M+H]^+$. MS, m/z = 385 (100), 387 (60) $[M+H]^+$. HRMS found: (M + H) 385.0653; $C_{16}H_{18}Cl_2N_4OS$ requires (M +H), 385.0651.

4-(3-Chloro-4-methylphenyl)-N-(5-chlorothiazol-2-yl)-3-oxopiperazine-1-carboxamide (**50**). General Procedure A was followed using **94** (9 mg, 0.040 mmol) and **121** (10 mg, 0.040 mmol) to obtain **50** as a solid (3.9 mg, 25%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.32 - 7.28 (m, 2H), 7.24 - 7.16 (m, 1H), 7.10 (dd, *J* 2.4, 8.1 Hz, 1H), 4.39 (s, 2H), 4.01 – 3.95 (m, 2H), 3.87 - 3.78 (m, 2H), 2.38 (s, 3H). MS, m/z = 385 (100), 387 (60) $[M+H]^+$. HRMS found: (M + H) 385.0286; $C_{15}H_{14}Cl_2N_4O_2S$ requires (M +H), 385.0288.

6-(3-Chloro-4-methylphenyl)-N-(5-methylthiazol-2-yl)-2,6-diazaspiro[3.3]heptane-2-carboxamide (**51**). General Procedure A was followed using **93** (12 mg, 0.051 mmol) and **122** (23 mg, 0.051 mmol) to obtain **51** as a solid (12 mg, 65%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.10 - 7.00 (m, 1H), 6.96 (d, *J* 1.3 Hz, 1H), 6.46 (d, *J* 2.4 Hz, 1H), 6.27 (dd, *J* 2.4, 8.1 Hz, 1H), 4.21 (s, 4H), 3.96 (s, 4H), 2.37 (d, *J* 1.32 Hz, 3H), 2.27 (s, 3H). ^{13}C NMR (75 MHz; $CDCl_3$): δ 154.8, 150.2, 134.7, 132.9, 131.2, 126.9, 125.4, 112.4, 110.4, 62.3, 59.6, 33.8, 18.9, 11.6 (1 quaternary C not observed). MS, m/z = 363 (100) [M+H]+, 365 (30). HRMS found: (M + H) 363.1042; $C_{17}H_{19}ClN_4OS$ requires (M +H), 363.1041.

6-(3-Chloro-4-methylphenyl)-N-(5-chlorothiazol-2-yl)-2,6-diazaspiro[3.3]heptane-2-carboxamide (**52**). General Procedure A was followed using **94** (13 mg, 0.051 mmol) and **122** (23 mg, 0.051 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **52** as a solid (2.2 mg, 11%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.17 (s, 1H), 7.06 (d, *J* 8.1 Hz, 1H), 6.47 (d, *J* 2.6 Hz, 1H), 6.29 (dd, *J* 2.5, 8.0 Hz, 1H), 4.38 - 4.30 (m, 4H), 4.07 – 3.94 (m, 4H), 2.27 (s, 3H). MS, m/z = 383 (100) [M+H]+, 385 (60). HRMS found: (M + H) 383.0491; $C_{16}H_{16}Cl_2N_4OS$ requires (M +H), 383.0495.

N-(5-Methylthiazol-2-yl)-4-phenylpiperazine-1-carboxamide (**53**). General Procedure A was followed using **93** (15 mg, 0.059 mmol) and 1-phenylpiperazine (20 µL, 0.13 mmol) to

obtain **53** as a solid (12 mg, 65%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.34 - 7.27 (m, 2H), 6.99 - 6.88 (m, 4H), 3.82 - 3.68 (m, 4H), 3.30 - 3.16 (m, 4H), 2.38 (s, 3H). MS, m/z = 323 (100) $[M+H]^+$. HRMS found: (M + H) 303.1271; $C_{15}H_{18}N_4OS$ requires (M +H), 303.1274.

4-(4-Chlorophenyl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**54**). General Procedure A was followed using **93** (60 mg, 0.258 mmol) and **123** (39 µL, 0.198 mmol) to obtain **54** as a solid (38 mg, 57%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.26 - 7.21 (m, 2H), 7.06 – 6.96 (m, 1H), 6.89 - 6.81 (m, 2H), 3.93 - 3.72 (m, 4H), 3.31 - 3.14 (m, 4H), 2.40 (s, 3H). MS, m/z = 337 (100) $[M+H]^+$, 339 (60). HRMS found: (M + H) 337.0880; $C_{15}H_{17}ClN_4OS$ requires (M +H), 337.0885.

4-(3,4-Dichlorophenyl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**55**). General Procedure A was followed using **93** (33 mg, 0.142 mmol) and **124** (30 mg, 0.130 mmol) to obtain **55** as a solid (36 mg, 75%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.31 (d, *J* 8.8 Hz, 1H), 7.00 – 6.92 (m, 2H), 6.76 (dd, *J* 2.9, 9.0 Hz, 1H), 3.83 - 3.69 (m, 4H), 3.30 - 3.16 (m, 4H), 2.38 (s, 3H). MS, m/z = 371 (100) $[M+H]^+$, 373 (60). HRMS found: (M + H) 371.0491; $C_{15}H_{16}Cl_2N_4OS$ requires (M +H), 371.0495.

4-(3-Cyanophenyl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**56**). General Procedure A was followed using **93** (21 mg, 0.090 mmol) and **125** (17 mg, 0.090 mmol) to obtain **56** as a solid (15 mg, 30%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.42 - 7.30 (m, 1H), 7.20 - 7.06 (m, 3H), 6.92 (d, *J* 1.8 Hz, 1H), 3.79 - 3.69 (m, 4H), 3.34 - 3.22 (m, 4H), 2.36 (d, *J* 1.3 Hz, 3H). MS, m/z = 328 (100) $[M+H]^+$. HRMS found: (M + H) 328.1223; $C_{16}H_{17}N_5OS$ requires (M +H), 328.1227.

4-(4-Chlorophenyl)-N-(5-chlorothiazol-2-yl)piperazine-1-carboxamide (**57**). General Procedure A was followed using **94** (57 mg, 0.224 mmol) and **123** (40 mg, 0.203 mmol) to obtain **57** as a solid (38 mg, 57%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.27 - 7.22 (m, 3H), 6.93 -

6.77 (m, 2H), 3.87 - 3.71 (m, 4H), 3.28 - 3.15 (m, 4H). MS, m/z = 357 (100) $[M+H]^+$, 359 (60). HRMS found: (M + H) 357.0335; $C_{17}H_{20}ClN_3O_2S$ requires (M +H), 357.0338.

4-(4-Chlorophenyl)-N-(5-cyanothiazol-2-yl)piperazine-1-carboxamide (**58**). General Procedure A was followed using **126** (55 mg, 0.224 mmol) and **123** (40 mg, 0.203 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **58** as a solid (7.1 mg, 10%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.29 - 7.20 (m, 3H), 6.90 (d, *J* 8.8 Hz, 2H), 3.84 - 3.65 (m, 4H), 3.34 - 3.15 (m, 4H). MS, m/z = 348 (100) $[M+H]^+$, 350 (30). HRMS found: (M + H) 348.0679; $C_{16}H_{17}Cl_2N_3O_2S$ requires (M +H), 348.0681.

4-(3-Chloro-4-methylphenyl)-N-(thiazol-2-yl)piperazine-1-carboxamide (**59**). General Procedure A was followed using **127** (23 mg, 0.11 mmol) and **106** (24 mg, 0.11 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **59** as a solid (2.0 mg, 5.6%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.36 (d, *J* 4.0 Hz, 1H), 7.13 (d, *J* 9.0 Hz, 1H), 6.93 (d, *J* 2.4 Hz, 2H), 6.75 (dd, *J* 2.6, 8.4 Hz, 1H), 3.88 - 3.68 (m, 4H), 3.32 - 3.15 (m, 4H), 2.30 (s, 3H). MS, m/z = 337 (100) [M+H]+, 339 (30). HRMS found: (M + H) 337.0884; $C_{15}H_{17}ClN_4OS$ requires (M +H), 337.0885.

4-(3-Chloro-4-methylphenyl)-N-(5-cyanothiazol-2-yl)piperazine-1-carboxamide (**60**). General Procedure A was followed using **126** (33 mg, 0.14 mmol) and **106** (26 mg, 0.12 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **60** as a solid (3.0 mg, 7%). ^{1}H NMR (300 MHz, d_6-Acetone): δ 8.06 (s, 1H), 7.19 (d, *J* 8.8 Hz, 1H), 7.02 (d, *J* 2.6 Hz, 1H), 6.91 (dd, *J* 8.5, 2.8 Hz, 1H), 3.88 - 3.78 (m, 4H), 3.33 - 3.22 (m, 4H), 2.26 (s, 3H). MS, m/z = 362

(100) $[M+H]^+$, 364 (30). HRMS found: (M + H) 362.0835; $C_{16}H_{16}ClN_5OS$ requires (M +H), 362.0837.

4-(3-Chloro-4-methylphenyl)-N-(5-(trifluoromethyl)thiazol-2-yl)piperazine-1-carboxamide (**61**). General Procedure A was followed using **128** (25 mg, 0.087 mmol) and **106** (18 mg, 0.087 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **61** as a solid (5.6 mg, 16%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.69 (s, 1H), 7.14 (d, *J* 8.4 Hz, 1H), 6.96 (d, *J* 2.4 Hz, 1H), 6.82 - 6.74 (m, 1H), 3.81 - 3.74 (m, 4H), 3.26 - 3.19 (m, 4H), 2.31 (s, 3H). MS, m/z = 405 (100) $[M+H]^+$. HRMS found: (M + H) 405.0759; $C_{16}H_{16}ClF_3N_4OS$ requires (M +H), 405.0758.

N-(5-Chlorothiazol-2-yl)-4-(3,4-dichlorophenyl)piperazine-1-carboxamide (**62**). General Procedure A was followed using **94** (36 mg, 0.143 mmol) and **124** (30 mg, 0.130 mmol) to obtain **62** as a solid (41 mg, 80%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.33 (d, *J* 9.0 Hz, 1H), 7.19 (s, 1H), 6.99 (d, *J* 2.9 Hz, 1H), 6.77 (dd, *J* 8.9, 3.0 Hz, 1H), 3.76 - 3.68 (m, 4H), 3.30 - 3.20 (m, 4H). ^{13}C NMR (75 MHz, $CDCl_3$) d 160.4, 152.5, 149.9, 133.1, 131.0, 130.7, 123.5, 120.6, 118.0, 115.9, 48.7, 43.8. MS, m/z = 391 (100) $[M+H]^+$, 393 (100). HRMS found: (M + H) 390.9947; $C_{14}H_{13}Cl_3N_4OS$ requires (M +H), 390.9949.

N-(5-Cyanothiazol-2-yl)-4-(3,4-dichlorophenyl)piperazine-1-carboxamide (**63**). General Procedure A was followed using **126** (35 mg, 0.143 mmol) and **124** (30 mg, 0.130 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **63** as a solid (3.6 mg, 7%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.94 (s, 1H), 7.37 - 7.30 (m, 1H), 7.00 (d, *J* 2.86 Hz, 1H), 6.78 (dd, *J* 8.9, 2.8 Hz, 1H), 3.79 - 3.70 (m, 4H), 3.32- 3.23 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d6) δ 165.6, 153.8, 150.4, 149.4, 131.5, 130.5, 120.0, 116.6, 115.6, 113.8, 96.1, 47.3, 43.2. MS, m/z = 382 (100)

$[M+H]^+$, 384 (60). HRMS found: (M + H) 382.0288; $C_{15}H_{13}C_{l2}N_5OS$ requires (M +H), 382.0291.

4-(4-Chloro-3-methylphenyl)-N-(5-cyanothiazol-2-yl)piperazine-1-carboxamide (**64**). General Procedure A was followed using **126** (38 mg, 0.16 mmol) and **92** (30 mg, 0.14 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **64** as a solid (3.2 mg, 6%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.93 (br. s., 1H), 7.52 - 7.41 (m, 1H), 6.95- 6.88 (m, 1H), 6.82 (d, *J* 8.6 Hz, 1H), 3.88 - 3.72 (m, 4H), 3.35 - 3.20 (m, 4H), 2.38 (s, 3H). MS, m/z = 362 (100) $[M+H]^+$, 364 (30). HRMS found: (M + H) 362.0832; $C_{16}H_{16}ClN_5OS$ requires (M +H), 362.0837.

N-(5-Chlorothiazol-2-yl)-4-(3-cyanophenyl)piperazine-1-carboxamide (**65**). General Procedure A was followed using **94** (23 mg, 0.090 mmol) and **125** (17 mg, 0.090 mmol) to obtain **65** as a solid (25 mg, 81%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.43 - 7.28 (m, 1H), 7.21 - 7.06 (m, 4H), 3.78 - 3.63 (m, 4H), 3.38 - 3.22 (m, 4H). MS, m/z = 348 (100) [M+H]+, 350 (30). HRMS found: (M + H) 348.0680; $C_{15}H_{14}ClN_5OS$ requires (M +H), 348.0681.

4-(5-Cyano-4-methylpyridin-2-yl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**66**). General Procedure A was followed using **93** (30 mg, 0.13 mmol) and **129** (31 mg, 0.15 mmol) to obtain **66** as a solid (25 mg, 77%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.36 (s, 1H), 6.95 (s, 1H), 6.47 (s, 1H), 3.85 - 3.69 (m, 8H), 2.44 (s, 3H), 2.38 (s, 3H). MS, m/z = 343 (100) $[M+H]^+$. HRMS found: (M + H) 343.1334; $C_{16}H_{18}N_6OS$ requires (M +H), 343.1336.

N-(5-Chlorothiazol-2-yl)-4-(5-cyano-4-methylpyridin-2-yl)piperazine-1-carboxamide (**67**). General Procedure A was followed using **94** (30 mg, 0.12 mmol) and **129** (29 mg, 0.14 mmol) to obtain **67** as a solid (36 mg, 84%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.36 (s, 1H), 6.95 (s, 1H), 6.47 (s, 1H), 3.85 - 3.76 (m, 4H), 3.76 – 3.62 (m, 4H), 2.45 (s, 3H). ^{13}C NMR

(75 MHz, DMSO-d6) δ 158.9, 153.7, 152.2, 150.0, 134.3, 121.6, 117.6, 116.1, 106.4, 96.8, 43.3, 42.7, 19.6,. MS, m/z = 363 (100) $[M+H]^+$, 365 (30). HRMS found: (M + H) 363.0788; $C_{15}H_{15}ClN_6OS$ requires (M +H), 363.0790.

4-(6-Cyano-5-methylpyridin-3-yl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**68**). General Procedure A was followed using **93** (25 mg, 0.10 mmol) and **130** (24 mg, 0.12 mmol) to obtain **68** as a solid (30 mg, 82%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.15 - 8.20 (m, 1H), 6.92 - 7.04 (m, 2H), 3.80 - 3.94 (m, 4H), 3.52 - 3.42 (m, 4H), 2.50 (s, 3H), 2.41 (s, 3H). MS, m/z = 343 (100) $[M+H]^+$. HRMS found: (M + H) 343.1336; $C_{16}H_{18}N_6OS$ requires (M +H), 343.1336.

N-(5-Chlorothiazol-2-yl)-4-(6-cyano-5-methylpyridin-3-yl)piperazine-1-carboxamide (**69**). General Procedure A was followed using **94** (25 mg, 0.098 mmol) and **130** (22 mg, 0.11 mmol) to obtain **69** as a solid (25 mg, 70%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.29 - 8.26 (m, 1H), 7.39 (s, 1H), 7.34 - 7.30 (m, 1H), 3.68 - 3.60 (m, 6H), 3.50 - 3.45 (m, 7H), 2.39 (s, 3H). MS, m/z = 363 (100) $[M+H]^+$, 365 (30). HRMS found: (M + H) 363.0790; $C_{15}H_{15}ClN_6OS$ requires (M +H), 363.0790.

4-(5-Chloro-4-(trifluoromethyl)pyridin-2-yl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**70**). General Procedure A was followed using **93** (30 mg, 0.13 mmol) and **131** (34 mg, 0.13 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **70** as a solid (13 mg, 25%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.26 (s, 1H), 6.94 (s, 1H), 6.86 (s, 1H), 3.77 - 3.65 (m, 8H), 2.37 (d, *J* 1.3 Hz, 3H). MS, m/z = 406 (100) [M+H]+, 408 (30). HRMS found: (M + H) 406.0711; $C_{15}H_{15}ClF_3N_5OS$ requires (M +H), 406.0711.

4-(5-Chloro-4-(trifluoromethyl)pyridin-2-yl)-N-(5-chlorothiazol-2-yl)piperazine-1-carboxamide (**71**). General Procedure A was followed using **94** (33 mg, 0.13 mmol) and **131**

(34 mg, 0.13 mmol) to obtain **71** as a solid (30 mg, 55%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.27 (s, 1H), 7.19 (s, 1H), 6.87 (s, 1H), 3.75 – 3.71 (m, 8H). MS, m/z = 426 (100) [M+H]+, 428 (60). HRMS found: (M + H) 426.0161; $C_{14}H_{12}Cl_2F_3N_5OS$ requires (M +H), 426.0165.

4-(5,6-Dichloropyridin-3-yl)-N-(5-methylthiazol-2-yl)piperazine-1-carboxamide (**72**). General Procedure A was followed using **93** (38 mg, 0.16 mmol) and **132** (38 mg, 0.16 mmol) to obtain **72** as a solid (13 mg, 20%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.98 (d, *J* 2.9 Hz, 1H), 7.30 (d, *J* 2.9 Hz, 1H), 6.93 (d, *J* 1.3 Hz, 1H), 3.81 - 3.72 (m, 4H), 3.33 - 3.23 (m, 4H), 2.38 (d, *J* 1.1 Hz, 3H). MS, m/z = 372 (100) $[M+H]^+$, 374 (60). HRMS found: (M + H) 372.0447; $C_{14}H_{15}Cl_2N_5OS$ requires (M +H), 372.0447.

N-(5-Cyanothiazol-2-yl)-4-(5,6-dichloropyridin-3-yl)piperazine-1-carboxamide (**73**). General Procedure A was followed using **126** (20 mg, 0.082 mmol) and **132** (21 mg, 0.090 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **73** as a solid (9.1 mg, 29%). ^{1}H NMR (300 MHz, d_6-Acetone): δ 8.13 (d, *J* 2.9 Hz, 1H), 8.07 (s, 1H), 7.63 (d, *J* 2.9 Hz, 1H), 3.93 - 3.80 (m, 4H), 3.54 - 3.40 (m, 4H). MS, m/z = 383 (100) $[M+H]^+$, 385 (60). HRMS found: (M + H) 383.0242; $C_{14}H_{12}Cl_2N_6OS$ requires (M +H), 383.0243.

N-(5-Chlorothiazol-2-yl)-4-(5,6-dichloropyridin-3-yl)piperazine-1-carboxamide (**74**). General Procedure A was followed using **94** (42 mg, 0.16 mmol) and **132** (38 mg, 0.16 mmol) to obtain **74** as a solid (30 mg, 47%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.97 (d, *J* 2.6 Hz, 1H), 7.30 (d, *J* 2.8 Hz, 1H), 7.22 - 7.17 (m, 1H), 3.83 - 3.67 (m, 4H), 3.37 - 3.24 (m, 4H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 159.6, 154.1, 146.8, 135.6, 135.1, 134.2, 128.9, 124.6, 116.7, 46.8, 42.9. MS, m/z = 392 (100) [M+H]+, 394 (100). HRMS found: (M + H) 391.9896; $C_{13}H_{12}Cl_3N_5OS$ requires (M +H), 391.9901.

4-(5,6-Dichloropyridin-3-yl)-N-(5-(trifluoromethyl)thiazol-2-yl)piperazine-1-carboxamide (**75**). General Procedure A was followed using **128** (25 mg, 0.087 mmol) and **132** (20 mg, 0.087 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **75** as a solid (9.6 mg, 26%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.07 – 7.93 (m, 1H), 7.76 – 7.62 (m, 1H), 7.33 - 7.28 (m, 1H), 3.98 - 3.73 (m, 4H), 3.44 - 3.29 (m, 4H). MS, m/z = 426 (100) $[M+H]^+$. HRMS found: (M + H) 426.0162; $C_{14}H_{12}Cl_2F_3N_5OS$ requires (M +H), 426.0165.

N-(5-Methylthiazol-2-yl)-4-(pyrimidin-5-yl)piperazine-1-carboxamide (**76**). General Procedure A was followed using **93** (36 mg, 0.15 mmol) and **133** (25 mg, 0.15 mmol) to obtain **76** as a solid (31 mg, 67%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.76 (s, 1H), 8.43 - 8.35 (m, 2H), 6.91 (d, *J* 1.1 Hz, 1H), 3.84 - 3.71 (m, 4H), 3.37 - 3.23 (m, 4H), 2.37 (d, *J* 1.3 Hz, 3H). MS, m/z = 305 (100) $[M+H]^+$. HRMS found: (M + H) 305.1177; $C_{13}H_{16}N_6OS$ requires (M +H), 305.1179.

N-(5-Chlorothiazol-2-yl)-4-(pyrimidin-5-yl)piperazine-1-carboxamide (**77**). General Procedure A was followed using **94** (38 mg, 0.15 mmol) and **133** (25 mg, 0.15 mmol) to obtain **77** as a solid (32 mg, 65%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.79 (s, 1H), 8.42 (s, 2H), 7.19 (s, 1H), 3.85 - 3.65 (m, 4H), 3.44 - 3.18 (m, 4H). MS, m/z = 325 (100) $[M+H]^+$, 327 (30). HRMS found: (M + H) 325.0632; $C_{12}H_{13}ClN_6OS$ requires (M +H), 325.0633.

General Procedure B: 2-(3-(3-Chloro-4-methylphenoxy)propyl)-6-methyl-3H-imidazo[4,5-b]pyridine (**78**). **135** (245 mg, 1.07 mmol) and **136** (120 mg, 0.97 mmol) were dissolved in $POCl_3$ (5 mL) and stirred at reflux for 16 h. The reaction was then cooled to 0 °C and quenched with saturated $NaHCO_3$ solution. The crude product was extracted with EtOAc (3 × 15 mL). The organic layers were combined and washed with water (2 × 20 mL) and brine (2 × 20 mL), then dried with anhydrous Na_2SO_4. The organic layer was then concentrated to a

volume of 5 mL, after which a precipitate formed. The precipitate was then filtered, washed with water and dried *in vacuo* to obtain **78** as a solid (103 mg, 33%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.16 (d, *J* 2.0 Hz, 1H), 7.76 (s, 1H), 7.23 (d, *J* 8.4 Hz, 1H), 6.95 (d, *J* 2.4 Hz, 1H), 6.80 (dd, *J* 8.4, 2.4 Hz, 1H), 4.06 (t, *J* 6.2 Hz, 2H), 3.06 - 2.95 (m, 2H), 2.41 (s, 3H), 2.30 – 2.16 (m, 5H). MS, m/z = 316 (100) $[M+H]^+$, 318 (30). HRMS found: (M + H) 316.1215l; $C_{17}H_{18}ClN_3O$ requires (M +H), 316.1211.

6-Chloro-2-(3-(3-chloro-4-methylphenoxy)propyl)-3H-imidazo[4,5-b]pyridine (**79**). General Procedure B was followed using **135** (88 mg, 0.38 mmol) and 5-chloropyridine-2,3-diamine (50 mg, 0.35 mmol). The crude residue was then purified by reverse phase preparatory HPLC using a gradient of 95% water/ACN to 100% ACN to obtain **79** as a solid (6.5 mg, 6%). ^{1}H NMR (300 MHz, d_6-Acetone): δ 8.23 (d, *J* 2.2 Hz, 1H), 7.92 (d, *J* 2.2 Hz, 1H), 7.19 (d, *J* 8.6 Hz, 1H), 6.94 (d, *J* 2.6 Hz, 1H), 6.82 (s, 1H), 4.14 (t, *J* 6.1 Hz, 2H), 3.14 (t, *J* 7.4 Hz, 2H), 2.42 - 2.28 (m, 2H), 2.25 (s, 3H). MS, m/z = 336 (100) $[M+H]^+$, 338 (60). HRMS found: (M + H) 336.0662; $C_{16}H_{15}Cl_2N_3O$ requires (M +H), 336.0665.

2-(3-(3,4-Dichlorophenoxy)propyl)-6-methyl-3H-imidazo[4,5-b]pyridine (**80**). General Procedure B was followed using **138** (101 mg, 0.41 mmol) and **136** (50 mg, 0.41 mmol) to obtain **80** as a solid (32 mg, 24%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.09 (br. s., 1H), 7.67 (br. s., 1H), 7.50 (d, *J* 8.8 Hz, 1H), 7.22 (d, *J* 2.9 Hz, 1H), 6.95 (dd, *J* 2.9, 9.0 Hz, 1H), 4.10 (t, *J* 6.1 Hz, 2H), 2.97 (t, *J* 7.3 Hz, 2H), 2.38 (s, 3H), 2.10 - 2.30 (m, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 157.9, 156.0, 143.1, 131.4, 130.7, 125.9, 122.2, 116.3, 115.3, 67.5, 26.3, 25.1, 17.9 (three quaternary C not observed due to tautomerism of imidazopyridine). MS, m/z = 336 (100) [M+H]+, 338 (60). HRMS found: (M + H) 336.0668; $C_{16}H_{15}Cl_2N_3O$ requires (M +H), 336.0665.

6-Chloro-2-(3-(3,4-dichlorophenoxy)propyl)-3H-imidazo[4,5-b]pyridine (**81**). General Procedure B was followed using **138** (130 mg, 0.52 mmol) and 5-chloropyridine-2,3-diamine (75 mg, 0.52 mmol) to obtain **81** as a solid (32 mg, 24%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (d, *J* 2.4 Hz, 1H), 8.03 (d, *J* 2.2 Hz, 1H), 7.50 (d, *J* 9.0 Hz, 1H), 7.20 (d, *J* 2.9 Hz, 1H), 6.94 (dd, *J* 2.9, 9.0 Hz, 1H), 5.75 (s, 1H), 4.11 (t, *J* 6.4 Hz, 2H), 3.00 (t, *J* 7.4 Hz, 3H), 2.23 (t, *J* 6.9 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 158.3, 157.8, 140.8, 131.4, 130.6, 123.8, 122.2, 116.3, 115.3, 67.5, 26.2, 25.2 (three quaternary C not observed due to tautomerism of imidazopyridine). MS, m/z = 356 (100) [M+H]+, 358 (100). HRMS found: (M + H) 356.0117; $C_{15}H_{12}Cl_3N_3O$ requires (M +H), 356.0119.

2-(3-((5,6-Dichloropyridin-3-yl)oxy)propyl)-6-methyl-3H-imidazo[4,5-b]pyridine (**82**). General Procedure B was followed using **140** (51 mg, 0.20 mmol) and **136** (25 mg, 0.20 mmol) to obtain **82** as a solid (18 mg, 26%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.01 - 8.18 (m, 2H), 7.90 - 7.80 (m, 1H), 7.72 (s, 1H), 4.28 - 4.14 (m, 2H), 3.04 – 2.91 (m, 2H), 2.39 (d, *J* 2.9 Hz, 3H), 2.25 (quin, *J* 6.9 Hz, 2H). ^{13}C NMR (75 MHz, DMSO-d_6) δ 156.1, 154.8, 143.3, 138.3, 136.1, 129.1, 126.2, 124.9, 68.4, 26.3, 25.2, 18.1 (three quaternary C not observed due to tautomerism of imidazopyridine). MS, m/z = 337 (100) [M+H]+, 339 (60).

6-Chloro-2-(3-((5,6-dichloropyridin-3-yl)oxy)propyl)-3H-imidazo[4,5-b]pyridine (**83**). General Procedure B was followed using **140** (52 mg, 0.21 mmol) and 5-chloropyridine-2,3-diamine (30 mg, 0.21 mmol) to obtain **83** as a solid (20 mg, 27%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.26 (s, 1H), 8.10 (d, *J* 2.6 Hz, 1H), 8.03 (br. s., 1H), 7.82 (d, *J* 2.6 Hz, 1H), 4.20 (t, *J* 6.2 Hz, 2H), 3.01 (t, *J* 7.5 Hz, 2H), 2.34 - 2.17 (m, 2H). MS, m/z = 357 (100) [M+H]+, 359 (100). HRMS found: (M + H) 357.0067; $C_{14}H_{11}C_{l3}N_4O$ requires (M +H), 357.0071.

2-(3-(3-Chloro-4-methylphenoxy)propyl)-6-methylthiazolo[5,4-b]pyridine (**84**). Phosphorus pentasulfide (87 mg, 0.20 mmol) was added to a stirred solution of **141** (21 mg, 0.065 mmol) and sodium carbonate (21 mg, 0.20 mmol) in dry THF (3 mL) and stirred for 72 h. 2M NaOH (1 mL) was then added and the reaction stirred for 2 h. The reaction mixture was then diluted with EtOAc (15 mL) and washed with water (10 mL) and brine (10 mL). The organic layer was then dried with anhydrous Na_2SO_4 and concentrated in vacuo. The crude residue was then purified by column chromatography gradient eluting with 100% CyHex to 20% EtOAc/CyHex to obtain **84** as a solid (12.3 mg, 44%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.41 (d, *J* 2.0 Hz, 1H), 8.02 (d, *J* 1.1 Hz, 1H), 7.10 (d, *J* 8.4 Hz, 1H), 6.91 (d, *J* 2.6 Hz, 1H), 6.71 (dd, *J* 2.6, 8.4 Hz, 1H), 4.06 (t, *J* 6.1 Hz, 2H), 3.32 (t, *J* 7.5 Hz, 2H), 2.51 (s, 3H), 2.46 - 2.32 (m, 2H), 2.30 (s, 3H). MS, m/z = 333 (100) $[M+H]_+$, 335 (30). HRMS found: (M + H) 333.0822; $C_{17}H_{17}ClN_2OS$ requires (M +H), 333.0823.

6-Chloro-2-(3-(3-chloro-4-methylphenoxy)propyl)thiazolo[4,5-b]pyridine (**85**). **144** (41 mg, 0.13 mmol) and **135** (37 mg, 0.16 mmol) were dissolved in $POCl_3$ (2 mL) and stirred at reflux for 16 h. The reaction was then cooled to 0 °C and basified with 2M NaOH until the solution was basic (pH 8). The crude product was extracted into EtOAc (2 × 10 mL). The organic layers were combined and washed with water (2 × 20 mL) and brine (2 × 20 mL), then dried with anhydrous Na_2SO_4 and concentrated. The crude residue was then purified by column chromatography gradient eluting with 100% CyHex to 20% EtOAc/CyHex to obtain **85** as a solid (5.4 mg, 12%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.64 (br s, 1H), 8.19 (d, *J* 2.0 Hz, 1H), 7.11 (d, *J* 8.6 Hz, 1H), 6.90 (d, *J* 2.6 Hz, 1H), 6.71 (dd, *J* 2.5, 8.3 Hz, 1H), 4.08 (t, *J* 5.8 Hz, 2H), 3.37 (t, *J* 7.37 Hz, 2H), 2.53 - 2.36 (m, 2H), 2.30 (s, 3H). MS, m/z = 353 (100) [M+H]+, 355 (60). HRMS found: (M + H) 353.0273; $C_{16}H_{14}Cl_2N_2OS$ requires (M +H), 353.0277.

General Procedure C: 2-(4-(3-Chloro-4-methylphenyl)piperazin-1-yl)-6-methyl-3H-imidazo[4,5-b]pyridine (**86**). **146** (20 mg, 0.12 mmol), **106** (25 mg, 0.12 mmol) and *N,N*-

diisopropylethylamine (35 µL, 0.20 mmol) were dissolved in DMSO (1 mL) and stirred in a pressure tube for 5 days at 80 °C. The reaction was then concentrated and lyophilized. The crude residue was then purified by column chromatography (100% CyHex to 100% EtOAc) to obtain **86** as a solid (9.0 mg, 22%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.79 (dd, *J* 0.7, 1.8 Hz, 1H), 7.54 (dd, *J* 0.7, 1.8 Hz, 1H), 7.19 (d, *J* 1.3 Hz, 1H), 7.15 - 7.08 (m, 1H), 6.97 (d, *J* 2.6 Hz, 1H), 6.79 (dd, *J* 2.5, 8.5 Hz, 1H), 3.99 - 3.81 (m, 4H), 3.39 - 3.21 (m, 4H), 2.42 (s, 3H), 2.32 - 2.27 (m, 3H). MS, m/z = 342 (100) [M+H]+, 343 (100). HRMS found: (M + H) 342.1478; $C_{18}H_{20}ClN_5$ requires (M +H), 342.1480.

6-Bromo-2-(4-(3-chloro-4-methylphenyl)piperazin-1-yl)-3H-imidazo[4,5-b]pyridine (**87**). General Procedure C was followed using **148** (32 mg. 0.14 mmol) and **106** (25 mg, 0.12 mmol) to obtain **87** (2.1 mg, 4%) as a solid. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.11 - 8.06 (m, 1H), 7.94 - 7.89 (m, 1H), 7.16 - 7.10 (m, 1H), 6.94 - 6.85 (m, 1H), 6.78 - 6.70 (m, 1H), 4.20 - 4.08 (m, 4H), 3.38 - 3.27 (m, 4H), 2.30 (s, 3H). MS, m/z = 406 (100) $[M+H]^+$. HRMS found: (M + H) 406.0430; $C_{17}H_{17}BrClN_5$ requires (M +H), 406.0429.

2-(4-(3,4-Dichlorophenyl)piperazin-1-yl)-6-methyl-3H-imidazo[4,5-b]pyridine (**88**). General Procedure C was followed using **146** (25 mg. 0.15 mmol) and **124** (52 mg, 0.22 mmol) to obtain **88** (2.0 mg, 4%) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.79 (s, 1H), 7.42 (d, *J* 9.0 Hz, 1H), 7.33 (s, 1H), 7.21 (d, *J* 2.9 Hz, 1H), 7.01 (dd, *J* 2.9, 9.0 Hz, 1H), 3.71 – 3.62 (m, 4H), 3.39 - 3.23 (m, 4H), 2.30 (s, 3H). MS, m/z = 362 (100) $[M+H]^+$, 364 (60). HRMS found: (M + H) 362.0937; $C_{17}H_{17}Cl_2N_5$ requires (M +H), 362.0934.

6-Chloro-2-(4-(3,4-dichlorophenyl)piperazin-1-yl)-3H-imidazo[4,5-b]pyridine (**89**). General Procedure C was followed using **150** (18 mg. 0.096 mmol) and **124** (33 mg, 0.14 mmol) to obtain **89** (2.0 mg, 5.5%) as a solid. ^{1}H NMR (300 MHz, d_6-Acetone): δ 8.08 – 8.02 (m, 0.5H), 7.88 – 7.84 (m, 0.5H), 7.52 – 7.47 (m, 1H), 7.40 (d, *J* 9.0 Hz, 1H), 7.20 (d, *J* 2.9 Hz,

1H), 7.09 - 7.00 (m, 1H), 3.89 – 3.82 (m., 4H), 3.38 - 3.49 (m, 4H). MS, m/z = 382 (100) [M+H]+, 384 (90). HRMS found: (M + H) 382.0388; $C_{16}H_{14}Cl_3N_5$ requires (M +H), 382.0388.

2-(4-(5,6-Dichloropyridin-3-yl)piperazin-1-yl)-6-methyl-3H-imidazo[4,5-b]pyridine (**90**). General Procedure C was followed using **146** (25 mg. 0.15 mmol) and **132** (52 mg, 0.22 mmol) to obtain **90** (5.2 mg, 10%) as a solid. ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.14 (d, *J* 2.9 Hz, 1H), 7.92 - 7.64 (m, 2H), 7.32 (br. s., 1H), 3.73 - 3.62 (m, 4H), 3.46 – 3.34 (m, 4H), 2.29 (s, 3H). MS, m/z = 363 (100) [M+H]+, 365 (60). HRMS found: (M + H) 363.0883; $C_{16}H_{16}Cl_2N_6$ requires (M +H), 363.0886.

6-Chloro-2-(4-(5,6-dichloropyridin-3-yl)piperazin-1-yl)-3H-imidazo[4,5-b]pyridine (**91**). General Procedure C was followed using **150** (18 mg. 0.096 mmol) and **132** (33 mg, 0.14 mmol) to obtain **91** (10 mg, 27%) as a solid. ^{1}H NMR (300 MHz, d_6-Acetone): δ 8.16 (br. s., 1H), 7.89 (br s, 1H), 7.66 (s, 1H), 7.50 (s, 1H), 3.94 – 3.82 (m, 4H), 3.54 (d, *J* 4.2 Hz, 4H). MS, m/z = 383 (100) [M+H]+, 385 (90). HRMS found: (M + H) 383.0341; $C_{15}H_{13}Cl_3N_6$ requires (M +H), 383.0340.

General Procedure D: 1-(4-Chloro-3-methylphenyl)piperazine (**92**). Nitrogen was purged through a stirred solution of 4-bromo-1-chloro-2-methylbenzene (258 µL, 1.95 mmol) in 1,4-dioxane (5 mL) for 30 min. rac-BINAP (363 mg, 1.95 mmol), caesium carbonate (1.27 g, 3.89 mmol), 1-Boc-piperazine (363 mg, 1.95 mmol) and $Pd(OAc)_2$ (87 mg, 0.039 µmol) and stirred at reflux for 14 h under N_2. The reaction was filtered through celite and concentrated *in vacuo*. The residue was dissolved in EtOAc (20 mL), filtered through celite and washed with additional EtOAc (50 mL). The organic layer was washed with water (2 × 20 mL) and brine (2 × 20 mL), dried with Na_2SO_4 and concentrated *in vacuo*. The crude residue was purified by column chromatography (100% CyHex to 10% EtOAc/CyHex) to obtain the

protected intermediate as a solid (413 mg, 68%). MS, m/z = 255 (100) [M-*t*Bu]. The intermediate was dissolved in a 1:3 mixture of TFA/DCM (4 mL) and stirred at 20 °C for 1 h. The solvent was evaporated in vacuo and the crude residue dissolved in EtOAc (10 mL) which was successively washed with a 10% solution of $NaHCO_3$ (10 mL), water (10 mL) and brine (10 mL). The organic layer dried with Na_2SO_4 and concentrated *in vacuo* to obtain **92** as a solid (211 mg, 76%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.23 (d, *J* 8.8 Hz, 1H), 6.80 (d, *J* 2.9 Hz, 1H), 6.71 (dd, *J* 3.0, 8.7 Hz, 1H), 3.35 - 3.19 (m, 8H), 2.35 (s, 3H). MS, m/z = 211 (100) $[M+H]^+$, 213 (30).

Phenyl (5-methylthiazol-2-yl)carbamate (**93**). 5-Methyl-2-aminothiazole (1.5 g, 13 mmol) was dissolved in pyridine (8 mL) and cooled to 0 °C under a N_2 atmosphere. Phenyl chloroformate (3.6 mL, 29 mmol) was then added dropwise and reaction stirred for 5 h at this temperature. The reaction was then quenched with water (10 mL) and the resulting precipitate filtered off. The crude solid was then purified by column chromatography gradient eluting with 100% DCM to obtain **93** as a solid (590 mg, 19%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.48 - 7.36 (m, 2H), 7.31 - 7.26 (m, 2H), 7.24 - 7.22 (m, 1H), 7.10 (d, *J* 1.3 Hz, 1H), 2.37 (d, *J* 1.1 Hz, 3H). MS, m/z = 235 $[M+H]^+$.

Phenyl (5-chlorothiazol-2-yl)carbamate (**94**). The procedure used for **93** was followed using 2-amino-5-chlorothiazole hydrochloride (700 mg, 4.09 mmol) and phenyl chloroformate (1.13 mL, 9.00 mmol) to obtain **94** as a solid (518 mg, 50%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.49 - 7.41 (m, 2H), 7.35 - 7.28 (m, 2H), 7.26 - 7.22 (m, 2H). MS, m/z = 255 (100) $[M+H]^+$, 257 (60).

4-(4-Chloro-3-methylphenoxy)piperidine (**95**). Triphenylphosphine (2.2 g, 8.4 mmol) was added to a stirred solution of DIAD (1.6 mL, 8.4 mmol) in THF at 0 °C for 15 min. 4-Chloro-3-methylphenol (600 mg, 4.2 mmol) was then added and the reaction warmed to 20 °C and

then stirred for 10 min. *N*-Boc-4-hydroxypiperidine (931 mg, 4.6 mmol) was then added in one portion and the solution was stirred for 16 h. The reaction was then concentrated *in vacuo* and dissolved in EtOAc (50 mL). The organic layers were then washed with saturated $NaHCO_3$ solution (2 × 30 mL), water (30 mL) and brine (30 mL), and then dried with Na_2SO_4, and concentrated. The crude solid was then purified by column chromatography gradient eluting with 100% CyHex to 10% EtOAc/CyHex to obtain the crude Boc-protected product as a clear oil (675 mg, 49%). The Boc-protected product was then dissolved in a mixture of 3:1 mixture of DCM/TFA (8 mL) and stirred for 1 h. The reaction was then concentrated in vacuo and dissolved in 1M HCl (30 mL) and washed with EtOAc (2 × 20 mL). The aqueous layer was basified (pH 9) with 2 M NaOH and washed with EtOAc (2 × 20 mL). The organic layers were then combined, washed successively with saturated $NaHCO_3$ (30 mL), water (30 mL) and brine (30 mL). The organic layer was then dried with Na_2SO_4 and concentrated to obtain **95** as an oil (80 mg, 9%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.25 - 7.18 (m, 1H), 6.79 (d, *J* 3.08 Hz, 1H), 6.69 (dd, *J* 2.97, 8.91 Hz, 1H), 4.25 - 4.45 (m, 1H), 3.27 - 3.05 (m, 2H), 2.63 - 2.86 (m, 2H), 2.34 (s, 2H), 2.15 - 2.03 (m, 1H), 1.98 (d, *J* 1.98 Hz, 2H), 1.84 - 1.57 (m, 2H). MS, m/z = 225 (100) $[M+H]^+$, 227 (30)

tert-Butyl 4-((4-chloro-3-methylphenyl)amino)piperidine-1-carboxylate (**96**). General Procedure D was followed using 4-bromo-1-chloro-2-methylbenzene (258 µL, 1.95 mmol) and *tert*-butyl 4-aminopiperidine-1-carboxylate (322 mg, 1.61 mmol) to obtain **96** as a solid (100 mg, 85%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.11 (d, *J* 8.6 Hz, 1H), 6.47 (d, *J* 2.9 Hz, 1H), 6.38 (dd, *J* 2.6, 8.4 Hz, 1H), 4.05 (d, *J* 12.5 Hz, 2H), 3.55 - 3.27 (m, 2H), 2.92 (t, *J* 11.2 Hz, 2H), 2.29 (s, 3H), 2.02 (d, *J* 10.6 Hz, 2H), 1.47 (s, 9H), 1.37 - 1.24 (m, 2H). MS, m/z = 269 [M-^{t}Bu].

N-(4-Chloro-3-methylphenyl)piperidin-4-amine (**97**). The deprotection step in General Procedure D was followed using **96** (25 mg, 0.074 mmol) to obtain **97** as an oil (13 mg,

74%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.09 (d, *J* 8.6 Hz, 1H), 6.46 (d, *J* 2.9 Hz, 1H), 6.37 (dd, *J* 2.9, 8.6 Hz, 1H), 3.38 - 3.24 (m, 1H), 3.11 (td, *J* 3.3, 12.7 Hz, 2H), 2.78 - 2.64 (m, 2H), 2.29 (s, 3H), 2.10 - 2.00 (m, 2H), 1.90 (br. s., 1H), 1.40 - 1.19 (m, 2H). MS, m/z = 225 $[M+H]^+$.

N-(4-Chloro-3-methylphenyl)-N-methylpiperidin-4-amine (**98**). Iodomethane (51 µL, 0.81 mmol) was added to a stirred solution of **96** (44 mg, 0.14 mmol) and potassium carbonate (56 mg, 0.41 mmol) in ACN (3 mL) and stirred at reflux 48 h. The reaction was then concentrated and dissolved in EtOAc (20 mL). The organic solution was washed with water (2 × 20 mL) and brine (2 × 20 mL), then dried with Na_2SO_4 and concentrated in vacuo. The crude residue was then purified by column chromatography (100% CyHex to 15% EtOAc/CyHex) to obtain the protected intermediate as a solid (27 mg, 60%). MS, m/z = 339 (100) $[M+H]^+$. The intermediate was then dissolved in a 1:3 mixture of TFA/DCM (4 mL) and stirred at 20 °C for 1 h. The solvent was then evaporated in vacuo and the crude residue dissolved in EtOAc (10 mL). The organic solution was then successively washed with a 10% $NaHCO_3$ solution (10 mL), water (10 mL) and brine (10 mL). The organic layer was then dried with Na_2SO_4 and concentrated in vacuo to obtain **98** as a solid (13 mg, 74%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.16 (d, *J* 8.58 Hz, 1H), 6.65 (d, *J* 2.86 Hz, 1H), 6.58 (dd, *J* 3.08, 8.80 Hz, 1H), 3.62 (td, *J* 7.84, 15.35 Hz, 1H), 3.24 (d, *J* 12.32 Hz, 2H), 2.89 (br. s., 1H), 2.79 - 2.70 (m, 5H), 2.34 (s, 3H), 1.80 - 1.68 (m, 4H). MS, m/z = 239 (100) [M+H]+

3-(4-Chloro-3-methylphenoxy)piperidine (**99**). The procedure used for **95** was followed using 4-chloro-3-methylphenol (600 mg, 4.2 mmol) (700 mg, 4.09 mmol) and *N*-Boc-3-hydroxypiperidine (1.1g, 5.4 mmol) to obtain **99** (12 mg, 1%) as a solid. ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.16 (d, *J* 8.58 Hz, 1H), 6.70 (d, *J* 2.86 Hz, 1H), 6.63 - 6.55 (m, 1H), 4.28 (dd, *J* 3.0, 6.5 Hz, 1H), 3.15 (d, *J* 12.8 Hz, 1H), 3.00 – 2.78 (m, 2H), 2.34 - 2.31 (m, 5H), 2.08 – 1.89 (m, 1H), 1.89 - 1.70 (m, 2H), 1.64 - 1.48 (m, 1H). MS, m/z = 225

(S)-N-(4-Chloro-3-methylphenyl)piperidin-3-amine (**100**). General Procedure D was followed using 4-bromo-1-chloro-2-methylbenzene (322 µL, 2.43 mmol) and (*S*)-3-amino-1-N-Boc-piperidine (536 mg, 2.68 mmol) to obtain **100** as a solid (80 mg, 15%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.09 (d, *J* 8.6 Hz, 1H), 6.52 (d, *J* 2.9 Hz, 1H), 6.42 (dd, *J* 2.8, 8.5 Hz, 1H), 3.66 - 3.58 (m, 1H), 3.23 (dd, *J* 3.2, 12.2 Hz, 1H), 3.02 – 2.87 (m, 2H), 2.74 (dd, *J* 6.71, 12.21 Hz, 1H), 2.28 (s, 3H), 1.97 - 1.82 (m, 2H), 1.76 - 1.55 (m, 2H). MS, m/z = 225 (100) $[M+H]^+$, 227 (30). MS, m/z = 225 (100) $[M+H]^+$, 227 (30).

(R)-N-(4-Chloro-3-methylphenyl)piperidin-3-amine (**101**). General Procedure D was followed using 4-bromo-1-chloro-2-methylbenzene (161 µL, 1.22 mmol) and (*R*)-3-amino-1-N-Boc-piperidine (268 mg, 1.34 mmol) to obtain **101** as a solid (100 mg, 37%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.09 (d, *J* 8.6 Hz, 1H), 6.56 (d, *J* 2.6 Hz, 1H), 6.44 (dd, *J* 2.8, 8.5 Hz, 1H), 4.50 (br s, 1H), 3.95 - 3.76 (m, 1H), 3.29 (dd, *J* 3.1, 12.8 Hz, 1H), 3.15 - 2.91(m, 3H), 2.27 (s, 3H), 2.18 - 2.06 (m, 1H), 2.00 – 1.65 (m, 3H). MS, m/z = 225 (100) $[M+H]^+$, 227 (30).

tert-Butyl 3-((4-chloro-3-methylphenyl)amino)pyrrolidine-1-carboxylate (**102**). General Procedure D was followed using 4-bromo-1-chloro-2-methylbenzene (452 µL, 3.41 mmol) and 1-Boc-3-aminopyrrolidine (745 µL, 4.09 mmol) to obtain **102** as a solid (111 mg, 10%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.13 (d, *J* 8.4 Hz, 1H), 6.48 (d, *J* 2.6 Hz, 1H), 6.39 (dd, *J* 8.58, 2.86 Hz, 1H), 3.99 (br s, 1H), 3.79 – 3.57 (m, 2H), 3.56 – 3.38 (m, 2H), 3.31 – 3.14 (m, 1H), 2.30 (s, 3H), 2.10 - 2.26 (m, 1H), 1.96 – 1.79 (m, 1H), 1.47 (s, 9H). MS, m/z = 255 (100) [M-56], 257 (30).

N-(4-Chloro-3-methylphenyl)pyrrolidin-3-amine (**103**). The deprotection step in General Procedure D was followed using **102** (50 mg, 0.16 mmol) to obtain **103** as an oil (32 mg, 94%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.11 (d, *J* 8.6 Hz, 1H), 6.47 (d, *J* 2.6 Hz, 1H), 6.38 (dd,

J 8.6, 2.9 Hz, 1H), 3.92 (br. s., 1H), 3.61 - 2.54 (m, 4H), 2.30 (s, 3H), 2.27 - 2.04 (m, 3H), 1.75 - 1.57 (m, 1H). MS, m/z = 211 (100) $[M+H]^+$, 213 (30).

3-(4-Chloro-3-methylphenoxy)pyrrolidine (**104**). The procedure used for **95** was followed using tert-butyl 3-hydroxypyrrolidine-1-carboxylate (250 mg, 1.34 mmol) and 4-chloro-3-methyl-phenol (209 mg, 1.47 mmol) to obtain **104** as a solid (35 mg, 13%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.21 (d, *J* 8.6Hz, 1H), 6.73 (d, *J* 2.9 Hz, 1H), 6.67 - 6.60 (m, 1H), 4.89 - 4.70 (m, 1H), 3.29 – 2.89 (m, 4H), 2.33 (s, 3H), 2.16 – 1.92 (m, 2H). MS, m/z = 212 (100) $[M+H]^+$, 214 (30).

N-(4-Chloro-3-methylphenyl)-N-methylpyrrolidin-3-amine (**105**). The procedure used for **98** was followed using **102** (53 mg, 0.17 mmol) to obtain **105** as a clear oil (20 mg, 52%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.16 (d, *J* 8.8 Hz, 1H), 6.69 (d, *J* 2.9 Hz, 1H), 6.61 (dd, *J* 3.0, 8.7 Hz, 1H), 4.32 - 4.19 (m, 1H), 3.21 - 3.04 (m, 2H), 3.04 – 2.96 (m, 1H), 2.96 - 2.86 (m, 1H), 2.80 (s, 3H), 2.33 (s, 3H), 2.13 – 1.97 (m, 1H), 1.79 (qd, *J* 7.0, 14.1 Hz, 1H). MS, m/z = 225 (100) $[M+H]^+$.

General Procedure E: 1-(3-Chloro-4-methylphenyl)piperazine (**106**). 2-Chloro-4-iodotoluene (250 µL, 1.78 mmol), 1-Boc-piperazine (398 mg, 2.14 mmol), $Pd_2(dba)_3$ (40.8 mg, 0.045 mmol), Xantphos (103 mg, 0.178 mmol) and potassium *tert*-butoxide (280 mg, 2.50 mmol) were dissolved in toluene (5 mL) and heated at reflux for 16 h under N_2. The reaction was then concentrated and dissolved in EtOAc (20 mL), filtered through celite and washed with additional EtOAc (50 mL). The organic layer was washed with water (2 × 20 mL) and brine (2 × 20 mL), then dried with Na_2SO_4 and concentrated in vacuo. The crude residue was then purified by column chromatography (100% CyHex to 10% EtOAc/CyHex) to obtain the protected intermediate as an oil (436 mg, 79%). MS, m/z = 311 (100) $[M+H]^+$, 313 (30). The intermediate was then dissolved in a 1:3 mixture of TFA/DCM (4 mL) and

stirred at 20 °C for 1 h. The solvent was then evaporated in vacuo and the crude residue dissolved in EtOAc (10 mL) which was then successively washed with a 10% $NaHCO_3$ solution (10 mL), water (10 mL) and brine (10 mL). The organic layer was then dried with Na_2SO_4 and concentrated in vacuo to obtain **106** as a solid (288 mg, 97%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.10 (dd, *J* 8.5, 0.6 Hz, 1H), 6.91 (d, *J* 2.6 Hz, 1H), 6.74 (dd, *J* 8.5, 2.6 Hz, 1H), 3.23 – 2.99 (m, 8H), 2.29 (s, 3H). MS, m/z = 211 (100) $[M+H]^+$, 213 (30)

Ethyl 1-(3-chloro-4-methylphenyl)piperidine-4-carboxylate (**107**). General Procedure E was followed using 2-chloro-4-iodotoluene (389 µL, 2.77 mmol) and ethyl isonipecotate (470 µL, 3.05 mmol) to obtain **107** as an oil (130 mg, 17%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.09 (d, *J* 8.4 Hz, 1H), 6.92 (br. s., 1H), 6.82 - 6.66 (m, 1H), 4.17 (q, *J* 7.0 Hz, 2H), 3.58 (dt, *J* 12.5, 3.4 Hz, 2H), 2.77 (t, *J* 12.1 Hz, 2H), 2.53 - 2.36 (m, 1H), 2.28 (s, 3H), 2.09 – 1.88 (m, 3H), 1.88 - 1.77 (m, 1H), 1.28 (t, *J* 7.2 Hz, 3H). MS, m/z = 382 (100) $[M+H]^+$, 384 (30)

1-(3-Chloro-4-methylphenyl)piperidine-4-carboxylic acid (**108**). **107** (130 mg, 0.46 mmol) and LiOH (33 mg, 1.4 mmol) in a solution of THF (2 mL) and water (2 mL) was stirred for 4 h at 20 ^{o}C. The solution was then acidified with 10% citric acid (pH 4) and extracted with EtOAc (2 × 10 mL). The organic layers were combined and washed with brine (20 mL), dried with Na_2SO_4, and concentrated in vacuo to obtain **108** as a solid (100 mg, 85%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.10 (d, *J* 8.1 Hz, 1H), 6.99 - 6.87 (m, 1H), 6.84 - 6.68 (m, 1H), 3.60 (dt, *J* 12.7, 3.4 Hz, 2H), 2.90 - 2.72 (m, 2H), 2.63 - 2.42 (m, 1H), 2.30 (s, 3H), 2.15 - 2.01 (m, 2H), 2.01 – 1.77 (m, 2H). MS, m/z = 252 (100) $[M-H]^-$, 254 (30)

tert-Butyl 3-((3-chloro-4-methylphenyl)amino)azetidine-1-carboxylate (**109**). General Procedure E was followed using 2-chloro-4-iodotoluene (700 µL, 0.39 mmol) and 3-amino-1-(*N*-Boc)azetidine (478 µL, 3.05 mmol) to obtain **109** as a solid (412 mg, 50%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.03 (d, *J* 7.9 Hz, 1H), 6.53 (d, *J* 2.4 Hz, 1H), 6.37 (dd, *J* 2.4, 8.1 Hz, 1H),

4.32 - 4.26 (m, 2H), 4.20 - 4.12 (m, 1H), 3.72 (dd, *J* 4.7, 9.4 Hz, 2H), 2.26 (s, 3H), 1.45 (s, 9H). MS, m/z = 241 (100) [M-*t*Bu].

N-(3-Chloro-4-methylphenyl)azetidin-3-amine (**110**). The deprotection step in General Procedure D was followed using **109** (100 mg, 0.34 mmol) to obtain **110** as a brown oil (60 mg, 91%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.04 (d, *J* 8.1 Hz, 1H), 6.56 (d, *J* 2.4 Hz, 1H), 6.41 (dd, *J* 2.4, 8.4 Hz, 1H), 4.22 - 4.01 (m, 3H), 4.01 – 3.87 (m, 2H), 2.26 (s, 3H). MS, m/z = 197 (100) $[M+H]^+$.

N-(3-Chloro-4-methylphenyl)-N-methylazetidin-3-amine (**111**). The procedure used for **98** was followed using **109** (120 mg, 0.40 mmol) to obtain **111** as a clear oil (48 mg, 57%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.06 (d, *J* 8.4 Hz, 1H), 6.71 (d, *J* 2.6 Hz, 1H), 6.53 (dd, *J* 2.6, 8.4 Hz, 1H), 4.40 - 4.23 (m, 1H), 3.90 - 3.58 (m, 4H), 2.83 (s, 3H), 2.56 (br s, 1H), 2.27 (s, 3H). MS, m/z = 211 (100) $[M+H]^+$.

tert-Butyl 3-((methylsulfonyl)oxy)azetidine-1-carboxylate (**112**). To a solution of *tert*-butyl 3-hydroxyazetidine-1-carboxylate (1.00 g, 5.77 mmol) and triethylamine (1.6 mL, 12 mmol) in DCM (10 mL) was added methanesulfonyl chloride (0.73 g, 6.35 mmol) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was diluted with brine (20 mL) and was extracted with DCM (15 mL × 2). The combined organic layers were dried with Na_2SO_4 and concentrated to obtain **112** as a solid (1.41 g, 97% yield). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 5.21 (tt, *J* 4.2, 6.7 Hz, 1H), 4.34 - 4.23(m, 2H), 4.14 - 4.04 (m, 2H), 3.07 (s, 3H).

3-(3-Chloro-4-methylphenoxy)azetidine (**113**). **112** (200 mg, 0.796 mmol), 3-chloro-4-methyl-phenol (113 mg, 0.796 mmol) and caesium carbonate (519 mg, 1.59 mmol) were dissolved in DMF (3 mL) and stirred at 90 °C for 12 h. The reaction was then quenched with water (10 mL) and reaction diluted with EtOAc (15 mL). The organic layer was then

separated and washed with brine (15 mL), dried with Na_2SO_4 and concentrated. The crude residue was then purified by column chromatography (100% CyHex to 25% EtOAc/CyHex) to obtain the protected intermediate as a clear oil (166 mg, 70% yield). MS, m/z = 242 (100) [M-*t*Bu]. The intermediate was then dissolved in a 1:3 mixture of TFA/DCM (4 mL) and stirred at 20 °C for 1 h. The solvent was then evaporated in vacuo and the crude residue dissolved in EtOAc (10 mL). The organic layer was successively washed with a 10% $NaHCO_3$ solution (10 mL), water (10 mL) and brine (10 mL). The organic layer dried with Na_2SO_4 and concentrated in vacuo to obtain **113** as a solid (110 mg, 99%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.13 - 7.08 (m, 1H), 6.77 (d, *J* 2.4 Hz, 1H), 6.59 (dd, *J* 2.6, 8.4 Hz, 1H), 4.97 - 4.83 (m, 1H), 4.06 – 3.55 (m, 4H), 2.30 (s, 3H). MS, m/z = 198 (100) [M+H]+, 200 (30).

1-Boc-homopiperazine (**114**). Homopiperazine (5.00 g, 49.92 mmol) was dissolved in MeOH (200 mL) and cooled to 0 °C. Boc anhydride (12 g, 55.0 mmol) in MeOH (100 mL) was added dropwise over 1 h and the reaction allowed to warm to room temperature after which the reaction was refluxed for 4 h. The reaction was concentrated *in vacuo* and dissolved in a 1M citric acid solution (150 mL). The aqueous layer was then washed with EtOAc (3 × 70 mL). The aqueous layer was then cooled to 0 °C basified with solid Na_2CO_3. The product was then extracted with EtOAc (3 × 100 mL), dried with Na_2SO_4 and concentrated in vacuo to obtain **114** as a clear oil (1.08 g, 11% yield). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 3.54 – 3.37 (m, 4H), 2.96 – 2.81 (m, 4H), 1.87 (br. s., 1H), 1.84 - 1.72 (m, 2H), 1.47 (s, 9H).

1-(3-Chloro-4-methylphenyl)-1,4-diazepane (**115**). General Procedure E was followed using 2-chloro-4-iodotoluene (139 µL, 0.99 mmol) and **114** (198 mg, 0.99 mmol) to obtain **115** as a solid (71 mg, 79%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.04 (dd, *J* 8.5, 0.6 Hz, 1H), 6.69 (d, *J* 2.6 Hz, 1H), 6.51 (dd, *J* 8.6, 2.6 Hz, 1H), 3.54 (t, *J* 6.1 Hz, 4H), 3.10 - 3.00 (m, 2H), 2.93 - 2.84 (m, 2H), 2.26 (s, 3H), 2.04 -1.90 (m, 2H). MS, m/z = 225 (100) $[M+H]^+$, 227 (30).

4-Benzyl 1-tert-butyl 2-methylpiperazine-1,4-dicarboxylate (**116**)**.** To a stirred solution of 2-methylpiperazine (2.0 g, 20 mmol) in DCM (15 mL) at 0 °C was added benzyl chloroformate (3.0 mL, 21 mmol) dropwise. The mixture was stirred at 0 °C for 1 h and then 20 °C for 2.5 h. The reaction was cooled to 0 °C and then *N*,*N*-diisopropylethylamine (4.5 mL, 26 mmol) and Boc anhydride (4.8 g, 22 mmol) was added. The solution was stirred at 20 °C for 14 h. The reaction was then concentrated in vacuo and residue dissolved in EtOAc (150 mL). The organic layer was then and washed with water (50 mL) and brine (50 mL), then dried with $Na_2SO_{4(S)}$ and concentrated. The crude solid was then purified by column chromatography gradient eluting with 100% CyHex to 10% EtOAc/CyHex to obtain **116** as a solid (5.4 g, 81%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.44 - 7.28 (m, 5H), 5.23 - 5.09 (m, 2H), 4.33 - 4.19 (m, 1H), 4.19 – 3.87 (m, 2H), 3.87 - 3.78 (m, 1H), 3.17 – 2.97 (m, 2H), 2.97 – 2.80 (m, 1H), 1.47 (s, 8H), 1.13 (d, *J* 5.3 Hz, 3H). MS, m/z = 235 (100) [M-Boc].

tert-Butyl 2-methylpiperazine-1-carboxylate (**117**)**. 116** (3.1 g, 9.3 mmol) was dissolved in MeOH (30 mL) and 200 mg of Pd/C added. The reaction was then stirred at 20 °C under an atmosphere of H_2 for 14 h. The reaction mixture was filtered through Celite, and then concentrated to obtain **117** as an oil (1.8 g, 97%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 4.25 - 4.10 (m, 1H), 3.86 - 3.73 (m, 1H), 3.08 – 2.85 (m, 3H), 2.83 - 2.62 (m, 2H), 1.49 - 1.45 (m, 9H), 1.25 - 1.19 (m, 3H). MS, m/z = 145 (100) [M-*t*Bu].

1-(3-chloro-4-methylphenyl)-3-methylpiperazine (**118**). General Procedure E was followed using 2-chloro-4-iodotoluene (194 µL, 1.39 mmol) and **117** (305 mg, 1.52 mmol) to obtain **118** as a solid (141 mg, 45%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.12 (dd, *J* 0.4, 8.4 Hz, 1H), 6.91 (d, *J* 2.6 Hz, 1H), 6.74 (dd, *J* 2.6, 8.4 Hz, 1H), 3.57 - 3.40 (m, 2H), 3.36 - 3.21 (m, 2H), 3.14 (dt, *J* 2.9, 11.8 Hz, 1H), 3.06 – 2.91 (m, 1H), 2.71 (dd, *J* 10.3, 12.5 Hz, 1H), 2.30 (s, 3H), 1.33 (d, *J* 6.6 Hz, 3H). MS, m/z = 225 (100) $[M+H]^+$, 227 (30)

tert-Butyl 3-methylpiperazine-1-carboxylate (**119**). Boc anhydride (2.7 g, 12.5 mmol) was added portion wise to a stirred solution of 2-methylpiperazine (2.5 g, 25 mmol) in DCM (40 mL). *N*,*N*-Diisopropylethylamine (7.5 mL, 56 mmol) was then added and the mixture stirred at 20 °C for 14 h. The reaction mixture was washed successively with saturated $NaHCO_3$ solution (30 mL), water (30 mL) and brine (30 mL), and then dried with anhydrous Na_2SO_4 and concentrated. The crude solid was purified by column chromatography gradient eluting with 100% CyHex to 30% EtOAc/CyHex to obtain **119** as a clear oil (1.0 g, 40%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 4.10 – 3.72 (m, 2H), 3.00 – 2.88 (m, 1H), 2.86 – 2.62 (m, 3H), 2.52 – 2.27 (m, 1H), 1.46 (s, 9H), 1.05 (d, *J* 6.4 Hz, 3H). MS, m/z = 201 $[M+H]^+$

1-(3-Chloro-4-methylphenyl)-2-methylpiperazine (**120**). General Procedure E was followed using 2-chloro-4-iodotoluene (194 µL, 1.39 mmol) and **119** (330 mg, 1.65 mmol) to obtain **120** as a solid (40 mg, 13%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 9.77 (br s, 1H), 7.17 (d, *J* 8.6 Hz, 1H), 7.04 (d, *J* 2.2 Hz, 1H), 6.86 (dd, *J* 2.3, 8.3 Hz, 1H), 3.78 - 3.56 (m, 1H), 3.46 - 3.15 (m, 5H), 3.13 – 2.98 (m, 1H), 2.33 (s, 3H), 1.07 (d, *J* 6.6 Hz, 3H). MS, m/z = 225 (100) $[M+H]^+$, 227 (60).

1-(3-Chloro-4-methylphenyl)piperazin-2-one (**121**). General Procedure E was followed using 2-chloro-4-iodotoluene (96 µL, 0.68 mmol) and 2-oxopiperazine (150 mg, 0.75 mmol) to obtain **121** as a solid (9 mg, 6%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.26 – 6.97 (m, 3H), 4.15 – 3.76 (m, 2H), 3.71 - 3.39 (m, 1H), 2.36 (m, 3.5H), 1.81 - 1.62 (m, 1H), 1.35 - 1.22 (m, 1H), 0.95 – 0.80 (m, 0.5H). MS, m/z = 225 (100) $[M+H]^+$, 227 (30).

2-(3-Chloro-4-methylphenyl)-2,6-diazaspiro[3.3]heptane bis-trifluoroacetate (**122**). General Procedure E was followed using 2-chloro-4-iodotoluene (55 µL, 0.39 mmol) and *tert*-butyl 2,6-diazaspiro[3.3]heptane-2-carboxylate (65 mg, 0.33 mmol) to obtain **122** as a solid (71 mg, 62%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.15 (d, *J* 8.4 Hz, 1H), 6.71 (d, *J* 2.4 Hz, 1H), 6.52

(dd, *J* 2.3, 8.3 Hz, 1H), 4.39 (br. s., 4H), 4.22 (s, 4H), 2.31 (s, 3H). MS, m/z = 223 (100) $[M+H]^+$.

1-(4-Chlorophenyl)piperazine (**123**). General Procedure D was followed using 1-bromo-4-chlorobenzene (625 mg, 3.26 mmol) and 1-Boc-piperazine (608 mg, 3.26 mmol) to obtain **123** as a solid (420 mg, 65%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.27 - 7.20 (m, 2H), 6.98 - 6.77 (m, 2H), 3.94 - 3.75 (m, 1H), 3.66 - 3.56 (m, 2H), 3.52 - 3.43 (m, 1H), 3.43 - 3.33 (m, 1H), 3.24 - 3.17 (m, 1H), 3.17 - 3.04 (m, 2H). MS, m/z = 197 (100) $[M+H]^+$.

1-(3,4-Dichlorophenyl)piperazine (**124**). General Procedure E was followed using 1,2-dichloro-4-iodobenzene (400 mg, 1.47 mmol) and 1-Boc-piperazine (300 mg, 1.61 mmol) to obtain **124** as a solid (260 mg, 76%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.30 – 7.28 (m, 1H), 6.96 (d, *J* 2.9 Hz, 1H), 6.75 (dd, *J* 2.9, 8.9 Hz, 1H), 3.18 - 3.10 (m, 4H), 3.10 - 3.00 (m, 4H). MS, m/z = 231 (100) $[M+H]^+$, 233 (60).

3-(Piperazin-1-yl)benzonitrile (**125**). General Procedure E was followed using 3-iodobenzonitrile (100 mg, 0.44 mmol) and 1-Boc-piperazine (98 mg, 0.52 mmol) to obtain **125** as a solid (55 mg, 67%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.39 - 7.29 (m, 1H), 7.18 - 7.05 (m, 3H), 3.31 - 3.21 (m, 4H), 3.17 - 3.05 (m, 4H). MS, m/z = 188 (100) $[M+H]^+$.

Phenyl (5-cyanothiazol-2-yl)carbamate (**126**). The procedure used for **93** was followed using 2-aminothiazole-5-carbonitrile (240 mg, 1.92 mmol) and phenyl chloroformate (0.48 mL, 3.84 mmol) to obtain **126** as a solid (230 mg, 49%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.98 (s, 1H), 7.48 - 7.43 (m, 2H), 7.37 - 7.29 (m, 2H), 7.26 - 7.22 (m, 1H). MS, m/z = 246 (100) $[M+H]^+$.

Phenyl thiazol-2-ylcarbamate (**127**). The procedure used for **93** was followed using thiazol-2-amine (200 mg, 2.00 mmol) and phenyl chloroformate (0.28 mL, 2.20 mmol) to obtain **127** as

a solid (253 mg, 58%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.59 - 7.40 (m, 3H), 7.37 - 7.23 (m, 3H), 6.98 (dd, *J* 1.8, 3.5 Hz, 1H). MS, m/z = 221 (100) $[M+H]^+$.

Phenyl (5-(trifluoromethyl)thiazol-2-yl)carbamate (**128**). The procedure used for **93** was followed using 5-(trifluoromethyl)thiazol-2-amine (60 mg, 0.36 mmol) and phenyl chloroformate (0.49 mL, 3.92 mmol) to obtain **128** as a solid (82 mg, 79%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.82 - 7.76 (m, 1H), 7.52 - 7.41 (m, 2H), 7.39 - 7.28 (m, 3H). MS, m/z = 289 (100) $[M+H]^+$.

4-Methyl-6-(piperazin-1-yl)nicotinonitrile (**129**). 6-Bromo-4-methylnicotinonitrile (200 mg, 1.02 mmol), 1-Boc-piperazine (208 mg, 1.12 mmol) and *N*,*N*-diisopropylethylamine (0.53 mL, 3.05 mmol) were dissolved in DMF (5 mL) and stirred at 130 °C for 3 h under N_2. The reaction was then cooled to 20 °C and concentrated. The crude residue was dissolved in EtOAc (20 mL), filtered through celite and washed with additional EtOAc (50 mL). The organic layer was then washed with water (2 × 20 mL) and brine (2 × 20 mL), dried with Na_2SO_4 and concentrated in vacuo. The crude residue was then purified by column chromatography (100% CyHex to 20% EtOAc/CyHex) to obtain the protected intermediate as a solid (292 mg, 95%). MS, m/z = 303 (100) $[M+H]^+$. The intermediate was then dissolved in a 1:3 mixture of TFA/DCM (4 mL) and stirred at 20 °C for 1 h. The solvent was then evaporated in vacuo and the crude residue dissolved in EtOAc (10 mL). The organic solution was successively washed with a 10% $NaHCO_3$ solution (10 mL), water (10 mL) and brine (10 mL). The organic layer was dried with Na_2SO_4 and concentrated in vacuo to obtain **129** as a solid (180 mg, 92%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.33 (s, 1H), 6.44 (s, 1H), 3.77 - 3.60 (m, 4H), 3.05 – 2.92 (m, 4H), 2.45 - 2.37 (m, 3H). MS, m/z = 203 (100) $[M+H]^+$.

3-Methyl-5-(piperazin-1-yl)picolinonitrile (**130**). General Procedure D was followed using 5-bromo-3-methylpicolinonitrile (200 mg, 1.02 mmol) and 1-Boc-piperazine (227 mg, 1.22

mmol) to obtain **130** as a solid (217 mg, 71%). ^{1}H-NMR (300 MHz, d_6-Acetone): δ 8.24 (d, *J* 2.9 Hz, 1H), 7.25 (d, *J* 2.9 Hz, 1H), 3.58 - 3.40 (m, 4H), 3.22 – 2.98 (m, 4H), 2.42 (s, 3H). MS, m/z = 203 (100) $[M+H]^+$.

1-(5-Chloro-4-(trifluoromethyl)pyridin-2-yl)piperazine (**131**). General Procedure E was followed using 2,5-dichloro-4-trifluoromethylpyridine (200 mg, 0.93 mmol) and 1-Boc-piperazine (190 mg, 1.02 mmol) to obtain **131** as a solid (134 mg, 56%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.24 (s, 1H), 6.85 (s, 1H), 3.65 - 3.56 (m, 4H), 3.07 – 2.97 (m, 4H). MS, m/z = 266 (100) $[M+H]^+$.

1-(5,6-Dichloropyridin-3-yl)piperazine (**132**). General Procedure E was followed using 5-bromo-2,3-dichloropyridine (200 mg, 0.88 mmol) and 1-Boc-piperazine (181 mg, 0.97 mmol) to obtain **132** as a solid (152 mg, 74%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.93 (d, *J* 2.9 Hz, 1H), 7.24 (d, *J* 2.9 Hz, 1H), 3.23 - 3.12 (m, 4H), 3.07 2.97 (m, 4H). MS, m/z = 232 (100) $[M+H]^+$, 234 (60).

5-(Piperazin-1-yl)pyrimidine (**133**). General Procedure E was followed using 5-bromopyrimidine (250 mg, 1.57 mmol) and 1-Boc-piperazine (351 mg, 1.89 mmol) to obtain **133** as an oil (196 mg, 75%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.69 (s, 1H), 8.36 (s, 2H), 3.27-3.18 (m, 4H), 3.13 – 2.98 (m, 4H), 1.93 (br. s., 1H). MS, m/z = 165 (100) $[M+H]^+$.

Ethyl 4-(3-chloro-4-methylphenoxy)butanoate (**134**). 3-Chloro-4-methylphenol (1.0 g, 7.01 mmol), ethyl bromobutyrate (1.21 mL, 8.42 mmol) and K_2CO_3 (2.91 g, 21.04 mmol) were dissolved in DMF (4 mL) and stirred at 90 °C for 16 h. The solvent was evaporated, and the crude residue dissolved in EtOAc (15 mL). the organic solution was then washed with water (10 mL) and brine (10 mL), dried with Na_2SO_4 and concentrated in vacuo. The crude residue was then purified by column chromatography gradient eluting with 100% CyHex to 10% EtOAc/CyHex to obtain **134** as a clear oil (1.7 g, 96%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.16 -

7.07 (m, 1H), 6.91 (d, *J* 2.4 Hz, 1H), 6.72 (dd, *J* 8.5, 2.5 Hz, 1H), 4.17 (q, *J* 7.3 Hz, 2H), 3.99 (t, J 7.3 Hz, 2H), 2.50 (m, 2H), 2.31 (s, 3H), 2.19 – 2.03 (m, 2H), 1.28 (t, J 7.2 Hz ,3H). MS, m/z = 257 (100) $[M+H]^+$, 259 (30).

4-(3-Chloro-4-methylphenoxy)butanoic acid (**135**). **134** (1.70 g, 6.62 mmol) and LiOH (476 mg, 19.9 mmol) in a solution of THF (10 mL) and water (10 mL) was stirred at 20 ^oC for 4 h. The solution was then acidified with 1 N HCl (pH 5) and extracted with Et_2O (2 × 10 mL). The combined organic layers were washed with brine (20 mL), dried with Na_2SO_4, and concentrated in vacuo to obtain **135** as a solid (1.42 g, 94%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.12 (dd, *J* 8.4, 0.44 Hz, 1H), 6.92 (d, *J* 2.6 Hz, 1H), 6.72 (dd, *J* 8.5, 2.75 Hz, 1H), 4.00 (t, *J* 6.1 Hz, 2H), 2.60 (t, *J* 7.3 Hz, 2H), 2.31 (s, 3H), 2.20 - 2.06 (m, 2H). MS, m/z = 226 (100) $[M-H]^-$.

5-Methylpyridine-2,3-diamine (**136**). Pd/C (50 mg, 0.47 mmol) was added to a solution of 2-amino-5-methyl-3-nitropyridine (500 mg, 3.27 mmol) in MeOH (7 mL). The reaction was then stirred under this atmosphere of H_2 at 20 °C for 5 h. The reaction was filtered through celite and washed with MeOH (30 mL). The solution was concentrated in vacuo to obtain **136** (395 mg, 98%). ^{1}H NMR (300 MHz, MeOD): δ 7.22 (dd, *J* 2.0, 0.9 Hz, 1H), 6.81 (dd, *J* 2.0, 0.7 Hz, 1H), 2.13 (t, *J* 0.7 Hz, 3H). MS, m/z = 124 (100).

Ethyl 4-(3,4-dichlorophenoxy)butanoate (**137**). The procedure used for **134** was followed using 3,4-dichlorophenol (500 mg, 3.07 mmol) and ethyl bromobutyrate (598 mg, 3.07 mmol) to obtain **137** as a clear oil (645 mg, 76%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.32 (d, *J* 8.9 Hz, 1H), 7.00 (d, *J* 2.9 Hz, 1H), 6.75 (dd, *J* 8.9 and 2.9 Hz, 1H), 4.16 (q, *J* 7.2 Hz, 2H), 3.99 (t, *J* 6.3 Hz, 2H), 2.51 (t, *J* 7.5 Hz, 2H), 2.16-2.07 (m, 2H), 1.27 (t, *J* 7.1 Hz, 3H).

4-(3,4-Dichlorophenoxy)butanoic acid (**138**). The procedure used for **135** was followed using **137** (570 mg, 2.06 mmol) to obtain **138** as a solid (490 mg, 96%). ^{1}H-NMR (300 MHz;

$CDCl_3$): δ 7.33 (d, *J* 9.0 Hz, 1H), 7.00 (d, *J* 2.7 Hz, 1H), 6.75 (dd, *J* 8.7 and 2.8 Hz, 1H), 4.01 (t, *J* 6.3 Hz, 2H), 2.60 (t, *J* 7.2 Hz, 2H), 2.17-2.10 (m, 2H).

Ethyl 4-((5,6-dichloropyridin-3-yl)oxy)butanoate (**139**). The procedure used for **134** was followed using 5,6-dichloropyridin-3-ol (210 mg, 1.28 mmol) and ethyl bromobutyrate (250 mg, 1.28 mmol) to obtain **139** as a clear oil (196 mg, 55%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.32 (d, *J* 8.9 Hz, 1H), 7.00 (d, *J* 2.9 Hz, 1H), 6.75 (dd, *J* 8.9 and 2.9 Hz, 1H), 4.16 (q, *J* 7.2 Hz, 2H), 3.99 (t, *J* 6.3 Hz, 2H), 2.51 (t, *J* 7.5 Hz, 2H), 2.16 - 2.07 (m, 2H), 1.27 (t, *J* 7.1 Hz, 3H).

4-((5,6-Dichloropyridin-3-yl)oxy)butanoic acid (**140**). The procedure used for **135** was followed using **139** (196 mg, 0.70 mmol) to obtain **140** as a solid (170 mg, 97%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 7.33 (d, *J* 9.0 Hz, 1H), 7.00 (d, *J* 2.7 Hz, 1H), 6.75 (dd, *J* 8.7 and 2.8 Hz, 1H), 4.01 (t, *J* 6.3 Hz, 2H), 2.60 (t, *J* 7.2 Hz, 2H), 2.17 - 2.10 (m, 2H).

4-(3-Chloro-4-methylphenoxy)-N-(2-chloro-5-methylpyridin-3-yl)butanamide (**141**). **135** (250 mg, 1.09 mmol) was dissolved in $SOCl_2$ (3 mL, 42 mmol) and stirred at reflux for 3 h. The reaction was then concentrated and azeotroped 3 times with toluene (10 mL) to give a crude residue. The residue was then dissolved in DCM (10 mL) and cooled to 0 °C. 2-Chloro-5-methylpyridin-3-amine (156 mg, 1.10 mmol) was then added followed by *N*,*N*-diisopropylethylamine (381 µL, 2.20 mmol) and warmed to 20 °C. The reaction mixture was then stirred at this temperature for 4 h under N_2. The organic layer was washed successively with 5% citric acid (10 mL), saturated $NaHCO_3$ (2 × 10 mL), water (10 mL) and brine (10 mL), and then dried with Na_2SO_4 and concentrated. The crude residue was then purified by column chromatography gradient eluting with 100% CyHex to 50% EtOAc/CyHex to obtain **141** as a solid (56 mg, 15%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.58 (s, 1H), 7.94 (br s, 1H), 7.64 (br s, 1H), 7.11 (d, *J* 8.1 Hz, 1H), 6.91 (d, *J* 2.6 Hz, 1H), 6.72 (dd, *J* 2.6, 8.4 Hz, 1H),

4.05 (t, *J* 5.9 Hz, 2H), 2.68 (t, *J* 7.2 Hz, 2H), 2.34 (s, 3H), 2.30 (s, 3H), 2.15 - 2.28 (m, 2H). MS, m/z = 353 (100) $[M+H]^+$, 355 (60).

N-(5-Chloropyridin-2-yl)pivalamide (**142**). 5-Chloropyridin-2-amine (700 mg, 5.44 mmol) and *N,N*-diisopropylethylamine (1.52 mL, 10.9 mmol) was dissolved in DCM (30 mL) and cooled to 0 °C. Pivaloyl chloride (1.00 mL, 8.16 mmol) was then added dropwise and the reaction stirred while warming to 20 °C. The mixture was stirred for an additional 1 h. The reaction mixture was washed successively with saturated $NaHCO_3$ solution (15 mL), water (15 mL) and brine (10 mL), then dried with anhydrous Na_2SO_4 and concentrated. The crude residue was then purified by column chromatography gradient eluting with 100% CyHex to 20% EtOAc/CyHex to obtain **142** as a solid (1.13 g, 97%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.21 (d, *J* 8.8 Hz, 1 H), 8.18 (d, *J* 1.6 Hz, 1 H), 8.01 (s, 1 H), 7.64 (dd, *J* 8.8 Hz, *J* 2.4 Hz, 1 H), 1.30 (s, 9 H). MS, m/z = 213 (100) $[M+H]^+$, 215 (30).

5-Chloro-2-pivalamidopyridin-3-yl diisopropylcarbamodithioate (**143**). *n*-Butyllithium (1.76 mL, 1.41 mmol) was added dropwise to a stirred solution of **142** (150 mg, 0.71 mmol) in anhydrous THF (3 mL) at -78 °C under N_2. The mixture was allowed to warm to -10 °C and stirred at this temperature for 2 h. The reaction was then cooled to -78 °C and to it was added dropwise a solution of thiuram disulfide (249 mg, 0.71 mmol) in dry THF (2 mL). The reaction mixture was then stirred at this temperature for 1 h and then warmed to room temperature and stirred for a further 30 min. The reaction was quenched with water (5 mL) and the crude product extracted into Et_2O (30 mL) and washed with water (15 mL) and brine (10 mL), then dried with Na_2SO_4 and concentrated. The crude residue was then purified by column chromatography gradient eluting with 100% CyHex to 20% EtOAc/CyHex to obtain **143** as a solid (105 mg, 38%). ^{1}H-NMR (300 MHz; $CDCl_3$): δ 8.57 (d, *J* 2.4 Hz, 1H), 8.53 (br s, 1H), 7.77 (d, *J* 2.4 Hz, 1H), 1.50 (br s, 12H), 1.30 (s, 9H). MS, m/z = 388 (100) [M+H]+, 390 (30).

2-Amino-5-chloropyridin-3-yl diisopropylcarbamodithioate (**144**). **143** (105 mg, 0.27 mmol) was stirred in 10% w/v NaOH in MeOH (5 mL) for 14 h. The product precipitated and was filtered and dried to obtain **144** as a solid (43 mg, 52%). ^{1}H-NMR (300 MHz, MeOD): δ 8.04 (s, 1H), 7.51 (s, 1H), 1.50 (br s, 12H). MS, m/z = 304 (100) [M+H]+, 306 (30).

6-Methyl-1H-imidazo[4,5-b]pyridin-2(3H)-one (**145**)**. 136** (360 mg, 2.92 mmol) and disuccinimido carbonate (749 mg, 2.92 mmol) were dissolved in chloroform (5 mL) and heated at reflux for 48 h under N_2. The reaction was then concentrated, and the residue dissolved in a 1:3 mixture of CyHex/EtOAc (200 mL) and heated to reflux for 10 min. The reaction was then cooled and the precipitate filtered and dried to obtain **145** as a solid (210 mg, 48%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.69 (dd, *J* 0.9, 1.8 Hz, 1H), 7.05 (dd, *J* 0.7, 2.0 Hz, 1H), 5.09 (br s, 1H), 2.25 (s, 3H). MS, m/z = 150 (100) [M+H]+.

2-Chloro-6-methyl-3H-imidazo[4,5-b]pyridine (**146**). **145** (210 mg, 1.41 mmol) was dissolved in $POCl_3$ (4 mL) and stirred at reflux for 12 h. The mixture was concentrated, and the residue was dissolved in EtOAc (30 mL). The organic layer was then washed successively with saturated $NaHCO_3$ (2 × 20 mL), water (20 mL) and brine (20 mL), dried with Na_2SO_4 and concentrated. The crude residue was then purified by column chromatography (100% CyHex to 80% EtOAc/CyHex) to obtain the **146** as a solid (71 mg, 30%). ^{1}H-NMR (300 MHz, d_6-Acetone): δ 8.23 (s, 1H), 7.80 (dd, *J* 0.8, 1.9 Hz, 1H), 2.46 (t, *J* 0.7 Hz, 3H) MS, m/z = 168 (100) [M+H]+.

6-Bromo-1H-imidazo[4,5-b]pyridin-2(3H)-one (**147**). The procedure used for **145** was followed using 2,3-Diamino-5-bromopyridine (400 mg, 2.13 mmol) to obtain **147** (275 mg, 60%). ^{1}H NMR (300 MHz, DMSO-d_6) δ 7.95 (s, 1H), 7.41 (s, 1H). MS, m/z = 214 (100) [M+H]+, 216 (100).

6-Bromo-2-chloro-3H-imidazo[4,5-b]pyridine (**148**). The procedure used for **146** was followed using **147** (100 mg, 0.47 mmol) to obtain **148** (64 mg, 59%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.43 (d, *J* 1.76 Hz, 1H), 8.27 (s, 1H). MS, m/z = 232 (100) $[M+H]^+$, 234 (100).

6-Chloro-1H-imidazo[4,5-b]pyridin-2(3H)-one (**149**). The procedure used for **145** was followed using 5-chloropyridine-2,3-diamine (400 mg, 2.78 mmol) to obtain **149** (108 mg, 23%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.88 (d, *J* 2.2 Hz, 1H), 7.31 (d, *J* 2.2 Hz, 1H). MS, m/z = 170 (100) [M+H]+, 172 (30).

2,6-Dichloro-3H-imidazo[4,5-b]pyridine (**150**). The procedure used for **146** was followed using **149** (108 mg, 0.64 mmol) to obtain **150** (36 mg, 30%). ^{1}H-NMR (300 MHz, d_6-Acetone): δ 8.33 (d, *J* 2.2 Hz, 1H), 8.02 (d, *J* 2.2 Hz, 1H). MS, m/z = 188 (100) [M+H]+, 190 (60).

ASSOCIATED CONTENT

Supporting Information. Supporting information contains, schemes showing the synthesis of specific analogues; HEK293 FlpIn.FM cellular assay and J.Lat10.6 cellular assay dose response data of selected analogues; in vitro metabolite identification data; and target panel screening data (PDF).

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ABBREVIATIONS

AIDS, acquired immunodeficiency syndrome; BET, bromodomain and extra terminal domain; ART, antiretroviral therapy; CA-US, CD4+ T cell associated unspliced; CBG, click beetle green; CBR, click beetle red; CD4, cluster of differentiation 4; CL_{int}, intrinsic clearance; CMV, cytomegalovirus; DSC, disuccinimidyl carbonate; GFP, green fluorescent protein; HDAC, histone deacetylase; HEK293, human embryonic kidney cells 293; HMT, histone methyltransferase; HIV, human immunodeficiency virus, IRES, internal ribosome entry site; J.Lat, Jurkat T lymphocyte latency line; LipE, lipophilic efficiency; LogP, partition coefficient; LRA, latency reversing agent; LTR, long terminal repeat; Nef, nucleotide exchange factor; PLWH, people living with HIV; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PSA, polar surface area; Tat, trans-activator of transcription protein.

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circumvent bioactivation liabilities associated with a nonpeptidyl thrombopoietin receptor agonist containing a 2-amino-4-arylthiazole motif. *Chem. Res. Toxicol.* **2007**, *20* (12), 1954-1965.

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FIGURES

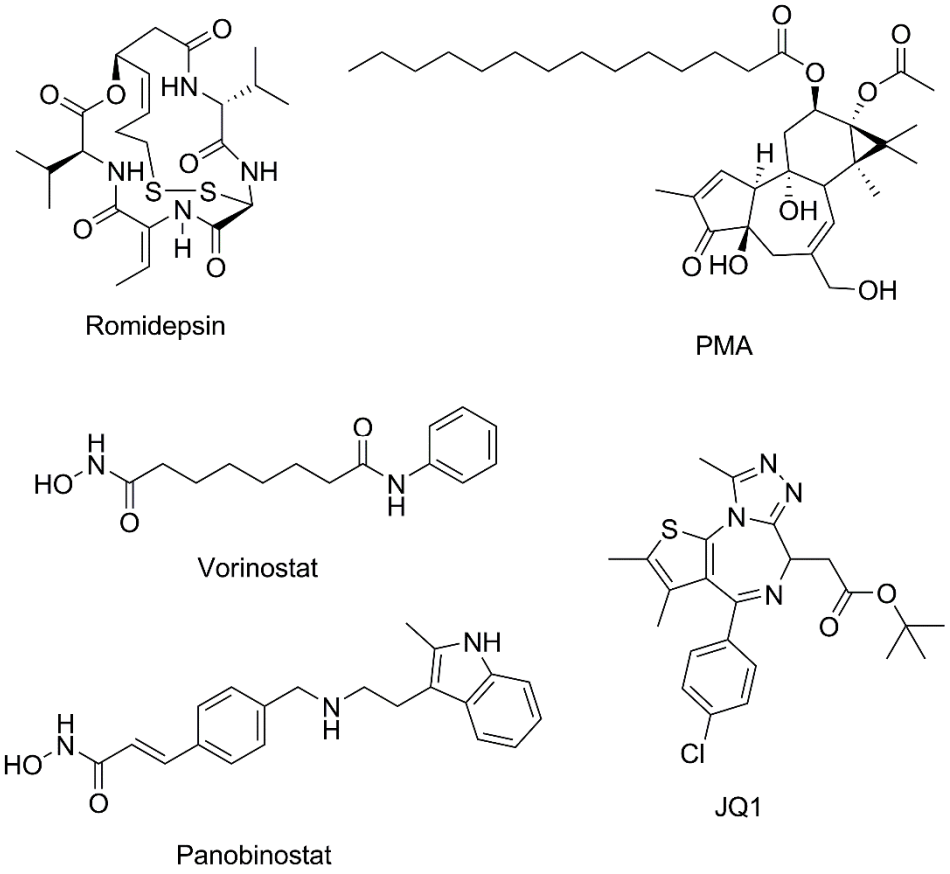


Figure 1. Structures of small molecules commonly used as latency reversing agents (LRA) in the "shock and kill" strategy.

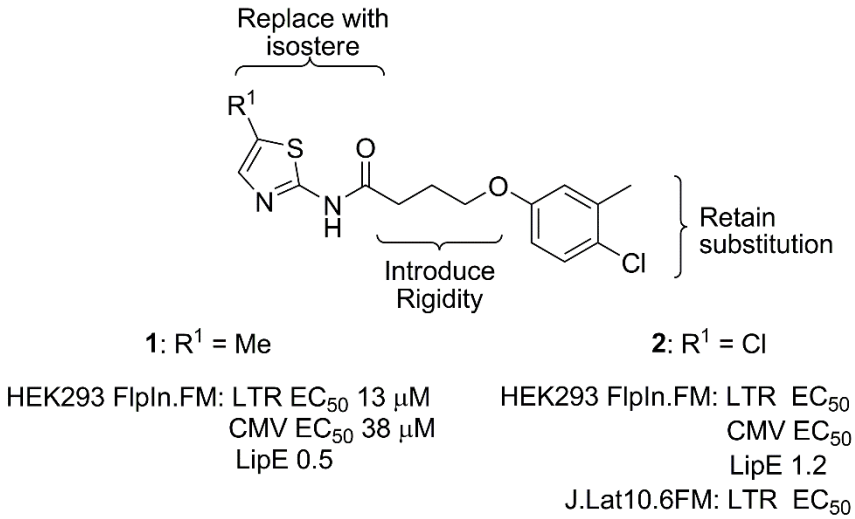


Figure 2. Design rationale of new analogues in this study and the previously reported activity of 2-acylaminothiazoles in the FlpIn.FM dual reporter and J.Lat10.6 cell assays.[23]

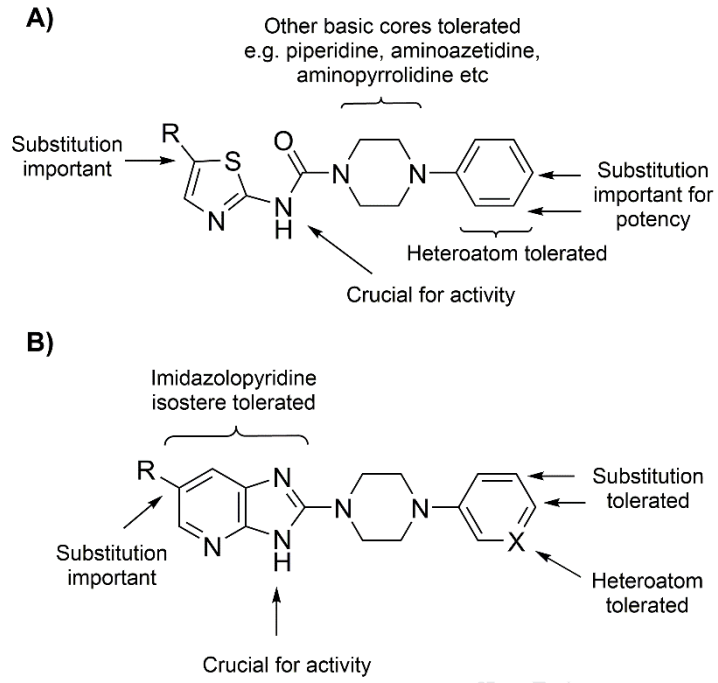
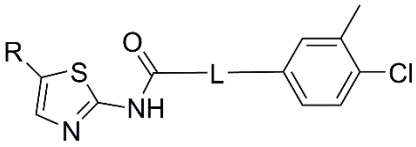
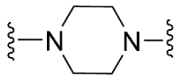
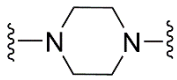
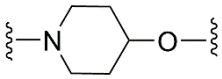
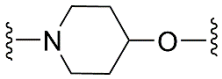
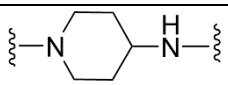
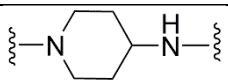
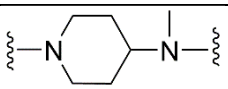
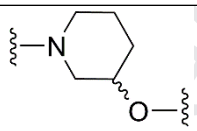
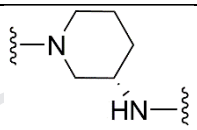
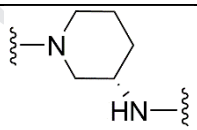
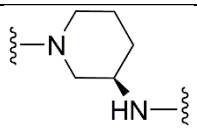
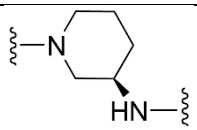
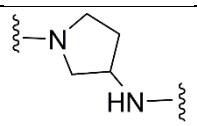
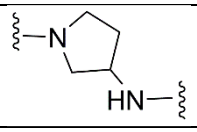
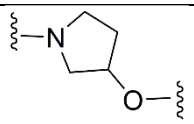
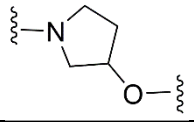
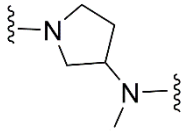
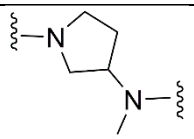


Figure 3. Summary of the structure activity relationship for the A) 5-substituted thiazolyl urea and B) 6-substituted imidazopyridine classes.

TABLES

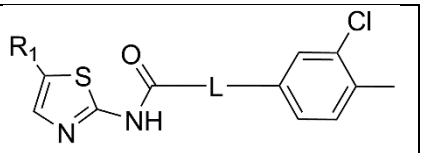
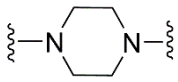
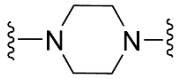
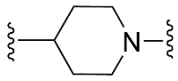
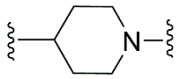
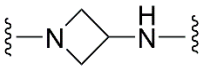
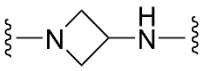
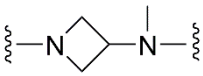
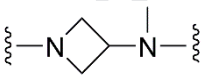
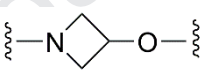
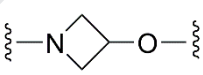
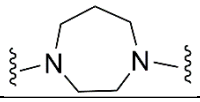
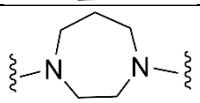
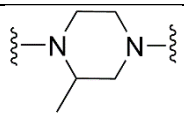
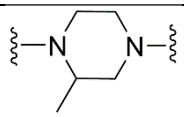
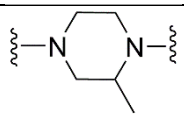
Table 1. Activity of Heterocyclic Analogues in the FlpIn.FM HEK293 Cell Assay.

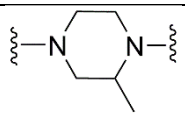
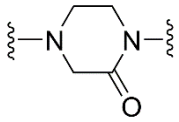
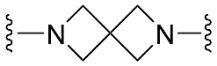
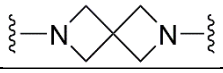
Cmpd							
	R	**L**	**LTR EC_{50} (SD) µM** [a]	**CMV EC_{50} (SD) µM** [a]	**cLog P** [b]	**PSA ($Å^2$)** [b]	**LipE** [c]
16	CH_3		0.60 (0.49)	2.3 (1.3)	4.2	48	2.0
17	Cl		0.40 (0.35)	0.89 (0.9)	4.3	48	2.1
18	CH_3		>40	~40	4.1	54	
19	Cl		20 (4.7)	20 (5.8)	4.2	54	0.5
20	CH_3		>40	~20	3.7	57	
21	Cl		>40	>40	3.8	57	
22	Cl		>40	>40	4.5	48	
23	Cl		>40	n.d.	4.7	54	
24	CH_3		>40	n.d.	4.2	57	
25	Cl		>40	n.d.	4.3	57	
26	CH_3		>40	>40	4.2	57	
27	Cl		>40	>40	4.3	57	
28	CH_3		6.2 (2.0)	14 (6.7)	3.7	57	1.5
29	Cl		0.79 (0.29)	1.8 (0.4)	3.8	57	2.3

30	CH_3		2.0 (1.3)	5.2 (0.24)	4.0	55	1.7
31	Cl		1.5 (0.74)	2.5 (0.03)	4.1	55	1.7
32	CH_3		>40	33 (5.7)	4.3	48	
33	Cl		11 (9.0)	20 (1.3)	4.4	48	0.6

[a] EC_{50} data represents means and SDs for three or more independent experiments measuring bioluminescence of the LTR and the CMV reporter using the HEK293 FlpIn.FM cell line following exposure to compounds in a 10-point dilution series for 72 h. The EC_{50} value is an extrapolated calculation and data points associated with cytotoxicity were excluded where LTR activity begins to decrease with increasing compound concentration. [b] Calculated using ChemAxon software.[37] [c] Calculated using the LTR EC_{50} value.

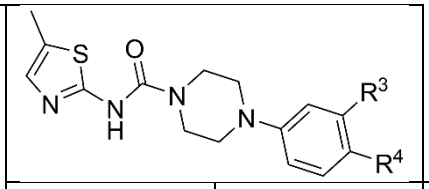
Table 2. Activity of Heterocyclic Analogues in the FlpIn.FM HEK293 Cell Assay.

Cmpd							
	R^1	**L**	**LTR EC_{50}** (SD) µM [a]	**CMV EC_{50}** (SD) µM [a]	**cLog P** [b]	**PSA** ($Å^2$) [b]	**LipE** [c]
34	CH_3		0.47 (0.33)	5.0 (3.7)	4.2	48	2.1
35	Cl		0.24 (0.20)	0.67 (0.28)	4.3	48	2.3
36	CH_3		2.5 (0.77)	>40	4.7	45	0.90
37	Cl		2.0 (1.0)	>40	4.8	45	0.90
38	CH_3		0.67 (0.34)	3.0 (1.5)	3.6	57	2.6
39	Cl		0.23 (0.04)	1.7 (1.9)	3.7	57	2.9
40	CH_3		12 (5.6)	16 (3.3)	4.2	48	0.7
41	Cl		0.93 (0.47)	2.8 (1.7)	4.4	48	1.6
42	CH_3		0.22 (0.11)	1.9 (0.88)	3.9	55	2.8
43	Cl		0.53 (0.16)	1.9 (0.10)	4.1	55	2.2
44	CH_3		10 (0.73)	21 (14)	4.2	48	0.80
45	Cl		22 (15)	19 (0.44)	4.4	48	0.26
46	CH_3		5.0 (2.1)	5.2 (0.78)	4.6	48	0.70
47	Cl		1.3 (1.0)	1.5 (0.27)	4.7	48	1.2
48	CH_3		8.9 (4.4)	6.5 (1.3)	4.6	48	0.45

49	Cl		0.82 (0.24)	3.7 (1.4)	4.7	48	1.4
50	Cl		0.64 (0.30)	1.3 (0.80)	3.2	66	3.0
51	CH_3		>40	>40	3.8	48	
52	Cl		16 (3.4)	19 (13)	4.0	49	0.8

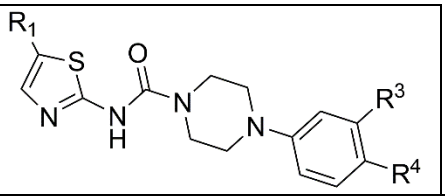
[a] EC_{50} data represents means and SDs for three or more independent experiments measuring bioluminescence of the LTR and the CMV reporter using the HEK293 FlpIn.FM cell line following exposure to compounds in a 10-point dilution series for 72 h. The EC_{50} value is an extrapolated calculation and data points associated with cytotoxicity were excluded where LTR activity begins to decrease with increasing compound concentration. [b] Calculated using ChemAxon software.[37] [c] Calculated using the LTR EC_{50} value.

Table 3. Activity of Aryl Substituted Analogues in the FlpIn.FM HEK293 Cell Assay.

Cmpd							
	R^3	**R^4**	**LTR EC_{50}** (SD) µM [a]	**CMV EC_{50}** (SD) µM [a]	**cLog P** [b]	**PSA** ($Å^2$) [b]	**LipE** [c]
16	CH_3	Cl	0.60 (0.49)	2.3 (1.3)	4.2	48	2.0
34	Cl	CH_3	0.47 (0.33)	5.0 (3.7)	4.2	48	2.1
53	H	H	1.7 (1.1)	5.0 (2.3)	3.1	48	2.7
54	H	Cl	2.7 (0.62)	5.5 (1.3)	3.7	48	1.9
55	Cl	Cl	0.93 (0.57)	0.49 (0.52)	4.3	48	1.7
56	CN	H	0.23 (0.01)	1.8 (0.78)	2.9	72	3.7

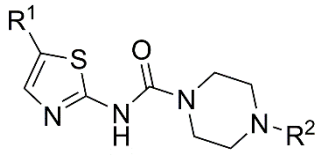
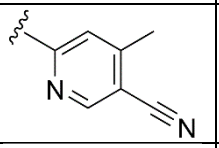
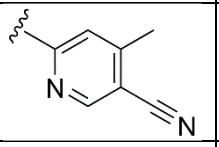
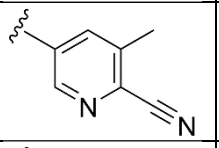
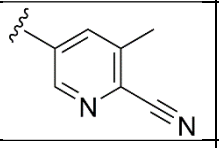
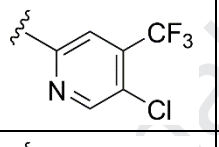
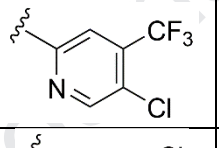
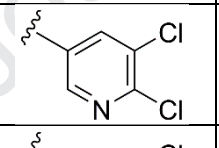
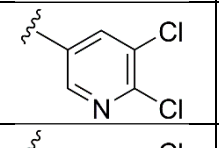
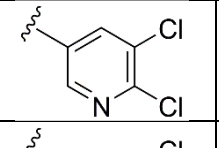
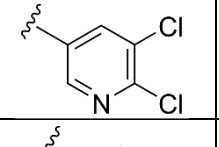
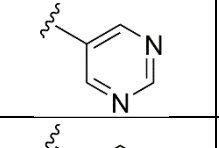
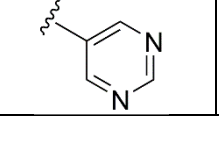
[a] EC_{50} data represents means and SDs for three or more independent experiments measuring bioluminescence of the LTR and the CMV reporter using the HEK293 FlpIn.FM cell line following exposure to compounds in a 10-point dilution series for 72 h. The EC_{50} value is an extrapolated calculation and data points associated with cytotoxicity were excluded where LTR activity begins to decrease with increasing compound concentration. [b] Calculated using ChemAxon software.[37] [c] Calculated using the LTR EC_{50} value. **16** and **34** are included for comparison.

Table 4. Effect of Aryl and Thiazolyl Substitutions on activity in the FlpIn.FM HEK293 Cell Assay.

Cmpd								
	R^1	**R^3**	**R^4**	**LTR EC_{50}** (SD) µM [a]	**CMV EC_{50}** (SD) µM [a]	**cLogP** [b]	**PSA** ($Å^2$) [b]	**LipE** [c]
57	Cl	H	Cl	0.35 (0.07)	1.64 (0.6)	3.8	48	2.7
58	CN	H	Cl	1.5 (0.37)	3.6 (2.9)	3.0	72	2.8
59	H	Cl	CH_3	3.4 (1.8)	4.0 (5.1)	3.5	48	2.0
35	Cl	Cl	CH_3	0.24 (0.16)	0.67 (0.28)	4.3	48	2.3
60	CN	Cl	CH_3	0.24 (0.12)	2.3 (0.9)	3.5	72	3.1
61	CF_3	Cl	CH_3	1.3 (0.81)	>2.0	4.5	48	1.4
62	Cl	Cl	Cl	0.31 (0.08)	0.80 (0.62)	4.4	48	2.1
63	CN	Cl	Cl	0.35 (0.16)	4.4 (4.1)	3.6	72	2.9
17	Cl	CH_3	Cl	0.40 (0.35)	0.89 (0.9)	4.3	48	2.1
64	CN	CH_3	Cl	0.71 (0.35)	2.3 (1.8)	3.5	72	2.6
65	Cl	CN	H	0.61 (0.49)	0.5 (0.4)	3.0	72	3.2

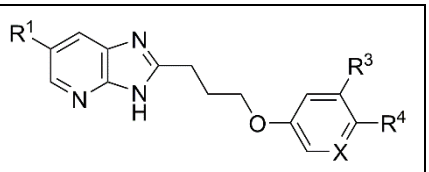
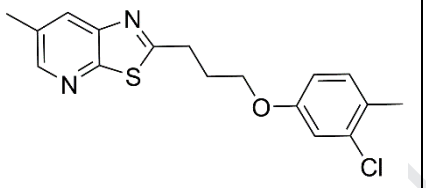
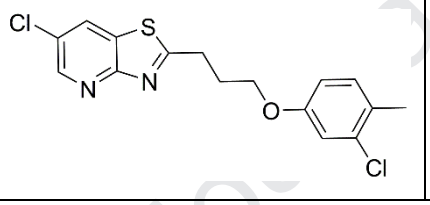
[a] EC_{50} data represents means and SDs for three or more independent experiments measuring bioluminescence of the LTR and the CMV reporter using the HEK293 FlpIn.FM cell line in a 10-point dilution series for 72 h. The EC_{50} value is an extrapolated calculation and data points associated with cytotoxicity were excluded where LTR activity begins to decrease with increasing compound concentration. [b] Calculated using ChemAxon software.[37] [c] Calculated using the LTR EC_{50} value. **17** and **35** are included for comparison.

Table 5. Activity of Heterocyclic Aryl Analogues in the FlpIn.FM HEK293 Cell Assay.

Cmpd							
	R^1	**R^2**	**LTR EC_{50}** (SD) µM [a]	**CMV EC_{50}** (SD) µM [a]	**cLogP** [b]	**PSA** ($Å^2$) [b]	**LipE** [c]
66	CH_3		4.2 (1.7)	>40	2.8	85	2.6
67	Cl		0.46 (0.07)	1.0 (0.96)	2.9	85	3.4
68	CH_3		10 (1.6)	21 (14)	2.6	85	2.4
69	Cl		0.21 (0.01)	0.83 (0.45)	2.7	85	4.0
70	CH_3		0.73 (0.52)	>40	3.9	61	2.2
71	Cl		0.92 (0.38)	1.8 (2.5)	4.0	61	2.0
72	CH_3		0.30 (0.30)	0.57 (0.50)	3.3	61	3.2
73	CN		0.39 (0.32)	0.15 (0.08)	2.6	85	3.8
74	Cl		0.08 (0.06)	0.07 (0.01)	3.4	61	3.7
75	CF_3		0.70 (0.09)	0.48 (0.64)	3.6	61	2.6
76	CH_3		>40	>40	1.1	74	
77	Cl		8.9 (5.2)	12 (4.7)	1.3	74	3.8

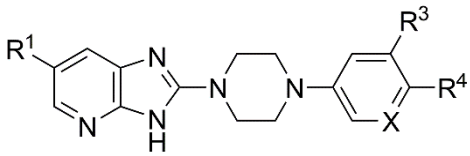
[a] EC_{50} data represents means and SDs for three or more independent experiments measuring bioluminescence of the LTR and the CMV reporter using the HEK293 FlpIn.FM cell line following exposure to compounds in a 10-point dilution series for 72 h. The EC_{50} value is an extrapolated calculation and data points associated with cytotoxicity were excluded where LTR activity begins to decrease with increasing compound concentration. [b] Calculated using ChemAxon software.[37] [c] Calculated using the LTR EC_{50} value.

Table 6. Activity of Imidazopyridine Analogues in the FlpIn.FM HEK293 Cell Assay.

Cmpd									
	R^1	**R^3**	**R^4**	**X**	**LTR EC_{50}** (SD) µM [a]	**CMV EC_{50}** (SD) µM [a]	**cLogP** [b]	**PSA** ($Å^2$) [b]	**LipE** [c]
78	CH_3	Cl	CH_3	C	12 (4.6)	24 (16)	4.2	51	0.7
79	Cl	Cl	CH_3	C	9.4 (3.1)	12 (10)	4.3	51	0.7
80	CH_3	Cl	Cl	C	14 (3.4)	28 (15)	4.3	51	0.6
81	Cl	Cl	Cl	C	3.3 (1.4)	1.2 (0.5)	4.4	51	1.1
82	CH_3	Cl	Cl	N	21 (11)	34 (9.9)	3.3	64	1.4
83	Cl	Cl	Cl	N	6.4 (4.1)	2.4 (0.1)	3.4	64	1.8
84					>40	>40	5.1	35	
85					>40	>40	5.1	35	

[a] EC_{50} data represents means and SDs for three or more independent experiments measuring bioluminescence of the LTR and the CMV reporter using the HEK293 FlpIn.FM cell line following exposure to compounds in a 10-point dilution series for 72 h. The EC_{50} value is an extrapolated calculation and data points associated with cytotoxicity were excluded where LTR activity begins to decrease with increasing compound concentration. [b] Calculated using ChemAxon software.[37] [c] Calculated using the LTR EC_{50} value.

Table 7. Activity of Imidazopyridine Piperazine analogues in the FlpIn.FM HEK293 Cell Assay.

Cmpd									
	R^1	**R^3**	**R^4**	**X**	**LTR EC_{50}** (SD) µM [a]	**CMV EC_{50}** (SD) µM [a]	**cLogP** [b]	**PSA** ($Å^2$) [b]	**LipE** [c]
86	CH_3	Cl	CH_3	C	3.2 (2.3)	2.8 (2.1)	4.6	48	0.9
87	Br	Cl	CH_3	C	0.32 (0.26)	>0.3	4.7	48	1.8
88	CH_3	Cl	Cl	C	0.75 (0.52)	0.82 (0.35)	4.7	48	1.4
89	Cl	Cl	Cl	C	0.98 (0.52)	2.6 (0.88)	4.8	48	1.2
90	CH_3	Cl	Cl	N	1.3 (1.1)	0.13 (0.07)	3.7	61	2.2
91	Cl	Cl	Cl	N	0.08 (0.08)	0.27 (0.17)	3.8	61	3.3

[a] EC_{50} data represents means and SDs for three or more independent experiments measuring bioluminescence of the LTR and the CMV reporter using the HEK293 FlpIn.FM cell line following exposure to compounds in a 10-point dilution series for 72 h. The EC_{50} value is an extrapolated calculation and data points associated with cytotoxicity were excluded where LTR activity begins to decrease with increasing compound concentration. [b] Calculated using ChemAxon software.[37] [c] Calculated using the LTR EC_{50} value.

Table 8. Physicochemical Properties and in vitro Metabolism of Selected Analogues.

	Aqueous solubility [a]		**Human liver microsomes**			**Mouse liver microsomes**		
Cmpd	**pH 2.0** (μM)	**pH 6.5** (μM)	**Half-life** (min)	***in vitro* CL_{int}** (μL/min/mg protein)	**Predicted E_H** [b]	**Half-life** (min)	***in vitro* CL_{int}** (μL/min/mg protein)	**Predicted E_H** [b]
1	< 1.1	1.1 - 2.3	10	175	0.87	2	815	n.d.
29	6.3 - 12.5	1.6 - 3.1	n.d.	n.d.	n.d.	11	151	0.76
35	< 1.6	< 1.6	n.d.	n.d.	n.d.	33	53	0.53
39	1.6 - 3.1	1.6 - 3.1	n.d.	n.d.	n.d.	36	49	0.51
50	1.6 - 3.1	1.6 - 3.1	n.d.	n.d.	n.d.	60	29	0.38
63	< 1.6	< 1.6	151	11	0.31	117	15	0.24
65	< 1.6	< 1.6	n.d.	n.d.	n.d.	46	38	0.45
73	< 1.6	< 1.6	n.d.	n.d.	n.d.	80	22	0.32
74	< 1.6	< 1.6	70	25	0.5	55	32	0.41
79	1.6 - 3.1	< 1.6	5	329	0.93	< 2	>866	>0.94
83	6.3 - 12.5	1.6 - 3.1	n.d.	n.d.	n.d.	14	122	0.72
86	50 - 100	< 1.6	15	115	0.82	8	224	n.d.
91	25 - 50	1.6 - 3.1	n.d.	n.d.	n.d.	13	132	0.74

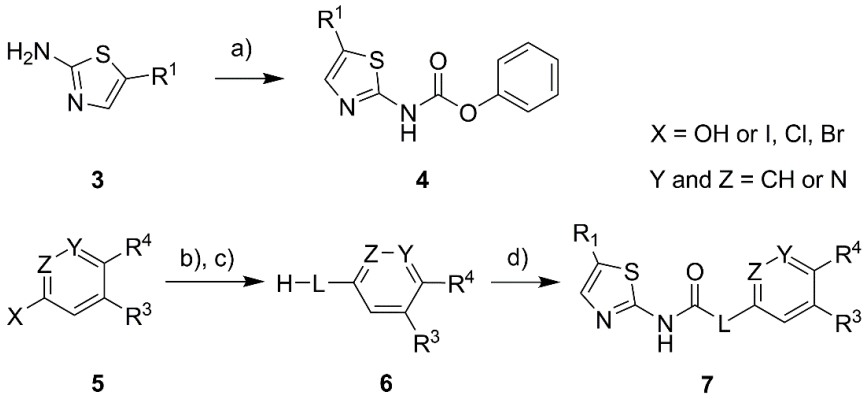
[a] Estimated by nephelometry. [b] Predicted hepatic extraction (E_H) ratio based on *in vitro* intrinsic clearance (CL_{int}); n.d. – not determined.

Table 9. Evaluation of Selected Analogues in Cellular Assays.

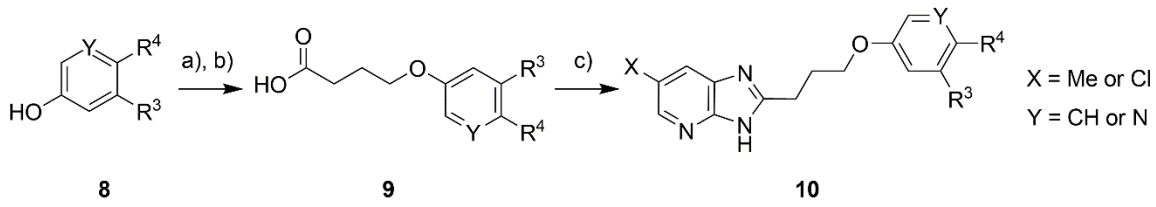
	HEK293 FlpIn.FM [a]			J.Lat10.6 [b]		HepG2 [c]
Cmpd	**LTR EC_{50}** (SD) µM	**CMV EC_{50}** (SD) µM	**Max LTR activity%** [at cmpd conc in µM]	**LTR EC_{50}** (SD) µM	**Max LTR activity%** [at cmpd conc in µM]	**CC_{50}** (SD) µM
2	1.9 (0.3)	>40	39 [1.3]	29 (3.9)	15 [20]	4.4 (0.5)
29	0.79 (0.29)	1.8 (0.4)	39 [0.63]	3.0 (0.48)	20 [2.0]	0.68 (0.17)
35	0.24 (0.20)	0.67 (0.28)	53 [0.25]	1.7 (0.2)	23 [1.0]	0.25 (0.05)
39	0.23 (0.04)	1.7 (1.9)	63 [0.25]	1.5 (0.15)	31 [1.0]	0.33 (0.12)
50	0.64 (0.30)	1.3 (0.8)	47 [0.31]	2.7 (0.27)	38 [2.0]	0.42 (0.07)
63	0.35 (0.16)	4.4 (4.1)	87 [5.0]	1.6 (0.21)	30 [1.0]	0.41 (0.02)
65	0.61 (0.49)	0.5 (0.4)	22 [0.31]	0.48 (0.1)	28 [0.25]	0.05 (0.02)
73	0.39 (0.32)	0.15 (0.08)	25 [0.16]	1.1 (0.31)	28 [0.25]	0.12 (0.02)
74	0.08 (0.06)	0.07 (0.01)	65 [0.13]	0.29 (0.09)	26 [0.13]	0.04 (0.02)
83	6.4 (4.1)	2.4 (0.09)	26 [0.13]	>4	11 [2]	1.9 (0.73)
91	0.08 (0.08)	0.27 (0.17)	57 [0.09]	0.24 (0.05)	28 [0.06]	0.04 (0.01)
Romidepsin [d]	0.001 (<0.001)	0.02 (<0.01)	19 [0.01]	0.22 (0.73)	61 [0.01]	0.01 (<0.01)
Vorinostat [d]	4.6 (1.2)	>40	22 [2.5]	2.3 (0.2)	60 [5]	12 (0.6)
Panobinostat [d]	1.8 (0.7)	0.06 (0.03)	31 [0.2]	0.13 (0.03)	59 [0.1]	<0.078
JQ1 (+) [d]	14 (0.7)	>40	33 [5]	8.1 (2.0)	34 [2.5]	9.2 (3.7)

[a] HEK293 FlpIn.FM values taken from other tables for comparison. [b] EC_{50} data represents means and SDs for three independent experiments measuring bioluminescence in the J.Lat10.6 GFP reporter cell line following exposure to compounds in a 10-point dilution series for 48 h. The EC_{50} value is an extrapolated calculation and data points associated with cytotoxicity were excluded where LTR activity begins to decrease with increasing compound concentration. Max LTR activity% refers to the highest percentage LTR reporter activity observed at the indicated concentration of compound. [c] CC_{50} data represents means and SDs for three HepG2 growth inhibition experiments in a 10-point dilution series over 48 h. Cell Titre-Glo was used to quantify cell growth inhibition. [d] Taken from previous results.[23]

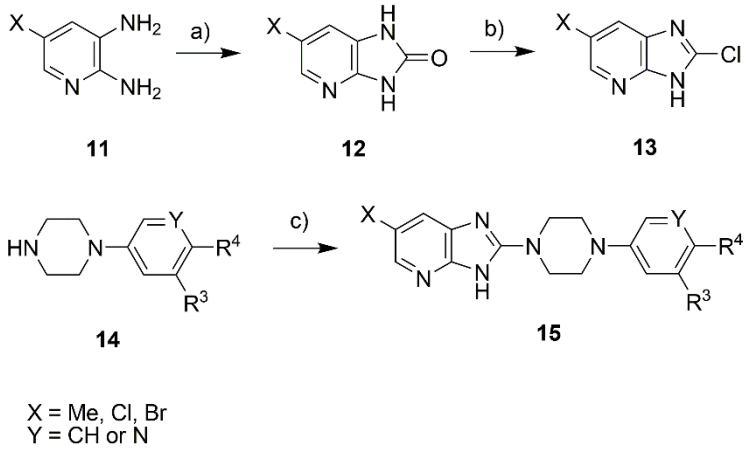
SCHEMES



Scheme 1. General Synthetic Pathway to Access Thiazolyl Ureas. *Reagents and conditions:* (a) phenyl chloroformate, pyridine, 0 °C; (b) Boc-L-H, For X = Br: rac-BINAP, $Pd(OAc)_2$, Cs_2CO_3, 1,4-dioxane, reflux; For X = I or Cl: $Pd_2(dba)_3$, Xantphos, *t*-BuOK, toluene, reflux; For X = OH: DIAD, PPh_3, THF; (c) TFA/DCM 20 °C; (d) **4**, Cs_2CO_3, 1,4-dioxane, reflux.

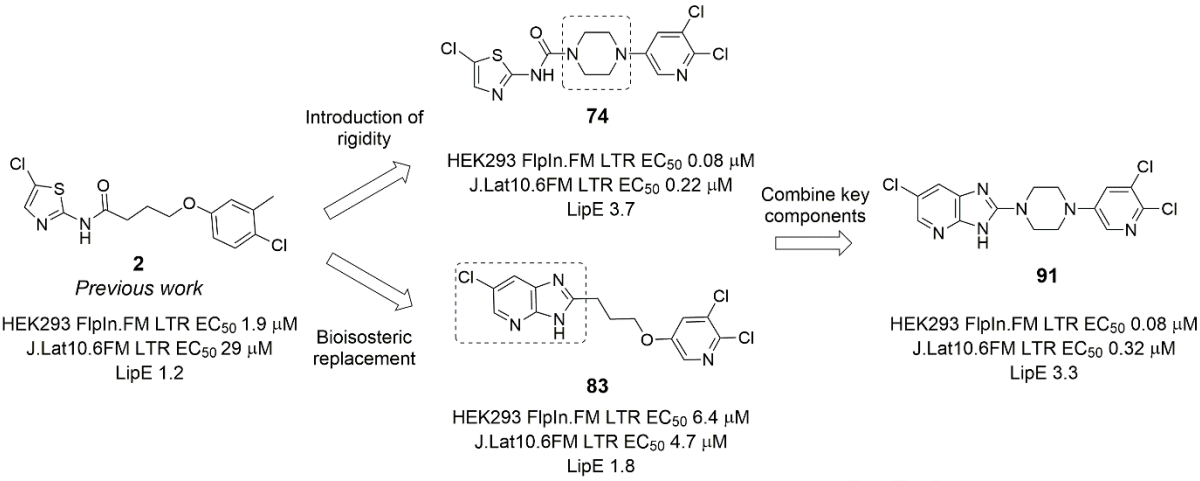


Scheme 2. General Synthesis of Imidazopyridine Analogues. *Reagents and conditions:* (a) Carboxy-protected 4-bromobutanoate, K_2CO_3, DMF; (b) LiOH, THF, H_2O, 20 °C; (c) substituted diaminopyridine, $POCl_3$, reflux.



Scheme 3. Synthetic Pathway to Access Imidazopyridine Analogues. *Reagents and conditions:* (a) disuccinimido carbonate, $CHCl_3$, reflux; (b) $POCl_3$, reflux; (c) **13**, DMSO, DIPEA, 80 °C.

GRAPHICAL ABSTRACT



HIGHLIGHTS:

- The optimized 2-acylaminothiazole class displays potent HIV LTR activity.
- Rigidification of the oxycarbon motif with piperazine enhances HIV gene expression.
- Imidazopyridine is a suitable bioisostere for the 2-acylaminothiazole motif.
- Lead compounds display potent HIV gene activation in the JLat10.6 cell line.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: