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Journal

Journal of Medicinal Chemistry, 62(2)

ISSN

0022-2623

Authors

Bachovchin, Kelly A
Sharma, Amrita
Bag, Seema
et al.

Publication Date

2019-01-24

DOI

10.1021/acs.jmedchem.8b01365

Peer reviewed

HHS Public Access

Author manuscript

J Med Chem. Author manuscript; available in PMC 2020 January 24.

Published in final edited form as:

J Med Chem. 2019 January 24; 62(2): 665–687. doi:10.1021/acs.jmedchem.8b01365.

Improvement of Aqueous Solubility of Lapatinib-derived Analogs: Identification of a Quinolinimine as a Lead for Human African Trypanosomiasis Drug Development

Kelly A. Bachovchin[1,ϕ], **Amrita Sharma**[2,ϕ], **Seema Bag**[1], **Dana M. Klug**[1], **Katherine M. Schneider**[1], **Baljinder Singh**[1], **Hitesh B. Jalani**[1], **Melissa J. Buskes**[1], **Naimee Mehta**[1], **Scott Tanghe**[3,4], **Jeremiah D. Momper**[5], **Richard J. Sciotti**[6], **Ana Rodriguez**[3,4], **Kojo Mensa-Wilmot**[2,*], **Michael P. Pollastri**[1], and **Lori Ferrins**[1,*]

[1]Department of Chemistry and Chemical Biology, Northeastern University, Boston, MA 02115

[2]Department of Cellular Biology, Center for Tropical and Emerging Global Diseases, University of Georgia, Athens, GA 30602

[3]New York University School of Medicine, Department of Microbiology, 430 E. 29th St. New York, NY 10016

[4]Anti-Infectives Screening Core, New York University School of Medicine, New York, NY 10016

[5]Skaggs School of Pharmacy and Pharmaceutical Sciences, University of California, San Diego, La Jolla, CA 92093

[6]Experimental Therapeutics, Walter Reed Army Institute for Research, 2460 Linden Lane, Silver Spring, MD, 20910

Abstract

Lapatinib, an approved EGFR inhibitor, was explored as a starting point for the synthesis of new hits against *Trypanosoma brucei*, the causative agent of human African trypanosomiasis (HAT). Previous work culminated in **1 (NEU-1953)**, which was part of a series typically associated with poor aqueous solubility. In this report, we present various medicinal chemistry strategies that were used to increase the aqueous solubility and improve the physicochemical profile without sacrificing anti-trypanosome potency. To rank trypanocidal hits, a new assay (summarized in a

*Corresponding Author: Lori Ferrins; l.ferrins@northeastern.edu; Kojo Mensa-Wilmot; mensawil@uga.edu.
ϕThese authors contributed equally

Supplementary Information

The supplementary information is available free of charge and includes:

Synthetic schemes and characterization data for compounds not presented directly in the manuscript (and not previously published)
LogD, pK_a and rat hepatocyte Cl_{int} data for all compounds disclosed in the manuscript
Calculated pK_a values for compounds **1** and **10e**
Comparison of LogP and *T. brucei* pEC_{50}
Crystal structure of compound **19a**
Curves obtained from the CEC_{50} assay
PK parameters and plasma concentration-time profile for compound **22a**
Human kinase profile of compounds **1** and **22a**
Ames testing results for **22a**
HitProfilingScreen panel results of **22a**
Molecular formula strings

cytocidal effective concentration (CEC_{50})) was established, as part of the lead selection process. Increasing the sp^3 carbon content of **1** resulted in **10e** (0.19 μM EC_{50} against *T. brucei* and 990 μM aqueous solubility). Further chemical exploration of **10e** yielded **22a**, a trypanocidal quinolinimine (EC_{50}: 0.013 μM, aqueous solubility: 880 μM, and CEC_{50}: 0.18 μM). Compound **22a** reduced parasitemia 10^9-fold in trypanosome-infected mice; it is an advanced lead for HAT drug development.

Introduction

Human African trypanosomiasis (HAT) is a neglected tropical disease that is caused by the protozoan parasite *Trypanosoma brucei*.[1] The two subspecies responsible for human infection, *T.b. gambiense* (western and central Africa) and *T.b. rhodesiense* (eastern and southern Africa), cause chronic and acute forms of the disease respectively, and infection is considered 100% fatal if left untreated.[2] HAT is a threat to over 70 million people in sub-Saharan Africa, where the tsetse fly vector can be found, and is endemic in many rural areas.[3, 4] In 2015, fewer than 3,000 cases were reported, largely due to disease elimination efforts set by the World Health Organization (WHO), which include vector control, case reporting, and availability of treatments.[4] Vaccines are not available for HAT prevention, due to extensive antigenic variation of the major surface protein, variant surface glycoprotein.

Chemotherapy for HAT is specific to the stage of disease as well as the subspecies of *T. brucei*. The disease occurs in two stages; the first stage starts with trypanosomes in the blood and lymphatic systems, causing influenza-like symptoms. Stage two of the disease is coincident with parasites crossing the blood-brain barrier, and is associated with disrupted sleep patterns, leading to coma and death.[2, 4, 5] Current drugs for HAT have significant shortcomings in terms of toxicity, efficacy, and convenience of dosing; treatment failure that could be a result of drug resistance has also emerged.[4]

Two orally-administered leads, fexinidazole[6–8] and acoziborole (**SCYX-7158**),[9] are in the midst of clinical trials. Because of well-documented high failure rates in drug development,[10–12] and the possibility of drug resistance,[13] we believe that it is prudent to continue populating the pipeline of anti-trypanosome leads with backup compounds that meet the target product profile (TPP) set by WHO and the Drugs for Neglected Diseases *initiative* (DND*i*).[14, 15]

To streamline our anti-trypanosome drug discovery effort, we used a target class repurposing approach to improve the efficacy against *T. brucei* of the U.S. Food and Drug Administration approved drug lapatinib.[16] A series of medicinal chemistry optimization campaigns produced the highly potent **NEU-617**;[1] subsequent work led to **1** (**NEU-1953**[17]), a compound that was moderately potent against *T. brucei*, but had limited aqueous solubility and high microsomal clearance (Table 1).[18, 19] Side-stepping the daunting task of simultaneously improving anti-trypanosome potency, aqueous solubility and metabolic clearance of these hits, we began efforts to improve physicochemical properties with a focus on increasing solubility of the compounds.

Several medicinal chemistry strategies can be employed to increase the aqueous solubility of compounds. These include increasing charge and polarity (through salt formation[22, 23] or introduction of hydrophilic functional groups), reducing planarity by increasing sp^3 carbon content,[24, 25] or reducing the aromatic ring content.[26, 27] We present our application of different strategies to improve the aqueous solubility and overall absorption, distribution, metabolism and excretion (ADME) profile of compound **1**, as part of an ongoing effort to produce an advanced lead that meets the criteria shown in Table 1. The progression of our hit-to-lead optimization process followed the schematic highlighted in Figure 1, wherein compounds were filtered through a series of selection parameters that were reflective of the TPP.[14, 15] Given that the TPP for HAT indicates a preference for compounds that act irreversibly against the trypanosome, we developed a cidality assay that we used to prioritize compounds that exhibited such an effect. Compounds were progressed into mouse model studies based upon these combined results (Figure 1).

Results and Discussion

Our goal was to improve the physicochemical properties, specifically focusing on the aqueous solubility, of the previously reported compound **1**[20] whose liabilities as an anti-trypanosome hit are presented in Table 1. We also paid close attention to the lipophilic ligand efficiency (LLE) of each analog, in comparison to **1**.[28] Calculated as the difference between the pEC_{50} and cLogP, LLE is now a frequently used metric in medicinal chemistry campaigns that normalizes the potency of an inhibitor to its lipophilicity; this allows easier comparison of compound quality as the optimization program proceeds.[29] Furthermore, given that compound lipophilicity can adversely impact pharmacokinetic properties, employment of LLE helps to ensure that the best balance of lipophilicity and potency is struck to identify molecules with a good ADME profile.

The approaches we pursued can be categorized as either increasing charge and polarity at biological pH or decreasing planarity of the hits. Thermodynamic solubility (extent to which a compound dissolves) is a more useful metric in guiding the optimization of compounds than kinetic solubility (the extent to which a compound precipitates from a solution).[30] Furthermore, thermodynamic solubility of a compound is more relevant in the biological assays used in the assessment of potency of our hits. As such, our hit optimization campaign tracked thermodynamic solubility throughout instead of the kinetic solubility of compounds, although we have assessed kinetic solubility where necessary.

Increasing charge and polarity

Salt screening.—A common strategy used to increase the kinetic aqueous solubility of a series is through the formation of salts at acidic or basic centers on compounds.[22, 23] Initially, a range of salts of **1** were studied (Table 2), taking advantage of the basic nitrogen atoms within the compound. The hydrochloride (**1a**) and sulfate (**1b**) salt forms remained unchanged in both potency and kinetic solubility, while the citrate salt (**1c**) showed a modest increase in kinetic solubility. The methanesulfonate salt (**1d**) demonstrated a 4-fold increase in the kinetic solubility. However, the thermodynamic solubility decreased 2-fold for the

methanesulfonate (**1d**) salt form, remained unchanged for the hydrochloride (**1a**) and sulfate (**1b**) salt forms and increased modestly for the citrate (**1c**) (25%).

Modification of ionizable groups.—A second strategy employed was introduction of functional groups that are charged at physiological pH. For this purpose, we added ionizable groups to the piperazine. A general synthetic scheme for these analogs is presented in Scheme 1. Briefly, 5-bromo-2-chloropyrimidine (**2**) was substituted with the appropriate amines in good to excellent yields. The resulting bromide intermediates **3a**-**3y** were subjected to Miyaura borylation to give the corresponding boronic acid pinacol ester **4a**-**4p** that, in a Suzuki reaction with **5**,[20] led to the desired products **6a**-**6j** and **10a**-**10j** in acceptable to excellent yields.

Replacement of the piperazine ring with ethylene diamine (**6a**, Table 3) led to a loss of activity against *T. brucei* and decreased aqueous solubility though the LLE remained high due to the reduced cLogP (0.81 compared with 2.1 for compound **1**). The synthetic precursor *tert*-butyl carbamate (**6b**) was predictably less soluble. The methylated, acyclic tail (**6d**) raised aqueous solubility but dropped anti-trypanosomal potency ~4-fold compared to **1**, however this was also accompanied by an improvement in the human liver microsome intrinsic clearance (HLM Cl_{int}: 45 μL/min/mg) though this was still outside of our targeted range. The demethylated piperazine derivative **6f** displayed a 6-fold loss in potency, the solubility remained unchanged and the HLM Cl_{int} was significantly improved (30 μL/min/mg) suggesting that demethylation of the piperazine is one of the major metabolites in this series. The *tert*-butyl carbamate precursor (**6e**) was far less soluble than **1**, with retention of moderate anti-trypanosome activity. Substitution of the piperazine with methylene carboxylic acid (**6h**, presumably present as a zwitterion *in vitro*), and the corresponding methyl ester (**6g**), made as a potentially more cell-permeable pro-drug of **6h**, lost potency and aqueous solubility compared to **1**. Finally, the piperazine was replaced by proline (**6i**) and its methyl ester derivative (**6j**); both were inactive against *T. brucei,* and no improvement in the ADME profile compared to **1** was evident. Thus, analogs that are charged at biological pH do not consistently have better aqueous solubility than **1**.

Reductions in planarity

Pseudo-ring analogs.—We looked to increase the conformational flexibility of the quinoline series by breaking one of the rings of the central core; we hoped to maintain *T. brucei* activity by taking advantage of a possible intramolecular hydrogen bond (Supplementary Information, Table S7). This scaffold also has the advantage of incorporating additional hydrogen bond donors, increasing its potential interactions with water. Applying this strategy to a compound related to **1**, compound **7**, led to compound **8** (Figure 2). However, despite breaking the bicyclic aromatic system (**8**) this strategy did not show any significant improvement to the aqueous solubility of the series or potency against *T. brucei*. An improvement in the metabolic stability was observed (24 μL/min/mg) and was representative of this series of analogs, however due to the lack of potency against *T. brucei* (and associated with a decreased LLE) this strategy was not pursued further.

Methylation of the quinoline core.—Methylation reduces planarity in biaryl motifs,[31] and we hypothesized that such a conformational change would increase solubility. Synthesis of compounds followed an analogous route to a previously reported procedure,[20] the details for which are presented in the Scheme S1, Supplementary Information. However, introduction of the methyl groups led to a significant decrease in the potency against *T. brucei* causing a reduction in the LLE of the compounds was was accompanied by increased toxicity toward HepG2 (human liver) cells, and a substantial decrease in aqueous solubility (Table 4).

Increased sp^3 atom content.—We explored increasing the sp^3 content of the tail group (Table 5), replacing the piperazine ring with groups that have increased three-dimensionality. Bridged piperazines in **10a** and **10b** improved aqueous solubility 20-fold, as compared to **1**, conferred a slight improvement in potency against *T. brucei*, exhibited an improved LLE, and a 4-fold increase in metabolic stability. Ethyl and propyl substituted piperazines (**10c** and **10d** respectively) were ineffective at improving aqueous solubility, though potencies were comparable or better than **1**. Swapping the piperazine moiety for alkyl-substituted homopiperazines (**10e**, **10f** and **10g**) yielded highly soluble compounds coupled with a 2-to-4-fold improvement in anti-trypanosome potency. In the case of **10f** and **10g**, there was a noticeable decrease in metabolic stability that makes these hits less attractive for anti-trypanosome drug development, compared to **10e**. Similarly, the spirocyclic derivatives **10i** and **10j** showed 2-to-3-fold improvement in potency compared with **1** and were both highly soluble. By far, the bridged piperazine (**10a**), the methylhomopiperazine (**10e**) and the spirocyclic derivatives (**10i** and **10j**) were the most improved compounds in terms of anti-trypanosome potency, LLE, metabolic stability, and aqueous solubility. For **10e** we also obtained calculated pK_a data and compared it with **1** (see Supplementary Information, Table S4), the aliphatic nitrogen on the homopiperazine was found to be between 0.8-1.2 log units more basic than **1**. Closer examination of the LogD values (see Supplementary Information, Table S3) for this set of analogs revealed that the addition of a one carbon bridge to the piperazine (**10a** and **10b**) and replacement with spirocyclic derivatives (**10i** and **10j**) led to a decrease in the LogD compared with **1**, which is consistent with a recent report from AstraZeneca.[32] We also observed a similar trend with two of the homopiperazine analogs (**10e** and **10f**), while **10g** had the same LogD value as **1**, despite the addition of 3 methylenes.

Saturated rings were also used to replace the pyrazine head group of **1** to explore the solubility effect of increasing sp^3 carbon content in this region. While the synthesis initially employed a Buchwald amination to install the saturated primary amines (required for the preparation of compounds **15b** and **15d**) (Scheme S2, Supplementary Information), subsequent optimization led to an alternative route where the dihalogenated intermediate (**11**)[20] was subjected to S_NAr conditions with various primary amines to give compounds **12a**-**12h**. A subsequent Suzuki coupling utilizing both the *N*-methylpiperazine pyrimidine boronic acid, pinacol ester (**14**, prepared from the bromo precursor **13**)[20] as well as the *N*-methylhomopiperazine pyrimidine boronic acid, pinacol ester (**4p**, prepared from **3m**) provided desired compounds **15a**, **15c**, **15e**-**15g** and **17a**-**17c**.

The analog bearing the cyclohexylamine, **15a** (Table 6), showed a modest improvement in solubility and potency over **1**, but was less metabolically stable and showed a reduction in LLE due to an increase in the cLogP (3.8 compared with 2.1 for compound **1**). Tertiary amines (**15b**, **15c** and **15d**) all exhibited improved solubility with moderate-to-excellent anti-trypanosome potencies. Compound **15d** is 20-fold more potent than **1** with a 10-fold improvement in aqueous solubility and significantly improved LLE (5.3). However, its high plasma protein binding (PPB; 100%) and toxicity against HepG2 cells are liabilities that make it less desirable for progression. Replacement of the headgroup with the tetrahydropyrans (**15e** and **15f**) maintained activity against *T. brucei*, when compared to **1**, and improved aqueous solubility 15-fold but again, increased toxicity against HepG2 cells.

In summary, modulation of the aqueous solubility of the headgroup of **1** could be achieved through increasing the sp^3 content. However, these analogs typically exhibited increased toxicity in HepG2 cells and were less metabolically stable than those presented in Table 5.

Insertion of -NH linker.—Addition of an -NH linker between ring systems in the molecule could be explored using a previously described synthetic route,[20] and increased aqueous solubility in a position-dependent manner (Table 7). Placing a nitrogen atom between the aromatic rings of the tail and core groups of the molecule increased the aqueous solubility. For example, compare **1** to the matched analog **16c**, which showed a ~12-fold reduction in potency, although a 3-fold improvement in aqueous solubility occurred. Moving the amine linker from the 5- to the 4-position of the pyrimidine (**16d**) restored some of the potency when compared to **16c**, but at a loss of aqueous solubility. Shifting the pyrimidine nitrogen (**16e**) restores some of the aqueous solubility that was lost in **16d** and retains potency compared to **1**. Inclusion of the nitrogen between the phenyl ring of the tail group and the quinoline core (**16b**), greatly improved the solubility whilst maintaining potency compared to **16a**. The addition of a nitrogen atom between the piperazine and pyrimidine rings (**16f**) increased solubility over 20-fold, decreased plasma protein binding to 51%, and increased metabolic stability 10-fold but dropped anti-trypanosomal potency 5-fold. Given the lack of broad applicability of this strategy to the series, we sought other avenues by which to increase the aqueous solubility.

At this point, **10e** (Table 5) emerged as the preferred chemotype for optimization because of its improved metabolic stability, aqueous solubility, plasma protein binding, and potent anti-trypanosomal activity. To further explore the SAR, we performed a methyl scan on the pyrazine headgroup (Table 8). These analogs are matched pairs with **10e** and were synthesized following the procedure outlined in Scheme 2. Addition of a methyl group at R^1 (**17a**) or R^3 (**17c**) was tolerated, whilst maintaining excellent aqueous solubility. Conversely, substitution with a methyl group at the R^2 position (**17b**) reduced anti-trypanosome potency 2-fold, compared to **10e**, and reduced aqueous solubility, although it is still significantly higher than our starting point (**1**) and the targeted threshold of 100 μM (Table 1). Finally, alkylation of the endocyclic nitrogen (**18**) was detrimental to anti-trypanosome potency, whilst the physicochemical features were relatively unchanged compared to **10e**.

Alkylation of the -NH was attempted using the conditions outlined in Scheme 3 however, alkylation occurred on the quinoline nitrogen (**19a**) rather than the endocyclic nitrogen as

expected. Alkylation on the quinoline nitrogen, and the E configuration of the imine was confirmed by x-ray crystallography (Figure S2, Supplementary Information). A compound with a related structure has previously been reported utilizing reaction conditions similar to those employed here[33] and there are only a few other examples of the quinolinimine scaffold available in the literature.[34–36] Further, analysis of the proton NMR reveals a characteristic chemical shift (~6.8 ppm) for the proton at the 3-position of the quinolinimine. A Suzuki coupling with the relevant boronic acid was used to obtain the final product **22a**, whose hydrolytic stability was confirmed under both acidic and basic conditions. Further, the stability of **22a** was assessed in the presence of a hard and soft nucleophile; it was found to be stable in the presence of both glutathione and cyanide (Supplementary Information). To access the originally desired analog (**18**, Scheme 3b), methylation of the 2-aminopyrazine (**23**) was readily achieved to give **24** which could be used to displace the chloro **11**, before a Suzuki coupling to provide **18** in good yield.

Alkylation of the quinoline nitrogen with the methyl, ethyl and propyl groups (**22a**, **22b** and **22c** respectively) maintained excellent aqueous solubility, reduced PPB, and increased metabolic stability. Compound **22a** demonstrated a significant boost in potency (~15-fold) when compared to **10e**, which translated to an improved LLE (5.4 compared with 4.6 for **10e**). The ethyl and propyl derivatives (**22b** and **22c**) proved to be more metabolically labile, although they were improved over our original compound **1**.

A comparison of the melting points of **1** (aqueous solubility: 44 μM), **10e** (aqueous solubility: 990 μM) and **22a** (aqueous solubility: 880 μM) (see Table S2, Supplementary Information) revealed a decrease in the melting point range with the increased solubility of **10e** and **22a**. This suggests that one of the factors influencing the increased solubility of **10e** and **22a** is the disruption of the crystal packing in these analogs.

Cidal effective concentration (CEC_{50}) can inform selection of hits for advancement

Given that the TPP for HAT indicates a preference for compounds that act irreversibly against the trypanosome,[15] an assessment of trypanocidality was undertaken for hits with $EC_{50} \leq 200$nM. Here trypanosomes (1×10^5/mL) were incubated with hit (concentration 25-times EC_{50}, *i.e.* $25\times EC_{50}$) for 6 h (Figure 3) or 12 h (data not shown). The number of trypanosomes used in this cidal assay was adjusted to maintain the ratio of trypanosome density-to-hit concentration as was used in the 48 h proliferation assay employed to determine EC_{50}. At the end of incubation, cell density was checked, compound was washed off, and trypanosomes were incubated in fresh drug-free medium at density of 10^4/mL. After 48 h culture in absence of hits, trypanosomes were enumerated using a hemocytometer.

Data presented on the left side of Figure 3 shows that in the first 6 h of incubation with hits, trypanosomes were viable. For cells treated with cidal hits, the expected density of trypanosomes is 50% of the control (*i.e.,* DMSO-treated) at the end of 48 h culture at the next stage of this cidal assay. Trypanosomes treated with **22c** (Table 9) had a density above 50% of the control value, meaning that the compound is not cidal at 6 h, based on data expected from the EC_{50} values. Trypanosomes treated with **22b** (Table 9) had densities that were approximately 50% of the controls, implying that the hit is cidal. The most

trypanocidal compounds, using the parameters presented above was **22a** (Table 9); for this compound trypanosome density was 25% that of the controls. In comparison to the clinical candidate, acoziborole (**SCYX-7158**), compound **22a** appears to exhibit less of a cidal effect. However, because the anti-trypanosomal potency of acoziborole (*T. brucei* EC_{50}: 0.29 μM) is higher than the other compounds, a significantly higher concentration of acoziborole (7.2 μM) was used in the cidal assay compared to **22a** (0.33 μM) making a direct comparison difficult.

To obtain a more robust measure of cidality, the study summarized in Figure 4 was performed. Using the protocol described in Figure 3, we tested several concentrations of hits around the amount used in the single-point assay ($25\times EC_{50}$), to arrive at CEC_{50}, the drug concentration at which 50% of trypanosomes are killed after a 6 h exposure (Figure 4). When comparing CEC_{50} with $25\times EC_{50}$ for multiple hits a linear relationship is expected in a plot, because the assumption behind the 48 h proliferation assay, used routinely in the field, is that trypanosome growth arrest occurs because a drug kills the parasite cells. For a drug development project, it is preferable that killing of the trypanosome takes place within one cell division cycle (6 h) of drug treatment. Compounds that kill in >6 h are revealed by significant deviation of a compound's data point from an idealized linear correlation between $25\times EC_{50}$ and CEC_{50} (see trend line in Figure 4). For **22a** and **22b** the CEC_{50} values fall on the trend line ($CEC_{50}/25\times EC_{50}$ is between 0.95-1.1), indicating that they are cidal after trypanosomes are exposed to them for 6 h.

Efficacy of 22a in a mouse model of HAT

Recognizing that it is difficult to attain a concentration in blood above 500 nM for most drugs administered orally to vertebrates, we considered **22a** as a candidate for testing in a murine model of HAT, because of its low CEC_{50} (175 nM), potency (*T. brucei* EC_{50} = 0.013 μM), selectivity over mammalian cells (selectivity index > 2000), low PPB (15%), excellent aqueous solubility (880 μM) and reasonable metabolic stability (22 μL/min/mg; Table 9). A preliminary toxicity study with **22a** showed no adverse effects when dosed orally to mice at 100 mg/kg. Pharmacokinetic (PK) parameters were measured in plasma following oral administration of 100 mg/kg of **22a** (see Table S6, Supplementary Information). To achieve efficacy in a mouse model of HAT, we aimed to exceed $10\times EC_{50}$ for 6-8 h (Table 1), and at this dose, we were in excess of 0.013 μM ($10\times EC_{50}$) for 13 h in blood (see Figure S4, Supplementary Information).

In a mouse efficacy study of **22a** (dose: 100 mg/kg, p.o., Figure 5) the compound was administered one day post-infection, once daily for five days, and then on day six they received an additional dose of 50 mg/kg of **22a**. In control (untreated) mice, parasitemia reached 10^8/mL (mean parasitemia = 4.1×10^8/mL) and they were sacrificed. For mice treated with **22a**, parasitemia decreased (compared to that recorded in control mice) from day two post-infection. On day four, the difference in parasitemia between untreated (control) and **22a**-treated animals was 100-fold, which met the benchmark for classification of **22a** as a lead compound for HAT.[37] In mice treated with **22a**, parasitemia peaked at day five (3.7×10^6/mL), and then declined to a mean of 2×10^4/mL, before becoming undetectable in three mice by day nine (cell density < 4×10^4/mL), although treatment was terminated on

day seven. Had the control (untreated) mice lived until day nine, the projected mean parasitemia would be 10^{13}/mL, as density of trypanosomes in blood rises 10-fold every 24 h. Thus, the estimated difference in parasitemia between **22a**-treated and control mice is 10^{9}-fold on day nine. However, as trypanosomes were detected in the blood of the treated mice on day 11, the mice were not deemed to have been cured in this study (which is defined as no parasitemia on day 30). Through this study, compound **22a** has been identified as a promising lead for further optimization. Future studies will attempt to find a dosing protocol that may lead to cure of the trypanosome infection in mice by **22a**.

Safety profiling of 22a

Given the promising outcome of the efficacy study into **22a**, we sought to gain a better understanding of the human kinase inhibition profile of this compound as compared to **1** (Figure S5, Supplementary Information). While **1** showed moderate to potent inhibition of 28 out of the 45 human kinases tested in the Eurofins ExpreS Diversity kinase panel, **22a** demonstrated potent inhibition of only three out of the 45 kinases tested. This indicates a significant improvement in the overall selectivity of this compound as compared to the starting point for this work (full data for **22a** presented in Table S9, Supplementary Information).

To assess the genotoxicity of **22a**, we used an Ames fluctuation assay offered by Eurofins to detect frameshift mutations and base-pair substitutions in *Salmonella typhimurium* strains treated with **22a** (full data presented in Table S10, Supplementary Information). We also tested major metabolites identified through metabolic activation using rat liver S9 fraction. Neither **22a** or its metabolites were found to be genotoxic under these conditions.

Finally, given the improved selectivity profile of **22a**, it was further assessed in a HitProfilingScreen (Eurofins) consisting of 30 GPCRs and ion channels. Significant responses (>50% inhibition at 10 μM) were recorded in five of these assays, against: muscarinic M2, muscarinic M3, nicotinic acetylcholine α1, hERG and the norepinephrine transporter (full data presented in Table S11, Supplementary Information). The activity of **22a** against these receptors is the major liability of this compound and is the subject of ongoing optimization efforts which will be reported in due course.

Conclusions

Previous studies focused on the optimization of lapatinib against *T. brucei* led to the identification of **1**, a potent and selective anti-trypanosomal compound. The work presented herein describes a number of strategies that were employed to improve the ADME profile of **1**, and it was found that increasing the sp^3 carbon content of the molecule was a highly successful approach. Increasing the sp^3 content in the tail portion of the molecule led to **10e** which reduced parasitemia *in vivo*. Further exploration of the **10e** chemotype led to **22a**, a quinolinimine that renders parasitemia undetectable *in vivo* in 75% of mice following seven days of treatment. Further profiling of **22a** reveals that it has an improved kinase selectivity profile versus the starting compound and neither it, nor its metabolites, are genotoxic. We have identified a hERG liability which we are conscious of correcting as our efforts to further optimize this chemotype for efficacy in the murine model of HAT continue. We

conclude that **22a** is a promising lead for HAT drug development, with an improved ADME profile and potent anti-trypanosomal activity over starting compound **1**.

Experimental

Biological assay protocols

Trypanosoma brucei proliferation.

New York University (NYU).: In a 96-well plate, compounds were added in triplicates at 50 μM and in serial dilutions 1:2 in HMI-9 medium. To each well, 2.5×10^3 *T. b. brucei* (strain 427) were added and incubated at 37 °C, 5% CO_2 for 48 h. Following incubation, 20 μL of PrestoBlue® were added to each well and incubated for additional 4 h. Fluorescence was read at 530 nm excitation and 590 nm emission. Suramin at 100 μM was used as positive control and reference for calculation of IC_{50}.

University of Georgia (UGA).: The high-throughput trypanosome proliferation inhibition assay was performed and analyzed as previously described.[38]

A comparison of the biological readouts from NYU and UGA revealed good correlation between the results (Supplementary Information, Table S1). As such, the readouts have not been distinguished in the text of the manuscript.

Mouse infection with *T.b. brucei*.: Bloodstream form (BSF) *T. brucei brucei* CA427 parasites were maintained at densities below 1×10^6 cells/mL in HMI-9 media supplemented with 10% fetal bovine serum (Atlanta Biologicals), 10% SERUM PLUS™ (Sigma), and 1% Antibiotic-Antimycotic Solution (Corning cellgro®) at 37 °C, 5% CO_2. Parasites were centrifuged at 5000×g for 3 min at room temperature and resuspended in cold 1×PBS containing 1% glucose to yield a suspension of 1×10^6 cells/mL. Correct cell density following resuspension was confirmed using a Z2 Coulter Counter (Beckman). Parasite viability was observed by motility with a Neubauer Bright-line hemocytometer. Cells were kept on ice until infection. Animal were infected with 100 μL of cell suspension (10^5 trypanosomes per mouse).

Compound 22a (NEU-4438) treatment of trypanosome-infected mice: Compound **22a** (NEU-4438) treatment was initiated one day after trypanosome infection and continued for 7 days. Mice in the treatment groups were dosed orally (by gavage) 100 mg/kg **22a** (NEU-4438) each day for 7 days, and an additional dose of 50 mg/kg on day 6. Control mice received vehicle (10% NMP and 90% PEG 300). Parasitemia was checked daily from day 2 to day 7, and on alternate days thereafter.

Mice were monitored daily for changes in body weight and overt signs of drug toxicity. To check parasitemia, three μl of blood obtained from a tail prick was diluted 8-fold into red blood cell (RBC) lysis solution (Qiagen), and the trypanosomes were counted with a hemocytometer. When parasitemia reached 10^7/mL, dilutions of blood were made with PBS/G (KH_2PO_4 0.144 g/L, NaCl 9 g/L, Na_2PO_4 0.795 g/L with glucose 10 mM, pH 7.4) to facilitate counting. Mice were euthanized for humane reasons, when parasitemia exceeded

10^8/mL. Data obtained were plotted with GraphPad Prism (GraphPad Software, La Jolla, CA).

Humane euthanasia by CO_2 overdose followed by incision to form a bilateral pneumothorax was conducted on mice at study termination. All animal experimental protocols were approved and performed in accordance with the guidelines of the Institutional Animal Care and Use Committee (IACUC) at the University of Georgia.

Trypanocidal effect of hits (cidal assay)

Methods: Compounds with anti-trypanosome proliferation potency $EC_{50} \leq 200$ nM (Table S3) were assessed for cidal activity. Mid-logarithmic growth phase parasites were seeded at 1×10^5 cell/mL in HMI-9 medium. Control parasites were treated with 0.1% DMSO (vehicle). Drug treatment was performed for 6 h or 12 h at 37 °C/5% CO_2. HMI-9 medium used for drug treatment was washed off by centrifuging trypanosomes (3,000×g, 5 min), and the trypanosome pellet was rinsed with drug-free HMI-9 medium. Trypanosomes were suspended (1×10^4 cells/mL) in drug-free HMI-9 medium, incubated for 48 h at 37 °C/5% CO_2, and counted with a hemocytometer. Data presented was obtained from three independent biological replicates each with duplicate determinations. Student's T-test was used to determine if the mean of cell densities in drug treated samples varied significantly ($p < 0.01$) from control samples. Compounds that were ≥50% trypanocidal (see Figure 3) in either of the assays were re-tested to determine drug concentrations that yielded 50% trypanocidality in 6 h (6 h CEC_{50}) or 12 h (12 h CEC_{50}) (Table 10).

To determine CEC_{50} a 2 mL culture of log-phase trypanosomes in HMI-9 medium was inoculated at 1×10^5/mL in 24-well plates and incubated with different concentration of hits for 6 or 12 h at 37 °C/5% CO_2. The treatment medium was washed off by centrifuging trypanosomes (3000×g) and aspiration of the supernatant. Trypanosomes were washed with HMI-9 medium, inoculated at 1×10^4/mL, incubated at 37 °C for 48 h, and cell density determined with a hemocytometer. Data presented was obtained from two independent biological samples (with duplicate determinations) and analyzed with GraphPad Prism. CEC_{50} values were obtained by non-linear regression analysis of the data.

Drug toxicity to HepG2 cells.[40]—HepG2 cells were cultured in complete Minimal Essential Medium prepared by supplementing MEM with 0.19% sodium bicarbonate, 10% heat inactivated FBS, 2 mM L-glutamine, 0.1 mM MEM non-essential amino, 0.009 mg/mL insulin, 1.76 mg/mL bovine serum albumin, 20 units/mL penicillin–streptomycin, and 0.05 mg/mL gentamycin. HepG2 cells cultured in complete MEM were first washed with 1× Hank's Balanced Salt Solution (Invitrogen #14175095), trypsinized using a 0.25% trypsin/EDTA solution, assessed for viability using trypan blue, and resuspended at 250,000 cells/mL. Using a Tecan EVO Freedom robot, 38.3 μL of cell suspension were added to each well of clear, cell culture-treated 384-well microtiter plates for a final concentration of 9570 liver cells per well, and plated cells were incubated overnight in 5% CO_2 at 37 °C. Drug plates were prepared with the Tecan EVO Freedom using sterile 96 well plates containing twelve duplicate 1.6-fold serial dilutions of each test compound suspended in DMSO. 4.25 μL of diluted test compound was then added to the 38.3 μL of media in each well providing

a 10%-fold final dilution of compound. Compounds were tested from a range of 57 ng/mL to 10,000 ng/mL for all assays. Mefloquine was used as a plate control for all assays with a concentration ranging from 113 ng/mL to 20,000 ng/mL. After a 48 h incubation period, 8 μL of a 1.5 mg/mL solution of MTT diluted in complete MEM media was added to each well. All plates were subsequently incubated in the dark for 1 h at room temperature. After incubation, the media and drugs in each well was removed by shaking the plate over sink, and the plates were left to dry in a fume hood for 15 mins. Next, 30 μL of isopropanol acidified by addition of HCl at a final concentration of 0.36% was added to dissolve the formazan dye crystals created by reduction of MTT. Plates are put on a 3-D rotator for 15-30 mins. Absorbance was determined in all wells using a Tecan iControl 1.6 Infinite plate reader. The 50% toxic concentrations (TC_{50}) were then generated for each toxicity dose response test using GraphPad Prism (GraphPad Software Inc., San Diego, CA) using the nonlinear regression (sigmoidal dose-response/variable slope) equation.

ADME experiment protocols

Aqueous pH 7.4 Solubility.—Compounds are dried down from 10 mM DMSO solutions using centrifugal evaporation technique. Phosphate buffer (0.1 M pH 7.4) added and StirStix inserted in the glass vials, shaking is then performed at a constant temperature of 25 °C for 20-24 h. This step is followed by double centrifugation with a tip wash in between, to ensure that no residues of the dried compound are interfering. The solutions are diluted before analysis and quantification using LC/MS/MS is performed.

Log $D_{7.4}$.—The distribution coefficient between 1-octanol and aqueous buffer, LogD, at pH 7.4, is based on the traditional shake flask technique, but with the modification of measuring compounds in mixtures of ten at a time using UPLC with quantitative MS to measure the relative octanol and aqueous concentrations of compounds. The buffer solution used is 10 mM sodium phosphate (pH 7.4). The method has been validated for Log $D_{7.4}$ ranging from −2 to 5.0.

Human Plasma Protein Binding (PPB).—PPB is determined using equilibrium dialysis (RED device) to separate free from bound compound. The amount of compound in plasma (1 mM initial concentration in DMSO) and in dialysis buffer (pH 7.4 phosphate buffer) is measured by LC-MS/MS after 18 h in a dialysis chamber at 37 °C. Sample levels are quantified using a 7-point calibration curve in plasma. The fraction unbound (fu%) is reported.

Human Liver Microsomal Cl_{int}.—*In vitro* intrinsic clearance determined from human liver microsomes using a standard approach.[41] Following incubation and preparation, the samples are analyzed using LC/MS/MS. Refined data are uploaded to IBIS and are displayed as Cl_{int} (intrinsic clearance) in μL/min/mg.

Rat Hepatocyte Cl_{int}.—*In vitro* intrinsic clearance was determined from rat han wistar hepatocytes at a final concentration of 1 million cells/mL. The cells were pre-incubated at 37 °C for 15 min before addition of test compound (as a 50 μM stock solution) to a final concentration of 1 μM. The samples were analyzed using LC/MSMS at predefined time

points. Refined data were uploaded to IBIS and are displayed as Cl_{int} (intrinsic clearance) μL/min/1 million cells.

Calculated LogP and LogD values.—Both LogP and LogD predictions are based on a modified version of the method[42] where the predicted partition coefficients are composed of the molecules' atomic increments.

Chemistry Experimental

General Chemistry.—All starting materials were commercially procured and were used without further purification, unless specified. Reaction solvents were purified by passage through alumina columns on a purification system manufactured by Innovative Technology (Newburyport, MA). For all microwave reactions a Biotage® Initiator+ was used and the absorbance was set in accordance with the recommendations set by the manufacturer. NMR spectra were obtained on Varian NMR systems, operating at 400 or 500 MHz for 1 H acquisitions. LCMS analysis was performed using a Waters Alliance reverse phase HPLC (columns Waters SunFire C18 4.6 × 50 mm, 3.5 μm, or Waters SunFire C8 4.6 × 50 mm, 3.5 μm), with single-wavelength UV–visible detector and LCT Premier time-of-flight mass spectrometer (electrospray ionization) or Waters Micromass ZQ detector (electrospray ionization). Optical rotations were obtained on a Jasco P-2000. Purification of intermediates and final compounds was performed using silica gel chromatography on a Biotage® Isolera™ One Flash purification system. Where required, final compounds were purified by preparative reverse phase HPLC (columns Waters Symmetry RP8 30 × 50 mm, 5 μm column, or OBD RP18 30 × 50 mm, 5 μm), with a single wavelength UV–visible detector and Waters Micromass ZQ (electrospray ionization). All final compounds have purities greater than 95% based upon LC/MS analysis.

General Procedure Gor Salt Formation

The qualitative solubility of **1** was determined in various solvents (methanol, ethanol, isopropanol, tetrahydrofuran, acetonitrile, ethyl acetate, and water). For each of the solvents, 2.0 mg of **1** as added to vials. Solvents were added in 200 μL increments until the solid was completely dissolved. With a solubility of about 2.75 mg/mL, ethanol was chosen as the solvent for the salt screen.

Compound **1** (20 mg, 0.05 mmol) was dissolved in ethanol (4 mL) using sonication and heat. Stock solutions of various acids were made (1M in ethanol). Acid solution was added to solution of **1** until a precipitate formed (different amounts needed for each acid). The precipitate was collected by vacuum filtration and washed with ethanol.

Hydrochloride salt (1a)—^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.04 (s, 2 H), 8.91 (s, 1 H), 8.81 - 8.86 (m, 1 H), 8.78 (d, J=5.9 Hz, 1 H), 8.38 - 8.44 (m, 2 H), 8.27 - 8.34 (m, 2 H), 8.08 - 8.13 (m, 1 H), 7.52 - 7.65 (m, 2 H), 2.79 (s, 3 H).

Sulfate salt (1b)—^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.79 - 11.01 (m, 1 H), 9.07 (s, 2 H), 8.86 - 8.94 (m, 3 H), 8.53 - 8.59 (m, 2 H), 8.50 (d, J=2.9 Hz, 1 H), 8.28 - 8.34 (m, 2 H), 3.34 - 3.40 (m, 2 H), 3.17 (s, 3 H), 2.88 (s, 3 H).

Citrate salt (1c)—^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.77 - 9.85 (m, 1 H), 8.98 (s, 2 H), 8.73 (s, 2 H), 8.57 (d, J=8.8 Hz, 1 H), 8.31 - 8.35 (m, 2 H), 8.23 - 8.26 (m, 1 H), 8.17 - 8.20 (m, 1 H), 7.96 - 8.02 (m, 1 H), 4.34 (br. s., 13 H), 3.85 - 3.96 (m, 4 H), 3.72 - 3.83 (m, 7 H), 3.40 - 3.48 (m, 8 H), 2.53 - 2.71 (m, 14 H), 2.42 (s, 4 H).

Methanesulfonate salt (1d)—^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.63 - 10.88 (m, 1 H), 9.62 - 9.94 (m, 1 H), 9.07 (s, 2 H), 8.77 - 8.96 (m, 4 H), 8.41 - 8.62 (m, 3 H), 8.30 (d, J=1.5 Hz, 2 H), 4.62 - 4.98 (m, 2 H), 2.87 (s, 4 H), 2.32 (s, 6 H).

General Procedure A

To a solution of 5-bromo-2-chloro-pyrimidine (**2**) in ethanol (0.2-0.3 M) was added triethylamine (3 eq) followed by appropriate amine (1.3 eq). The reaction mixture was stirred at ambient temperature overnight. Reaction monitored by LCMS analysis.

General Procedure B

Compounds **3a-3d**, **3f**, **3h-3m**, **3p-3r**, **3u**, **3×** or **3y**, bis(pinacolato)diboron (1.5 eq), potassium acetate (3.5 eq) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.05 eq) were combined in a microwave vial that was filled with nitrogen and evacuated three times. Anhydrous dioxane (0.1 M) was added and the reaction mixture was degassed. The vial was transferred to the microwave at 145 °C (low absorbance) for 1 h. Reaction monitored by LCMS analysis and, once complete the reaction mixture was filtered through celite and all of the volatiles were removed *in vacuo*. The crude material was used directly without further purification unless otherwise specified.

General Procedure C

The appropriate boronate (1.2 eq) was dissolved in a 3:1 dioxane/water (0.13 M) mixture. To this was added **5**, **12a**, **12f**, **25**, **19a-19c**, or 7-bromoquinolin-4(1*H*)-one (1 eq), potassium carbonate (3 eq) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.05 eq) and the reaction mixture was degassed before being transferred to the microwave for 30 min at 130 °C (high absorbance). Reaction monitored by LCMS analysis and, once complete the reaction mixture was filtered through celite and the crude material was purified by column chromatography, eluting with the specified mobile phase.

General Procedure D

Compound **3b** or **3m**, bis(pinacolato)diboron (1.5 eq), potassium acetate (3.5 eq) and $PdCl_2(dppf)\cdot CH_2Cl_2$ (0.05 eq) were combined in a microwave vial that was filled with nitrogen and evacuated three times. Anhydrous dioxane (0.13 M) was added and the reaction mixture was degassed. The vial was transferred to the microwave at 145 °C (low absorbance) for 1 h. Reaction monitored by LCMS analysis.

Once complete, compound **5**, **12g** or **12h** (1.0 eq) was added directly to the reaction mixture along with a 1.8 M aqueous potassium carbonate solution (0.33 M). The reaction mixture was degassed again before being returned to the microwave at 130 °C for 10 min (high absorbance). Reaction monitored by LCMS analysis and, once complete the reaction

mixture was filtered through celite and the crude material was purified by column chromatography, eluting with the specified mobile phase.

General Procedure E

To a solution of 5-bromo-2-chloro-pyrimidine (**2**) in *tert*-butanol (0.2-0.3 M) was added diisopropylethylamine (1.3 - 3 eq) followed by appropriate amine (or appropriate dihydrobromide salt where appropriate) (1.2 eq). The reaction mixture was stirred and heated in the microwave at 150 °C for 30 mins. Reaction monitored by LCMS analysis.

General Procedure F

The appropriate boronate (1.2 eq) was dissolved in a 1:2 water/ethanol (0.13 M) mixture. To this was added **5**, **31**, **37** or **12b-12e** (1 eq), cesium carbonate (4 eq), and tetrakis(triphenylphosphine)palladium(0) (5 mol%) and the reaction mixture was degassed before being transferred to the microwave for 10 min at 130 °C (high absorbance). Reaction monitored by LCMS analysis and, once complete the reaction mixture was filtered through celite and the crude material was purified by column chromatography, eluting with the specified mobile phase.

General Procedure G

To a suspension of sodium hydride (3.5 eq) in anhydrous dimethylformamide (1 M) was added appropriate aryl primary amine (2.4 eq) under nitrogen, upon which the reaction mixture changed color. To this, was added a solution of **11**, **30** or **36** (1 eq) in anhydrous dimethylformamide (0.4 M), which was also accompanied by a color change. The reaction was monitored by LCMS and, upon completion was quenched with a saturated aqueous ammonium chloride solution. A precipitate was observed and collected by vacuum filtration (washed with water).

General Procedure H

Compound **11** was dissolved in *n*-butanol (0.1 M). Appropriate saturated primary amine (4 eq) and diisopropylethylamine (10 eq) were added. The reaction was heated in the microwave at 200 °C for 3 h. The *n*-butanol was removed *in vacuo* and the reaction mixture was purified by column chromatography eluting with specified mobile phase.

General Procedure I

Compound **5**, **12h-12j** or 2-aminopyrazine (**23**) was added to a cooled suspension of sodium hydride (2.9 eq) in anhydrous tetrahydrofuran (0.16 M concentration to the relative amine). After 15 mins, the appropriate iodoalkane (1.7 eq) was added to the reaction mixture which was allowed to gradually warm to ambient temperature. Reaction mixture left to stir under nitrogen and monitored by LCMS analysis. Upon completion, the sodium hydride was neutralized by addition of methanol until no further effervescence was observed. All the volatiles were removed *in vacuo* and the crude material was purified by column chromatography, eluting with the gradient specified.

***tert*-Butyl (2-((5-bromopyrimidin-2-yl)amino)ethyl)carbamate (3a)—**Prepared via *General Procedure A*. The crude material was purified by column chromatography, eluting with 1-5% methanol/dichloromethane to obtain the title compound as a colorless solid (297 mg, 72%). LCMS $[M+H]^+$ 317.0 m/z (Br^{79}), 319.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.35 (s, 2 H), 7.40 (s, 1 H), 6.85 (s, 1 H), 3.26 (q, *J*=6.4 Hz, 2 H), 3.07 (q, *J*=6.5 Hz, 2 H), 1.36 (s, 9 H).

***tert*-Butyl (2-((5-bromopyrimidin-2-yl)amino)ethyl)(methyl)carbamate (3b)—**Prepared via *General Procedure A*. The crude material was purified by column chromatography, eluting with 1% methanol/dichloromethane to obtain the title compound as a colorless solid (123 mg, 32%). LCMS $[M+H]^+$ 331.0 m/z (Br^{79}), 333.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.36 (s, 2 H), 7.39 - 7.60 (m, 1 H), 3.34 (br. s., 2 H), 2.76 (s, 2 H), 1.36 (br. s., 3 H), 1.23 (s, 6 H).

***N*^{1}-(5-Bromopyrimidin-2-yl)-*N*^{1},*N*^{2},*N*^{2}-trimethylethane-1,2-diamine (3c)—**Prepared via *General Procedure A*. All of the volatiles were removed and the residue was dissolved in ethyl acetate and washed with an aqueous solution of 1M sodium hydroxide. The aqueous phase was back extracted with ethyl acetate three times and the organic phases were combined, washed with a saturated solution of sodium chloride and dried with magnesium sulfate. The material was then purified by column chromatography, eluting with 1% methanol/dichloromethane to obtain the title compound as a yellow oil (302 mg, 68%). LCMS $[M+H]^+$ 259.0 m/z (Br^{79}), 261.0 m/z (Br^{81}); ^1H NMR (399 MHz, DMSO-d_6) δ ppm 8.41 (d, *J*=5.1 Hz, 2 H), 3.65 (td, *J*=7.3, 5.9 Hz, 2 H), 3.07 (d, *J*=4.4 Hz, 3 H), 2.40 (dt, *J*=7.3, 5.9 Hz, 2 H), 2.16 (d, *J*=4.4 Hz, 6 H).

***tert*-Butyl 4-(5-bromopyrimidin-2-yl)piperazine-1-carboxylate (3d)—**Prepared via *General Procedure E*. All of the volatiles were removed *in vacuo* and the crude material was purified by column chromatography, eluting 0-5% ethyl acetate/hexanes to obtain the title compound as a colorless solid (819 mg, 92%). LCMS $[M+H]^+$ 343.0 m/z (Br^{79}), 345.0 m/z (Br^{81}); ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.30 (s, 2 H), 3.76 (t, *J*=4.9 Hz, 4 H), 3.48 (t, *J*=5.4 Hz, 4 H), 1.48 (s, 9 H).

5-Bromo-2-(piperazin-1-yl)pyrimidine (3e)—*tert*-Butyl 4-(5-bromopyrimidin-2-yl)piperazine-1-carboxylate (**3d**) (819 mg, 2.39 mmol) was dissolved in 4.0 M hydrochloric acid in dioxane (6.0 mL) and stirred at ambient temperature. After 5 mins the reaction mixture became thick with precipitate. TLC after 1 h indicated no starting material remained. The volatiles were removed *in vacuo* and the residue was re-dissolved in water, Si-carbonate was added and the reaction mixture was stirred at ambient temperature for 3 h. Si-carbonate removed by filtration and the volatiles were removed *in vacuo* to obtain the title compound as a colorless solid (501 mg, 86%). LCMS $[M+H]^+$ 243.0 m/z (Br^{79}), 245.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.93 (br. s., 2 H), 8.55 (s, 2 H), 3.91 (t, *J*=5.4 Hz, 4 H), 3.18 (br. s., 4 H).

Ethyl 2-(4-(5-bromopyrimidin-2-yl)piperazin-1-yl)acetate (3f)—5-Bromo-2-(piperazin-1-yl)pyrimidine (**3e**) (250 mg, 1.03 mmol) was dissolved in acetonitrile (20 mL, 0.05 M) prior to the addition of potassium carbonate (171 mg, 1.23 mmol) and ethyl

bromoacetate (137 μl, 1.23 mmol). The resulting reaction mixture was heated to reflux and monitored by TLC and LCMS. LCMS analysis after 8 h revealed no remaining starting material. The volatiles were removed *in vacuo* and the resulting residue was dissolved in ethyl acetate and washed with water (three times) and a saturated aqueous sodium chloride solution (once) before being dried with magnesium sulfate, filtered and the volatiles removed *in vacuo*. The crude material was purified by column chromatography, eluting with 10-15% ethyl acetate/hexanes to obtain the title compound as a pale-yellow solid (314 mg, 93%). LCMS $[M+H]^+$ 329.0 m/z (Br^{79}), 331.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.45 (s, 2 H), 4.08 (q, *J*=7.1 Hz, 2 H), 3.70 (t, *J*=5.1 Hz, 2 H), 3.27 (s, 2 H), 2.55 (t, *J*=5.1 Hz, 4 H), 1.19 (t, *J*=7.1 Hz, 3 H).

(5-Bromopyrimidin-2-yl)proline (3g)—5-Bromo-2-chloropyrimidine (**2**) (500 mg, 2.58 mmol) was dissolved in ethanol (100 ml) prior to the addition of D-proline (327 mg, 2.84 mmol) and triethylamine (1.08 ml, 7.75 mmol). The resultant mixture was heated to 75 °C and the reaction was monitored by LCMS. After 8 h only product was evident by LCMS analysis. The reaction mixture was concentrated *in vacuo* before the residue was partitioned between 1M hydrochloric acid and ethyl acetate. The aqueous phase was back extracted with ethyl acetate a further 2 times. The organic phases were combined, washed once with a saturated aqueous solution of sodium chloride and dried with sodium sulfate. The volatiles were removed *in vacuo* to obtain the title compound as a colorless solid (609 mg, 87%). LCMS $[M+H]^+$ 272.0 m/z (Br^{79}), 274.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.37 (s, 2 H), 4.52 (dd, *J*=8.1, 2.7 Hz, 1 H), 3.70 (ddd, *J*=11.0, 7.3, 3.2 Hz, 1 H), 3.59 (ddd, *J*=10.6, 9.2, 7.1 Hz, 1 H), 2.48 (ddt, *J*=12.1, 5.9, 2.9, 2.9 Hz, 1 H), 2.01 - 2.23 (m, 4 H).

Methyl (5-bromopyrimidin-2-yl)prolinate (3h)—Compound **3g** (100 mg, 0.368 mmol) was dissolved in anhydrous methanol/toluene (4:1, 500 μL) and cooled to 0 °C under nitrogen. To this was added dropwise a solution of TMS-diazomethane in hexanes (550 μL, 1.10 mmol). The reaction mixture was allowed to gradually warm to ambient temperature overnight. LCMS analysis revealed no remaining starting material and a mass consistent with the desired product. The reaction mixture was concentrated *in vacuo* and the crude material purified by column chromatography, eluting with 10% ethyl acetate/hexanes to obtain the title compound as a colorless solid (90 mg, 86%). LCMS $[M+H]^+$ 286.0 m/z (Br^{79}), 288.1 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.50 (br. s., 1 H), 8.43 (br. s., 1 H), 4.48 (dd, *J*=8.8, 3.4 Hz, 1 H), 3.51 - 3.66 (m, 5 H), 2.27 - 2.40 (m, 1 H), 1.88 - 2.05 (m, 3 H).

(1*S*,4*S*)-2-(5-Bromopyrimidin-2-yl)-5-methyl-2,5-diazabicyclo[2.2.1]heptane (3i)—Prepared via *General Procedure E* using (1*S*,4*S*)-2-methyl-2,5-diazabicyclo[2.2.1]heptane dihydrobromide. All the volatiles were removed *in vacuo* and the crude material was purified by column chromatography, eluting first with 20-50% ethyl acetate/hexanes before switching to 0-30% methanol/dichloromethane to obtain the title compound as a yellow solid (205 mg, 97%). $[\alpha]_D^{27}$ −134 ± 0.34 (*c* 0.45, CH_3OH); LCMS $[M+H]^+$ 269.0 m/z (Br^{79}), 271.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.34 (s, 2 H), 4.81

(br. s, 1 H), 3.65 - 3.73 (m, 2 H), 3.44 (dd, *J*=11.2, 1.9 Hz, 1 H), 2.91 - 2.97 (m, 1 H), 2.84 (dd, *J*=10.2, 1.5 Hz, 1 H), 2.51 (s, 3 H), 2.03 - 2.11 (m, 1 H), 1.90 - 1.96 (m, 1 H).

(1*R*,4*R*)-2-(5-Bromopyrimidin-2-yl)-5-methyl-2,5-diazabicyclo[2.2.1]heptane (3j)—Prepared via *General Procedure E* using (1*R*,4*R*)-2-methyl-2,5-diazabicyclo[2.2.1]heptane dihydrobromide. The reaction mixture was stirred and heated in the microwave at 150 °C, for 0.5 hr. Upon cooling, a precipitate was observed and collected by vacuum filtration (washed with MeOH) to afford the title compound as a tan solid (134 mg, 57%). $[\alpha]_D^{27}$ +27 ± 0.42 (*c* 0.56, CH_3OH); LCMS $[M+H]^+$ 268.99 m/z (Br^{79}), 271.01 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 9.55 - 9.86 (m, 1 H), 8.54 (s, 2 H), 4.87 (s, 1 H), 4.37 (br. s., 1 H), 3.72 (d, *J*=12.7 Hz, 1 H), 3.59 (dd, *J*=12.2, 1.9 Hz, 2 H), 2.85 (br. s., 3 H), 2.17 (d, *J*=9.8 Hz, 1 H), 1.31 - 1.38 (m, 2 H).

5-Bromo-2-(4-ethylpiperazin-1-yl)pyrimidine (3k)—Compound **3e** (280 mg, 1.15 mmol) was dissolved in methanol (4.6 ml, 0.25M), to this was added triethylamine (1.61 ml, 11.52 mmol) and iodoethane (461 µl, 5.76 mmol). The reaction mixture was stirred at ambient temperature for 43 h. LCMS analysis revealed no remaining starting material and a mass consistent with the desired product. All the volatiles were removed *in vacuo* and the crude material was purified by column chromatography, eluting with 1% methanol (+5% ammonium hydroxide)/dichloromethane. The fractions were combined and the volatiles removed before the material was re-dissolved in dichloromethane, washed with water (twice), and washed with a saturated aqueous solution of sodium chloride (twice). The organic phase was collected, dried with magnesium sulfate and concentrated *in vacuo* to obtain the title compound as a pale-yellow solid (201 mg, 64%). LCMS $[M+H]^+$ 271.1 m/z (Br^{79}), 273.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.57 (s, 2 H), 4.64 (d, *J*=14.2 Hz, 2 H), 3.56 (d, *J*=11.7 Hz, 2 H), 3.26 (t, *J*=12.5 Hz, 2 H), 3.16 (qd, *J*=6.3, 4.9 Hz, 2 H), 3.02 (td, *J*=11.2, 9.8 Hz, 2 H), 1.24 (t, *J*=7.3 Hz, 3 H).

5-Bromo-2-(4-propylpiperazin-1-yl)pyrimidine (3l)—Compound **3e** (280 mg, 1.15 mmol) was dissolved in methanol (4.6 mL, 0.25M). To this was added triethylamine (1.61 mL, 11.52 mmol) and 1-bromopropane (520 µL, 5.76 mmol). The reaction mixture was stirred at ambient temperature and monitored by TLC and LCMS analysis. Once the reaction was complete all the volatiles were removed *in vacuo* and the residue was re-dissolved in dichloromethane. The organic layer was washed with water (twice) and dried with magnesium sulfate before being concentrated *in vacuo* to obtain the title compound as a pale-yellow solid (279 mg, 85%). LCMS $[M+H]^+$ 285.0 m/z (Br^{79}), 287.1 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.44 (s, 2 H), 3.68 (t, *J*=4.9 Hz, 4 H), 2.38 (t, *J*=5.1 Hz, 4 H), 2.25 (t, *J*=7.3 Hz, 2 H), 1.45 (sxt, *J*=7.4 Hz, 2 H), 0.86 (t, *J*=7.3 Hz, 3 H).

1-(5-Bromopyrimidin-2-yl)-4-methyl-1,4-diazepane (3m)—Prepared via *General Procedure E*. All volatiles were removed and the crude material was dissolved in dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate. The aqueous phase was back extracted with dichloromethane and the organic phases were combined, washed once with a saturated solution of sodium chloride and dried with magnesium sulfate before being concentrated *in vacuo* to obtain the title compound as an

off-white solid (1.28 g, 91%). LCMS $[M+H]^+$ 271.1 m/z (Br^{79}), 273.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.50 (s, 2 H), 3.95 (br. s., 2 H), 3.74 (br. s., 2 H), 3.16 (d, *J*=4.9 Hz, 4 H), 2.66 (br. s., 3 H), 2.13 (br. s., 2 H).

***tert*-Butyl 4-(5-bromopyrimidin-2-yl)-1,4-diazepane-1-carboxylate (3n)—**Prepared via *General Procedure E*. All the volatiles were removed *in vacuo* and the crude material was purified by column chromatography, eluting 0-10% ethyl acetate/hexanes to obtain the title compound as a colorless solid (810 mg, 88%). LCMS $[M+H]^+$ 357.0 m/z (Br^{79}), 359.1 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.45 (s, 2 H), 8.44 (s, 2 H), 3.78 (td, *J*=5.9, 2.9 Hz, 4 H), 3.68 (q, *J*=6.3 Hz, 4 H), 3.52 (t, *J*=5.9 Hz, 2 H), 3.46 (t, *J*=5.6 Hz, 2 H), 3.30 (t, *J*=5.6 Hz, 2 H), 3.25 (t, *J*=5.9 Hz, 2 H), 1.77 (quin, *J*=6.0 Hz, 2 H), 1.70 (quin, *J*=5.6 Hz, 2 H), 1.30 (s, 9 H), 1.26 (s, 9 H).

*The HNMR contains two rotamers in a 1:1 ratio.

1-(5-Bromopyrimidin-2-yl)-1,4-diazepane, trifluoroacetic acid salt (3o)—Compound **3n** (810 mg, 2.27 mmol) was dissolved in dichloromethane (11.4 mL, 0.2M) and trifluoroacetic acid (1.74 mL, 22.67 mmol) added. The reaction mixture was stirred at ambient temperature and monitored by TLC and LCMS analysis. Once complete, all the volatiles were removed *in vacuo* and the material was used directly, without further purification. LCMS $[M+H]^+$ 257.0 m/z (Br^{79}), 250.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.79 (br. s., 2 H), 8.50 (s, 2 H), 3.93 (t, *J*=4.9 Hz, 2 H), 3.79 (t, *J*=6.1 Hz, 2 H), 3.26 (quin, *J*=5.0 Hz, 2 H), 3.12 - 3.22 (m, 2 H), 2.00 (dt, *J*=11.4, 5.8 Hz, 2 H).

1-(5-Bromopyrimidin-2-yl)-4-ethyl-1,4-diazepane (3p)—Compound **3o** (291 mg, 1.13 mmol) was dissolved in methanol (4.5 ml, 0.25M). To this was added triethylamine (1.58 ml, 11.32 mmol) and iodoethane (453 µl, 5.66 mmol). The reaction mixture was stirred at ambient temperature and monitored by TLC and LCMS analysis. Once the reaction was complete all the volatiles were removed *in vacuo* and the crude material purified by column chromatography, eluting with 1% methanol (+5% ammonium hydroxide)/dichloromethane. Material was dissolved in dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate. The aqueous phase back extracted with dichloromethane and the organic phases were combined, washed once with a saturated solution of sodium chloride and dried with magnesium sulfate before being concentrated *in vacuo* to obtain the title compound as a colorless solid (85 mg, 26%). LCMS $[M+H]^+$ 285.0 m/z (Br^{79}), 287.1 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.51 (s, 2 H), 4.19 (dd, *J*=15.9, 3.7 Hz, 1 H), 3.69 - 3.79 (m, 3 H), 3.58 (d, *J*=13.7 Hz, 1 H), 3.47 (dd, *J*=12.9, 5.6 Hz, 1 H), 3.12 - 3.19 (m, 4 H), 1.99 - 2.22 (m, 2 H), 1.19 (t, *J*=7.3 Hz, 3 H).

1-(5-Bromopyrimidin-2-yl)-4-propyl-1,4-diazepane (3q)—Compound **3o** (291 mg, 1.13 mmol) was dissolved in methanol (4.5 ml, 0.25M). To this was added triethylamine (1.58 ml, 11.32 mmol) and 1-bromopropane (511 µl, 5.66 mmol). The reaction mixture was stirred at ambient temperature and monitored by TLC and LCMS analysis. Once the reaction was complete all the volatiles were removed *in vacuo* and the residue was re-dissolved in dichloromethane. The organic layer was washed with water (twice), a saturated aqueous solution of sodium bicarbonate (once), a saturated solution of sodium chloride (once) and

dried with magnesium sulfate. All the volatiles were removed *in vacuo* to obtain the title compound as a pale-yellow oil (172 mg, 51%). LCMS $[M+H]^+$ 299.1 m/z (Br^{79}), 301.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.42 (s, 2 H), 3.75 (t, *J*=5.4 Hz, 2 H), 3.69 (t, *J*=6.1 Hz, 2 H), 2.68 (br. s., 2 H), 2.54 (br. s., 2 H), 2.37 (t, *J*=6.3 Hz, 2 H), 1.80 (quin, *J*=5.7 Hz, 2 H), 1.39 (sxt, *J*=7.3 Hz, 2 H), 0.80 (t, *J*=7.3 Hz, 3 H).

1-(5-Bromopyrimidin-2-yl)azepane (3r)—Prepared via *General Procedure E.* All the volatiles were removed *in vacuo* and the crude material was purified by column chromatography, eluting first with 50% ethyl acetate/hexanes before switching to 0-20% methanol/dichloromethane to obtain the title compound as a colorless solid (605 mg, 91%). LCMS $[M+H]^+$ 256.0 m/z (Br^{79}), 258.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.32 - 8.46 (m, 2 H), 3.66 (t, *J*=5.9 Hz, 4 H), 1.61 - 1.77 (m, 4 H), 1.47 (dt, *J*=6.5, 2.9 Hz, 4 H).

***tert*-Butyl 6-(5-bromopyrimidin-2-yl)-2,6-diazaspiro[3.4]octane-2-carboxylate (3s)—**Prepared via *General Procedure E.* The crude material was purified by column chromatography, eluting with 20% ethyl acetate/hexanes to afford the title compound as an off-white solid (268 mg, 94%). LCMS $[M+H]^+$ 369.1 m/z (Br^{79}), 371.1 m/z (Br^{81}); ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.31 (s, 2 H) 3.93 (d, *J*=8.3 Hz, 2 H) 3.88 (d, *J*=8.8 Hz, 2 H) 3.69 (s, 2 H) 3.59 (t, *J*=6.8 Hz, 2 H) 2.20 (t, *J*=6.8 Hz, 2 H) 1.46 (s, 9 H).

6-(5-Bromopyrimidin-2-yl)-2,6-diazaspiro[3.4]octane (3t)—*tert*-Butyl 6-(5-bromopyrimidin-2-yl)-2,6-diazaspiro[3.4]octane-2-carboxylate (**3s**) (268 mg, 0.726 mmol) was taken up in 4.0 M HCl in dioxane (1.0 mL, 4.00 mmol). The reaction was stirred at room temperature overnight. All volatiles were removed *in vacuo* to afford a white solid. The crude material was used directly in the next reaction without further purification. LCMS $[M+H]^+$ 269.03 m/z (Br^{79}), 271.05 m/z (Br^{81}).

6-(5-Bromopyrimidin-2-yl)-2-methyl-2,6-diazaspiro[3.4]octane (3u)—6-(5-Bromopyrimidin-2-yl)-2,6-diazaspiro[3.4]octane (**3t**) (195 mg, 0.724 mmol) was dissolved in methanol (3.0 mL, 0.25 M) and triethylamine (1.0 mL, 7.17 mmol) was added, followed by the addition of methyl iodide (68 μL, 1.09 mmol). The reaction was stirred overnight at ambient temperature. All volatiles were removed *in vacuo* and the resulting residue was dissolved in dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate. The aqueous layer was extracted once more with dichloromethane, then the combined organic layers were washed once with a saturated solution of sodium chloride and dried with sodium sulfate. The crude material was purified by column chromatography, eluting with 5-10-20% methanol (+10% ammonium hydroxide)/ethyl acetate to afford the title compound as an orange oil (20 mg, 10%). LCMS $[M+H]^+$ 283.1 m/z (Br^{79}), 285.1 m/z (Br^{81}); ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.29 (s, 2 H) 3.61 (s, 2 H) 3.54 (t, *J*=7.1 Hz, 2 H) 3.30 (d, *J*=7.8 Hz, 2 H) 3.16 (d, *J*=7.8 Hz, 2 H) 2.35 (s, 3 H) 2.19 (t, *J*=6.8 Hz, 2 H).

***tert*-Butyl 7-(5-bromopyrimidin-2-yl)-2,7-diazaspiro[4.4]nonane-2-carboxylate (3v)—**Prepared via *General Procedure E.* The crude material was purified by column chromatography, eluting with 20% ethyl acetate/hexanes to afford the title compound as a

colorless solid (554 mg, 93%). LCMS $[M+H]^+$ 383.1 m/z (Br^{79}), 385.1 m/z (Br^{81}); ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.31 (s, 2 H) 3.58 - 3.71 (m, 2 H) 3.25 - 3.56 (m, 6 H) 1.83 - 2.08 (m, 4 H) 1.47 (s, 9 H).

2-(5-Bromopyrimidin-2-yl)-2,7-diazaspiro[4.4]nonane (3w)—*tert*-Butyl 7-(5-bromopyrimidin-2-yl)-2,7-diazaspiro[4.4]nonane-2-carboxylate (**3v**) (554 mg, 1.45 mmol) was taken up in 4.0 M HCl in dioxane (1.8 mL, 7.20 mmol). The reaction was stirred at room temperature overnight. All volatiles were removed *in vacuo* to afford a white solid. The crude material was used directly in the next reaction without further purification. LCMS $[M+H]^+$ 283.1 m/z (Br^{79}), 285.1 m/z (Br^{81}).

2-(5-Bromopyrimidin-2-yl)-7-methyl-2,7-diazaspiro[4.4]nonane (3×)—2-(5-Bromopyrimidin-2-yl)-2,7-diazaspiro[4.4]nonane (**3w**) (350 mg, 1.24 mmol) was dissolved in methanol (5.0 mL, 0.25 M) and triethylamine (1.7 mL, 12.20 mmol) was added, followed by the addition of methyl iodide (115 μL, 1.85 mmol). The reaction was stirred overnight at ambient temperature. All volatiles were removed *in vacuo* and the resulting residue was dissolved in dichloromethane and washed with a saturated aqueous solution of sodium bicarbonate. The aqueous layer was extracted once more with dichloromethane, then the combined organic layers were washed once with a saturated solution of sodium chloride and dried with sodium sulfate. The crude material was purified by column chromatography, eluting with 5-10-20% methanol (+10% ammonium hydroxide)/ethyl acetate to afford the title compound as an orange oil (56 mg, 15%). LCMS $[M+H]^+$ 297.1 m/z (Br^{79}), 299.1 m/z (Br^{81}); ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.29 (s, 2 H) 3.52 - 3.63 (m, 3 H) 3.46 (d, *J*=11.2 Hz, 1 H) 2.72 (q, *J*=7.8 Hz, 1 H) 2.55 - 2.64 (m, 2 H) 2.51 (d, *J*=9.8 Hz, 1 H) 2.37 (s, 3 H) 2.02 (tdd, *J*=19.1, 19.1, 12.3, 7.1 Hz, 2 H) 1.87 (sxt, *J*=6.6 Hz, 2 H).

5-Bromo-*N*-(4-methylpiperazin-1-yl)pyrimidin-2-amine (3y)—1-Amino-4-methylpiperazine (404 μL, 3.36 mmol) was added to a solution of 5-bromo-2-chloro-pyrimidine (**2**) (500 mg, 2.58 mmol) and triethylamine (1.01 mL, 7.24 mmol) in ethanol (10 mL). The reaction mixture was stirred at ambient temperature overnight. LCMS analysis revealed no remaining starting material and a mass consistent with the desired product. The crude material was purified by column chromatography, eluting with 1-5% methanol (+5% ammonium hydroxide)/dichloromethane. The material was further purified by column chromatography, eluting first with 50-95% ethyl acetate/hexanes, 0-5% methanol/dichloromethane and, finally 5% methanol (+5% ammonium hydroxide)/dichloromethane to obtain the title compound as a colorless solid (57 mg, 8%). LCMS $[M+H]^+$ 272.0 m/z (Br^{79}), 274.0 m/z (Br^{81}); ^1H NMR (500 MHz, DMSO-d_6) δ ppm 8.41 (s, 1 H), 8.40 (s, 2 H), 2.78 (t, *J*=4.9 Hz, 4 H), 2.30 - 2.48 (m, 4 H), 2.18 (s, 3 H).

***tert*-Butyl pyrimidin-2-yl)amino)ethyl)carbamate boronic acid, pinacol ester (4a)—**Prepared via *General Procedure B*. The crude material was re-dissolved in ethyl acetate and washed with water and then a saturated aqueous solution of sodium chloride. The material was used directly in the next reaction without further purification. LCMS $[M+H]^+$ 365.2 m/z (Boronic acid, pinacol ester), 283.1 m/z (Boronic acid).

(2-((2-(Dimethylamino)ethyl)(methyl)amino)pyrimidin-5-yl)boronic acid, pinacol ester (4b)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 307.2 m/z (Boronic acid, pinacol ester), 225.0 m/z (Boronic acid).

(2-(4-(*tert*-Butoxycarbonyl)piperazin-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4c)—Prepared via *General Procedure B* (heating time was 3 h instead of 1 h). The crude material was purified by column chromatography, eluting with 5-10% ethyl acetate/hexanes to obtain the title compound as a colorless solid (140 mg, 49%). LCMS $[M+H]^+$ 391.3 m/z (Boronic acid pinacol ester), 309.2 m/z (Boronic acid); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.35 - 8.40 (m, 2 H), 3.71 (t, J=4.9 Hz, 4 H), 3.39 (t, J=5.4 Hz, 5 H), 1.42 (s, 12 H), 1.17 (s, 9 H).

(2-(4-(2-Ethoxy-2-oxoethyl)piperazin-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4d)—Prepared via *General Procedure B* (heating time was 15 min). The reaction mixture was filtered through celite and purified by column chromatography, eluting with 30-80% ethyl acetate/hexanes to obtain the title compound as a yellow solid (206 mg, 72%). LCMS $[M+H]^+$ 377.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.47 (s, 2 H), 4.08 (q, J=7.0 Hz, 2 H), 3.79 (t, J=5.4 Hz, 4 H), 3.27 (s, 2 H), 2.55 (t, J=5.4 Hz, 4 H), 1.27 (s, 12 H), 1.18 (t, J=7.1 Hz, 3 H).

(2-(2-(Methoxycarbonyl)pyrrolidin-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4e)—Prepared via *General Procedure B* (heating time was 3 h instead of 1h). The crude material was purified by column chromatography, eluting with 10-25% ethyl acetate/hexanes to obtain the title compound as a colorless solid (102 mg, 99%). LCMS $[M+H]^+$ 334.2 m/z (Boronic acid, pinacol ester), 252.1 m/z (Boronic acid); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.51 (d, J=2.9 Hz, 1 H), 8.40 (d, J=2.4 Hz, 1 H), 4.55 (dd, J=8.8, 3.4 Hz, 1 H), 3.60 - 3.70 (m, 5 H), 2.28 - 2.38 (m, 1 H), 1.99 - 2.04 (m, 1 H), 1.91 - 1.98 (m, 2 H), 1.27 (d, J=2.4 Hz, 12 H).

(2-((1*S*,4*S*)-5-Methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-5-yl)boronic acid, boronic acid (4f)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 317.1 m/z.

(2-((1*R*,4*R*)-5-Methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4g)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 317.2 m/z.

(2-(4-Ethylpiperazin-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4h)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 319.2 m/z (Boronic acid, pinacol ester), 237.1 m/z (Boronic acid).

(2-(4-Propylpiperazin-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4i)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 333.2 m/z (Boronic acid pinacol ester), 251.1 m/z (Boronic acid).

(2-(4-Ethyl-1,4-diazepan-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4j)— Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 333.2 m/z (Boronic acid, pinacol ester), 251.1 m/z (Boronic acid).

(2-(4-Propyl-1,4-diazepan-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4k)— Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 347.3 m/z (Boronic acid, pinacol ester), 265.2 m/z (Boronic acid).

(2-(Azepan-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4l)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 222.1 m/z (Boronic acid).

(2-(2-Methyl-2,6-diazaspiro[3.4]octan-6-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4m)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 249.1 m/z (Boronic acid).

(2-(7-Methyl-2,7-diazaspiro[4.4]nonan-2-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4n)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 263.2 m/z (Boronic acid).

(2-((4-Methylpiperazin-1-yl)amino)pyrimidin-5-yl)boronic acid, pinacol ester (4o)—Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 238.1 m/z (Boronic acid).

(2-(4-Methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)boronic acid, pinacol ester (4p)— Prepared via *General Procedure B* and used directly in the next reaction without purification. LCMS $[M+H]^+$ 319.3 m/z (Boronic acid, pinacol ester), 237.1 m/z (Boronic acid).

***N*^{1}-(5-(4-(Pyrazin-2-ylamino)quinolin-7-yl)pyrimidin-2-yl)ethane-1,2-diamine (6a)—**Compound **6b** (37.2 mg, 0.081 mmol) was dissolved in dichloromethane (3 mL) and trifluoroacetic acid (93 μL, 1.22 mmol) was added. The reaction mixture was stirred at ambient temperature overnight. TLC and LCMS analysis showed incomplete conversion to the desired product. Additional trifluoroacetic acid (186 μL, 2.44 mmol) was added and the reaction mixture stirred at ambient temperature for 3 h. LCMS analysis revealed no remaining starting material and a mass consistent with the desired product. All of the volatiles were removed *in vacuo* and the crude material was purified by prep HPLC (method: 95-5_6p5min) to obtain the title compound as a pale-yellow solid (22 mg, 76%). LCMS $[M+H]^+$ 359.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.76 - 10.89 (m, 1 H), 8.95 (s, 2 H), 8.90 (d, *J*=1.0 Hz, 1 H), 8.84 - 8.88 (m, 2 H), 8.51 - 8.56 (m, 1 H), 8.46 (d, *J*=2.4 Hz, 1 H), 8.29 (d, *J*=2.0 Hz, 1 H), 8.26 (d, *J*=8.3 Hz, 1 H), 7.85 (br. s., 2 H), 7.76 (t, *J*=5.6 Hz, 1 H), 3.62 (q, *J*=6.3 Hz, 2 H), 3.06 (dq, *J*=11.9, 5.8 Hz, 2 H).

***tert*-Butyl (2-((5-(4-(pyrazin-2-ylamino)quinolin-7-yl)pyrimidin-2-yl)amino)ethyl)carbamate (6b)—**Prepared via *General Procedure C*. The crude material was purified by column chromatography eluting with a step gradient of 2-5-7-10% methanol/dichloromethane to obtain the title compound as a brown solid (50 mg, 41%).

LCMS $[M+H]^+$ 459.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.81 (s, 1 H), 8.86 (s, 2 H), 8.73 (d, *J*=1.0 Hz, 1 H), 8.72 (d, *J*=5.4 Hz, 1 H), 8.56 (d, *J*=9.3 Hz, 1 H), 8.33 (m, 2 H), 8.20 (d, *J*=1.0 Hz, 1 H), 8.18 (d, *J*=2.9 Hz, 1 H), 7.97 (dd, *J*=8.8, 1.5 Hz, 1 H), 7.43 (t, *J*=5.9 Hz, 1 H), 6.91 (t, *J*=5.6 Hz, 1 H), 3.39 (q, *J*=6.3 Hz, 2 H), 3.16 (dd, *J*=7.1, 5.6 Hz, 2 H), 1.38 (s, 9 H).

***tert*-Butyl methyl(2-((4-(4-(pyrazin-2-ylamino)quinolin-7-yl)phenyl)amino)ethyl)carbamate (6c)—**Prepared via *General Procedure D*. The initial Miyaura was complete in 15 min, and the subsequent Suzuki reaction was complete in 20 min. The crude material was purified by column chromatography, eluting with 1-10% methanol/dichloromethane. Further purification was required, and was performed by column chromatography, eluting with 1-7% methanol/dichloromethane to obtain the title compound as a yellow solid (29 mg, 41% over two steps). LCMS $[M+H]^+$ 473.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.80 (s, 1 H), 8.87 (s, 2 H), 8.73 (d, *J*=1.5 Hz, 1 H), 8.72 (d, *J*=4.9 Hz, 1 H), 8.56 (d, *J*=8.8 Hz, 1 H), 8.31 - 8.35 (m, 2 H), 8.18 (d, *J*=2.9 Hz, 2 H), 7.96 (d, *J*=8.3 Hz, 1 H), 7.43 - 7.59 (m, 1 H), 3.44 (d, *J*=7.8 Hz, 4 H), 2.79 - 2.89 (m, 3 H), 1.24 - 1.41 (m, 9 H).

***N*^{1},*N*^{1},*N*^{2}-Trimethyl-*N*^{2}-(5-(4-(pyrazin-2-ylamino)quinolin-7-yl)pyrimidin-2-yl)ethane-1,2-diamine (6d)—**Prepared via *General Procedure C*. The crude material was purified by column chromatography, eluting with 10-20% methanol (+5% ammonium hydroxide)/ethyl acetate to obtain the title compound as a yellow solid (82 mg, 64%). LCMS $[M+H]^+$ 401.1 m/z; ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 9.82 (br. s., 2 H), 8.97 (s, 2 H), 8.75 (s, 1 H), 8.72 (d, *J*=5.1 Hz, 1 H), 8.59 (d, *J*=8.8 Hz, 2 H), 8.33 (br. s., 2 H), 8.23 (s, 1 H), 8.18 (d, *J*=2.2 Hz, 1 H), 7.99 (d, *J*=8.8 Hz, 1 H), 3.93 (t, *J*=6.2 Hz, 2 H), 3.21 (s, 3 H), 2.91 - 3.08 (m, 2 H), 2.60 (br. s., 6 H).

***tert*-Butyl 4-(5-(4-(pyrazin-2-ylamino)quinolin-7-yl)pyrimidin-2-yl)piperazine-1-carboxylate (6e)—**Prepared via *General Procedure F.* The crude material was purified by column chromatography, eluting with 1-5% methanol/dichloromethane to obtain the title compound as a pale-yellow solid (108 mg, 74%). LCMS $[M+H]^+$ 485.3 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.80 (s, 1 H), 8.97 (s, 2 H), 8.66 - 8.82 (m, 2 H), 8.57 (d, *J*=8.8 Hz, 1 H), 8.33 (dd, *J*=2.7, 1.2 Hz, 2 H), 8.24 (d, *J*=1.5 Hz, 1 H), 8.18 (d, *J*=2.9 Hz, 1 H), 7.99 (dd, *J*=8.8, 1.5 Hz, 1 H), 3.82 (dd, *J*=6.3, 4.4 Hz, 4 H), 3.45 (t, *J*=5.4 Hz, 4 H), 1.44 (s, 9 H).

7-(2-(Piperazin-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (6f)—Compound **6e** (110 mg, 0.227 mmol) was suspended in 4.0 M hydrochloric acid in dioxane (4 mL) and was stirred at ambient temperature. After 1 h the reaction was complete by LCMS and TLC analysis. All of the volatiles were removed and the crude material was re-dissolved in water (2 mL) and Si-carbonate was added. The suspension was stirred at ambient temperature for ~2 h. The Si-carbonate was collected by vacuum filtration and the filtrate was concentrated *in vacuo* to obtain the desired product as a yellow solid (70 mg, 80%). LCMS $[M+H]^+$ 385.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.16 (br. s, 1 H), 9.03 (s, 2 H), 8.94 - 8.99 (m, 2 H), 8.85 (d, *J*=6.3 Hz, 1 H), 8.52 (s, 1 H), 8.51 (dd, *J*=3.9, 1.5

Hz, 1 H), 8.43 (d, *J*=2.4 Hz, 1 H), 8.33 (d, *J*=2.0 Hz, 1 H), 8.21 - 8.26 (m, 1 H), 4.08 (t, *J*=5.4 Hz, 4 H), 3.23 (br. s., 4 H).

Methyl 2-(4-(5-(4-(pyrazin-2-ylamino)quinolin-7-yl)pyrimidin-2-yl)piperazin-1-yl)acetate (6g)—Prepared via *General Procedure F.* The crude material was purified by column chromatography, eluting with 1-5% methanol/dichloromethane to obtain the title compound as a pale-yellow solid (24 mg, 64%). LCMS $[M+H]^+$ 457.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.81 - 9.86 (m, 1 H), 8.95 (s, 2 H), 8.73 (d, *J*=1.5 Hz, 2 H), 8.55 - 8.60 (m, 1 H), 8.33 (dd, *J*=2.9, 1.5 Hz, 2 H), 8.23 (s, 1 H), 8.19 (d, *J*=2.9 Hz, 1 H), 7.96 - 8.02 (m, 1 H), 3.81 - 3.87 (m, 4 H), 3.63 (s, 3 H), 2.61 (t, *J*=4.9 Hz, 4 H).

*Note that the methyl ester was obtained following purification

2-(4-(5-(4-(Pyrazin-2-ylamino)quinolin-7-yl)pyrimidin-2-yl)piperazin-1-yl)acetic acid (6h)—Prepared via *General Procedure F.* The crude material was purified by column chromatography, eluting with 5-10% methanol/dichloromethane to obtain the title compound as a yellow solid (28 mg, 99%). LCMS $[M+H]^+$ 443.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.83 (br. s., 1 H), 8.95 (s, 2 H), 8.69 - 8.76 (m, 2 H), 8.57 (d, *J*=8.8 Hz, 1 H), 8.33 (dd, *J*=2.4, 1.5 Hz, 2 H), 8.23 (d, *J*=1.5 Hz, 1 H), 8.18 (d, *J*=2.9 Hz, 1 H), 7.98 (dd, *J*=8.8, 1.5 Hz, 1 H), 3.85 (t, *J*=4.9 Hz, 4 H), 3.18 (s, 2 H), 2.64 (t, *J*=4.9 Hz, 4 H).

*the carboxylic acid peak is too rapidly exchanging to be seen in the HNMR.

(5-(4-(Pyrazin-2-ylamino)quinolin-7-yl)pyrimidin-2-yl)proline (6i)—Prepared via *General Procedure F.* The crude material was purified by column chromatography eluting with 40-80% ethyl acetate/hexanes before switching to 0-40% methanol/dichloromethane to obtain the title compound as a yellow solid (27 mg, 53%). LCMS $[M+H]^+$ 414.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.90 (br. s., 1 H), 8.91 (br. s., 1 H), 8.87 (br. s., 2 H), 8.74 (s, 1 H), 8.70 (d, *J*=5.4 Hz, 1 H), 8.57 (d, *J*=8.8 Hz, 1 H), 8.32 (dd, *J*=2.4, 1.5 Hz, 2 H), 8.19 (s, 1 H), 8.17 (d, *J*=2.4 Hz, 1 H), 7.94 (d, *J*=8.8 Hz, 1 H), 4.50 (dd, *J*=8.5, 3.2 Hz, 1 H), 3.59 - 3.76 (m, 2 H), 2.20 - 2.33 (m, 2 H), 2.02 - 2.11 (m, 1 H), 1.92 - 2.02 (m, 2 H).

*the carboxylic acid peak is too rapidly exchanging to be seen in the HNMR

**Note ester hydrolysis was observed under the reaction conditions

Methyl (5-(4-(pyrazin-2-ylamino)quinolin-7-yl)pyrimidin-2-yl)prolinate (6j)—Prepared via *General Procedure F* (except that the reaction was run in anhydrous dioxane). The crude material was purified by column chromatography, eluting first with 40-80% ethyl acetate/hexanes before switching to 0-5% methanol/dichloromethane to obtain the title compound as a pale-yellow solid (20 mg, 38%). LCMS $[M+H]^+$ 428.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.81 (br. s., 1 H), 8.98 (br. s., 2 H), 8.92 (br. s., 2 H), 8.69 - 8.76 (m, 2 H), 8.57 (d, *J*=9.3 Hz, 1 H), 8.31 - 8.37 (m, 2 H), 8.23 (s, 1 H), 8.18 (d, *J*=2.4 Hz, 1 H), 7.99 (d, *J*=8.3 Hz, 1 H), 4.61 (dd, *J*=8.5, 3.2 Hz, 1 H), 3.68 - 3.79 (m, 2 H), 3.66 (s, 3 H), 2.38 (dd, *J*=11.7, 7.8 Hz, 1 H), 1.93 - 2.10 (m, 3 H).

6-Methyl-7-(2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (9a)—Prepared via *General Procedure F.* The reaction mixture was concentrated and ethyl acetate was added, the suspension was filtered. The filtrate was washed with water, brine and dried with sodium sulfate. The crude product was purified via prep HPLC in basic mode using water (+0.1% ammonium hydroxide):acetonitrile (30-100%) to obtain the title compound as a pale yellow solid (17 mg, 43%). LCMS $[M+H]^+$ 413.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.74 (s, 1H), 8.72 (s, 1H), 8.67 (d, *J*=5.0 Hz, 1H), 8.55 (s, 2H), 8.39 (s, 1H), 8.32 (s, 1H), 8.31 (d, *J*=5.5 Hz, 1H), 8.17 (d, *J*=2.5 Hz, 1H), 7.81 (s, 1H), 3.81 (t, *J*=5.0 Hz, 4H), 2.51 (d, *J*=6.0 Hz, 3H), 2.40 (t, *J*=5.0 Hz, 4H), 2.24 (s, 3H).

8-Methyl-7-(2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (9b)—Prepared via *General Procedure F.* The reaction mixture was then concentrated and ethyl acetate was added, the suspension was filtered. The filtrate was washed with water, brine and dried with sodium sulfate. The crude product was purified *via* prep HPLC in basic mode using water 30-100% acetonitrile (+0.1% ammonium hydroxide)/ water to obtain the title compound as a yellow solid (16 mg, 61%). LCMS $[M+H]^+$ 413.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.77 (s, 1H), 8.79 (d, *J*=5.5 Hz, 1H), 8.72 (s, 1H), 8.54 (s, 2H), 8.40 (d, *J*=9.0 Hz, 1H), 8.36 (d, *J*=5.0 Hz, 1H), 8.32 (dd, *J*=1.5, 2.5 Hz, 1H), 8.17 (d, *J*=3.0 Hz, 1H), 7.54 (d, *J*=9.0 Hz, 1H), 3.82 (t, *J*=5.0 Hz, 4H), 2,70 (s, 3H), 2.44 (t, *J*=5.0 Hz, 4H), 2.26 (s, 3H).

7-(2-((1*S*,4*S*)-5-Methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10a)—Prepared via *General Procedure C* (except that the reaction mixture was heated conventionally at 100 °C overnight). The crude material was purified by column chromatography, eluting with 1-20% methanol (+10% ammonium hydroxide)/dichloromethane. A second purification was necessary, and this was done by column chromatography, eluting first with 20-25% methanol/dichloromethane, before switching to 5-20% methanol (+10% ammonium hydroxide)/dichloromethane to afford the title compound as a yellow solid (16 mg, 21%). $[\alpha]_D^{22}$ −77 ± 3.8 (*c* 0.38, CH_3OH); LCMS $[M+H]^+$ 411.1 m/z; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.67 (s, 2 H) 8.58 (d, *J*=5.4 Hz, 1 H) 8.55 (d, *J*=1.5 Hz, 1 H) 8.37 (d, *J*=5.4 Hz, 1 H) 8.33 (d, *J*=8.8 Hz, 1 H) 8.29 (dd, *J*=2.4, 1.5 Hz, 1 H) 8.10 (d, *J*=2.9 Hz, 1 H) 7.97 (d, *J*=2.0 Hz, 1 H) 7.73 (dd, *J*=8.8, 2.0 Hz, 1 H) 4.86 (s, 1 H) 3.76 (dd, *J*=10.7, 1.5 Hz, 1 H) 3.62 (s, 1 H) 3.47 (dd, *J*=11.0, 2.2 Hz, 1 H) 3.35 (s, 5 H) 2.91 (dd, *J*=10.0, 2.2 Hz, 1 H) 2.75 (d, *J*=10.3 Hz, 1 H) 2.45 (s, 3 H) 2.04 (d, *J*=9.8 Hz, 1 H) 1.91 (d, *J*=9.8 Hz, 1 H)*The -NH peak is too rapidly exchanging to be seen in the HNMR.

7-(2-((1*R*,4*R*)-5-Methyl-2,5-diazabicyclo[2.2.1]heptan-2-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10b)—Prepared via *General Procedure C* (except that the reaction mixture was heated conventionally at 100 °C overnight). The crude material was purified by column chromatography, eluting first with 50-100% ethyl acetate/hexanes, before switching to 0-30% methanol/dichloromethane. A second purification was necessary, and this was done by column chromatography, eluting with 20% methanol (+10% ammonium hydroxide)/dichloromethane to afford the title compound as an orange-yellow

solid (63 mg, 35%). $[\alpha]_D^{27}$ +17 ± 0.42 (*c* 0.46, CH_3OH); LCMS $[M+H]^+$ 411.1 m/z; ^1H NMR (500 MHz, METHANOL-d_4) δ ppm 8.82 (s, 2 H), 8.68 (d, *J*=5.4 Hz, 1 H), 8.61 (s, 1 H), 8.49 (d, *J*=8.8 Hz, 1 H), 8.46 (d, *J*=5.4 Hz, 1 H), 8.35 (dd, *J*=2.4, 1.5 Hz, 1 H), 8.15 (d, *J*=2.4 Hz, 1 H), 8.12 (d, *J*=1.5 Hz, 1 H), 7.88 (d, *J*=7.8 Hz, 1 H), 5.01 (s, 1 H), 3.91 (br. s., 1 H), 3.84 (d, *J*=11.7 Hz, 1 H), 3.63 (d, *J*=12.2 Hz, 1 H), 3.09 (s, 2 H), 2.64 (s, 3 H), 2.18 (d, *J*=10.7 Hz, 1 H), 2.08 (d, *J*=11.2 Hz, 1 H).

*The -NH peak is too rapidly exchanging to be seen in the HNMR.

7-(2-(4-Ethylpiperazin-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10c)—Prepared via *General Procedure C* (except that the reaction mixture was heated conventionally at 100 °C overnight). The crude material was purified by column chromatography, eluting with 2-5-10-15% methanol (+5% ammonium hydroxide)/ethyl acetate to obtain the title compound as a yellow solid (25 mg, 40%). LCMS $[M+H]^+$ 413.2 m/z; ^1H NMR (500 MHz, DMSO-d_6) δ ppm 9.80 (s, 1 H), 8.95 (s, 2 H), 8.72 (d, *J*=5.4 Hz, 2 H), 8.56 (d, *J*=8.8 Hz, 1 H), 8.33 (d, *J*=4.9 Hz, 2 H), 8.23 (d, *J*=1.5 Hz, 1 H), 8.18 (d, *J*=2.4 Hz, 1 H), 7.98 (dd, *J*=8.8, 2.0 Hz, 1 H), 3.82 (t, *J*=4.9 Hz, 4 H), 2.45 (t, *J*=4.9 Hz, 4 H), 2.38 (q, *J*=7.2 Hz, 2 H), 1.05 (t, *J*=7.1 Hz, 3 H).

7-(2-(4-Propylpiperazin-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10d)—Prepared via *General Procedure C* (except 0.1 eq of catalyst was used instead of 0.05 eq). The crude material was purified by column chromatography, eluting with 2-5-10% methanol (+5% ammonium hydroxide)/ethyl acetate to obtain the title compound as a gold solid (64 mg, 51%). LCMS $[M+H]^+$ 427.2 m/z; ^1H NMR (500 MHz, DMSO-d_6) δ ppm 9.80 (s, 1 H), 8.94 (s, 2 H), 8.69 - 8.76 (m, 2 H), 8.56 (d, *J*=8.8 Hz, 1 H), 8.30 - 8.36 (m, 2 H), 8.23 (d, *J*=2.0 Hz, 1 H), 8.18 (d, *J*=2.9 Hz, 1 H), 7.98 (dd, *J*=9.0, 1.7 Hz, 1 H), 3.82 (t, *J*=4.9 Hz, 4 H), 2.44 (t, *J*=4.4 Hz, 4 H), 2.29 (t, *J*=7.3 Hz, 2 H), 1.49 (sxt, *J*=7.3 Hz, 2 H), 0.88 (t, *J*=7.3 Hz, 3 H).

7-(2-(4-Methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10e)—Prepared via *General Procedure D*. The crude material was purified by column chromatography, eluting with 1-5% methanol/dichloromethane to obtain the title compound as a yellow solid (73 mg, 48% over two steps). LCMS $[M+H]^+$ 413.1 m/z; ^1H NMR (500 MHz, DMSO-d_6) δ ppm 9.82 (s, 1 H), 8.94 (s, 2 H), 8.75 (d, *J*=1.0 Hz, 1 H), 8.72 (d, *J*=5.4 Hz, 1 H), 8.58 (d, *J*=8.8 Hz, 1 H), 8.30 - 8.34 (m, 2 H), 8.22 (d, *J*=2.0 Hz, 1 H), 8.17 (d, *J*=2.9 Hz, 1 H), 7.98 (dd, *J*=9.0, 1.7 Hz, 1 H), 3.92 (t, *J*=4.4 Hz, 2 H), 3.83 (t, *J*=6.1 Hz, 2 H), 2.72 (br. s., 2 H), 2.58 (br. s., 2 H), 1.91 - 1.97 (m, 2 H), 1.90 (s, 3 H).

7-(2-(4-Ethyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10f)—Prepared via *General Procedure C*. eluting with 2-5% methanol (+5% ammonium hydroxide)/dichloromethane. Further purification was required and was performed by prep HPLC (method: 99-90_6p5min) to obtain the title compound as a yellow solid (32 mg, 30%). LCMS $[M+H]^+$ 427.1 m/z; ^1H NMR (399 MHz, DMSO-d_6) δ ppm 9.83 (br. s., 1 H), 8.93 (s, 2 H), 8.73 (s, 1 H), 8.71 (d, *J*=5.1 Hz, 1 H), 8.57 (d, *J*=8.8 Hz, 1 H), 8.30 - 8.38 (m, 2 H), 8.26 - 8.30 (m, 1 H), 8.22 (d, *J*=1.5 Hz, 1 H), 8.17 (d, *J*=2.9 Hz, 1 H), 7.98 (dd, *J*=8.8, 1.5 Hz, 1 H), 3.89 (t, *J*=5.1 Hz, 2 H), 3.83 (t, *J*=6.2 Hz, 2 H), 2.74 (t, *J*=5.1 Hz, 2 H), 2.58 (t,

J=5.1 Hz, 2 H), 1.88 (qd, *J*=6.6, 5.1 Hz, 2 H), 0.99 (t, *J*=7.0 Hz, 3 H). ^{1}H NMR (399 MHz, METHANOL-d_4) δ ppm 8.68 (br. s., 2 H), 8.52 - 8.61 (m, 2 H), 8.36 (d, *J*=5.1 Hz, 1 H), 8.29 (br. s., 1 H), 8.10 (d, *J*=2.2 Hz, 1 H), 7.94 (s, 1 H), 7.72 (br. s., 1 H), 4.03 (br. s., 2 H), 3.88 (t, *J*=6.2 Hz, 2 H), 3.10 (br. s., 2 H), 2.99 (t, *J*=4.4 Hz, 1 H), 2.88 (q, *J*=7.1 Hz, 2 H), 2.11 (td, *J*=6.6, 4.4 Hz, 2 H), 1.22 (t, *J*=7.3 Hz, 3 H).

*In DMSO one of the methylenes is overlapping with the solvent signal

7-(2-(4-Propyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10g)—Prepared via *General Procedure C*. The crude material was purified by column chromatography eluting with 2-5-10-15-20% methanol (+5% ammonium hydroxide)/ethyl acetate. Further purification was performed by column chromatography, eluting with 20% acetonitrile (+5% ammonium hydroxide)/dichloromethane to obtain the title compound as a pale-brown solid (27 mg, 22%). LCMS $[M+H]^+$ 441.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.81 (s, 1 H), 8.93 (s, 2 H), 8.74 (s, 1 H), 8.72 (d, *J*=5.4 Hz, 1 H), 8.57 (d, *J*=8.8 Hz, 1 H), 8.33 (s, 2 H), 8.22 (s, 1 H), 8.17 (d, *J*=2.9 Hz, 1 H), 7.98 (dd, *J*=8.8, 1.5 Hz, 1 H), 3.86 - 3.91 (m, 2 H), 3.83 (t, *J*=6.1 Hz, 2 H), 2.71 - 2.77 (m, 2 H), 2.54 - 2.60 (m, 2 H), 2.39 (t, *J*=7.3 Hz, 2 H), 1.86 (quin, *J*=5.7 Hz, 2 H), 1.42 (sxt, *J*=7.3 Hz, 2 H), 0.83 (t, *J*=7.3 Hz, 3 H).

7-(2-(Azepan-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10h)—Prepared via *General Procedure C*. The crude material was purified by column chromatography eluting with 2-5-7-10% methanol/dichloromethane. Further purification was required by prep HPLC (method: 95-5_6p5min) to obtain the title compound as yellow solid (18 mg, 14%). LCMS $[M+H]^+$ 398.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.81 (br. s., 1 H), 8.92 (s, 2 H), 8.73 (s, 1 H), 8.71 (d, *J*=4.9 Hz, 1 H), 8.56 (d, *J*=8.8 Hz, 1 H), 8.32 (dd, *J*=2.4, 1.5 Hz, 2 H), 8.21 (d, *J*=1.5 Hz, 1 H), 8.17 (d, *J*=2.9 Hz, 1 H), 7.97 (dd, *J*=8.8, 2.0 Hz, 1 H), 3.80 (t, *J*=5.9 Hz, 4 H), 1.70 - 1.81 (m, 4 H), 1.52 (quin, *J*=2.4 Hz, 4 H).

7-(2-(2-Methyl-2,6-diazaspiro[3.4]octan-6-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10i)—Prepared via *General Procedure C* with one exception, the reaction was run at 0.1 M. The crude material was purified by column chromatography, eluting with 10-20% methanol (+10% ammonium hydroxide)/ethyl acetate (step gradient) to afford the title compound as a yellow solid (8 mg, 28%). LCMS $[M+H]^+$ 425.2 m/z; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.83 (s, 2 H) 8.69 (d, *J*=5.4 Hz, 1 H) 8.62 (d, *J*=1.0 Hz, 1 H) 8.51 (d, *J*=8.8 Hz, 1 H) 8.47 (d, *J*=5.4 Hz, 1 H) 8.36 (s, 1 H) 8.17 (d, *J*=2.4 Hz, 1 H) 8.14 (d, *J*=2.0 Hz, 1 H) 7.90 (dd, *J*=8.8, 2.0 Hz, 1 H) 4.12 (q, *J*=9.3 Hz, 4 H) 3.91 (s, 2 H) 3.73 (t, *J*=7.1 Hz, 2 H) 2.91 (s, 3 H) 2.38 (t, *J*=7.1 Hz, 2 H).

7-(2-(7-Methyl-2,7-diazaspiro[4.4]nonan-2-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (10j)—Prepared via *General Procedure C* with one exception, the reaction was run at 0.1 M. The crude material was purified by column chromatography, eluting with 10-20% methanol (+10% ammonium hydroxide)/ethyl acetate (step gradient) to afford the title compound as a tan solid (23 mg, 32%). LCMS $[M+H]^+$ 439.3 m/z; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.79 (s, 2 H), 8.68 (d, *J*=5.4 Hz, 1 H), 8.61 (s, 1 H), 8.48 (d, *J*=8.8 Hz, 1 H), 8.45 (d, *J*=5.4 Hz, 1 H), 8.35 (s, 1 H), 8.15 (d, *J*=2.4 Hz, 1 H), 8.12

(s, 1 H), 7.87 (dd, *J*=8.8, 1.5 Hz, 1 H), 3.58 - 3.74 (m, 4 H), 2.76 (t, *J*=7.1 Hz, 2 H), 2.68 (td, *J*=9.8, 4.9 Hz, 2 H), 2.42 (s, 3 H), 2.02 - 2.15 (m, 2 H), 1.95 (quind, *J*=5.9, 5.9, 5.9, 5.9, 4.4 Hz, 2 H).

7-Bromo-*N*-cyclohexylquinolin-4-amine (12a)—Prepared via *General Procedure H*. The crude material was purified by column chromatography eluting with 1-20% methanol/dichloromethane to afford the title compound as an off-white solid (26 mg, 41%). LCMS $[M+H]^+$ 305.1 m/z (Br^{79}), 307.1 m/z (Br^{81}); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.49 (d, *J*=5.9 Hz, 1 H), 8.12 (d, *J*=1.5 Hz, 1 H), 7.57 (d, *J*=8.8 Hz, 1 H), 7.46 (dd, *J*=9.0, 1.7 Hz, 1 H), 6.44 (d, *J*=5.4 Hz, 1 H), 4.93 (d, *J*=6.8 Hz, 1 H), 3.43 - 3.56 (m, 1 H), 2.08 - 2.21 (m, 2 H), 1.78 - 1.89 (m, 2 H), 1.66 - 1.76 (m, 1 H), 1.39 - 1.52 (m, 2 H), 1.22 - 1.38 (m, 3 H).

N^1-(7-Bromoquinolin-4-yl)-N^4,N^4-dimethylcyclohexane-1,4-diamine (12b)—Prepared via *General Procedure H*. The crude material was purified by column chromatography, eluting with 1-20% methanol/dichloromethane to afford the title compound as an off-white solid (15.8 mg, 22%). LCMS $[M+H]^+$ 348.1 m/z (Br^{79}), 350.1 m/z (Br^{81}); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.51 (d, *J*=4.9 Hz, 1 H), 8.13 (d, *J*=2.0 Hz, 1 H), 7.56 (d, *J*=8.8 Hz, 1 H), 7.48 (dd, *J*=8.8, 2.0 Hz, 1 H), 6.45 (d, *J*=5.4 Hz, 1 H), 4.83 (d, *J*=6.8 Hz, 1 H), 3.39 - 3.49 (m, 1 H), 2.33 (s, 6 H), 2.24 - 2.32 (m, 2 H), 2.12 - 2.22 (m, 1 H), 2.04 (d, *J*=12.7 Hz, 2 H), 1.27 - 1.51 (m, 4 H).

7-Bromo-*N*-(tetrahydro-2*H*-pyran-4-yl)quinolin-4-amine (12c)—Prepared via *General Procedure H*. The crude material was purified by column chromatography eluting with 1-20% methanol/dichloromethane to afford the title compound as a yellow solid (46 mg, 74%). LCMS $[M+H]^+$ 307.0 m/z (Br^{79}), 309.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.28 (d, *J*=5.4 Hz, 1 H), 7.95 (d, *J*=2.0 Hz, 1 H), 7.73 (d, *J*=9.3 Hz, 1 H), 7.42 (dd, *J*=8.8, 2.0 Hz, 1 H), 6.39 (d, *J*=5.9 Hz, 1 H), 3.98 (dt, *J*=11.5, 3.3 Hz, 2 H), 3.75 (s, 1 H), 3.66 (tt, *J*=10.7, 4.2 Hz, 1 H), 3.50 (td, *J*=11.8, 2.2 Hz, 2 H), 1.98 - 2.05 (m, 2 H), 1.56 - 1.67 (m, 2 H).

7-Bromo-*N*-((tetrahydro-2*H*-pyran-4-yl)methyl)quinolin-4-amine (12d)—Prepared via *General Procedure H*. The crude material was purified by column chromatography eluting with 5-15% methanol/dichloromethane. The resulting residue was washed with hexanes, after which the title compound was collected by vacuum filtration as a yellow solid (47 mg, 71%). LCMS $[M+H]^+$ 321.1 m/z (Br^{79}), 323.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.37 (d, *J*=5.4 Hz, 1 H), 8.22 (d, *J*=8.8 Hz, 1 H), 7.93 (d, *J*=2.0 Hz, 1 H), 7.55 (dd, *J*=8.8, 2.0 Hz, 1 H), 7.39 (t, *J*=5.4 Hz, 1 H), 6.52 (d, *J*=5.4 Hz, 1 H), 3.85 (dd, *J*=11.2, 2.9 Hz, 2 H), 3.24 - 3.30 (m, 2 H), 1.91 - 2.01 (m, 1 H), 1.68 (d, *J*=12.7 Hz, 2 H), 1.25 (qd, *J*=12.2, 4.4 Hz, 2 H), 1.07 (s, 2 H).

(1*R*,2*R*)-2-((7-Bromoquinolin-4-yl)amino)cyclohexan-1-ol (12e)—Prepared via *General Procedure H*, heating temperature was 180 °C instead of 200 °C. The crude material was purified by column chromatography eluting with 1-8% methanol (+10% ammonium hydroxide)/dichloromethane, to afford the product compound as an off-white solid (95 mg, 98%). $[\alpha]_D^{21}$ −0.79 ± 0.38 (*c* 1.1, 1:5 CH_3OH/DCM); LCMS $[M+H]^+$ 321.3 m/z (Br^{79}),

323.1 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.58 (d, *J*=9.3 Hz, 1 H), 8.41 (d, *J*=6.8 Hz, 2 H), 8.11 (d, *J*=2.0 Hz, 1 H), 7.76 (dd, *J*=9.3, 2.0 Hz, 1 H), 6.86 (d, *J*=6.3 Hz, 1 H), 5.00 (br. s., 1 H), 3.53 - 3.66 (m, 2 H), 2.84 - 3.06 (m, 1 H), 1.86 - 1.97 (m, 2 H), 1.64 - 1.76 (m, 2 H), 1.28 - 1.51 (m, 3 H).

7-Bromo-*N*-(3-methylpyrazin-2-yl)quinolin-4-amine (12f)—Prepared via *General Procedure G*. There was no precipitate evident so the aqueous phase was extracted twice with dichloromethane. The organic layers were combined and washed once with a saturated aqueous sodium chloride solution before being dried with magnesium sulfate. All the volatiles were removed *in vacuo* and the crude material was purified by column chromatography, eluting with 50-65% ethyl acetate/hexanes before switching to 0-10% methanol/dichloromethane to obtain the title compound as an orange solid (264 mg, 41%). LCMS $[M+H]^+$ 314.9 m/z (Br^{79}), 317.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.87 (s, 1 H), 8.70 (d, *J*=4.9 Hz, 1 H), 8.21 (d, *J*=9.3 Hz, 1 H), 8.12 - 8.18 (m, 3 H), 7.71 (dd, *J*=9.0, 2.2 Hz, 1 H), 7.61 (d, *J*=4.9 Hz, 1 H), 2.63 (s, 3 H).

7-Bromo-*N*-(5-methylpyrazin-2-yl)quinolin-4-amine (12g)—Prepared via *General Procedure G*. The solid was collected and purified by column chromatography, eluting with 0-15% methanol/dichloromethane to obtain the title compound as a pale-orange solid (250 mg, 38%). LCMS $[M+H]^+$ 314.9 m/z (Br^{79}), 317.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.73 (s, 1 H), 8.68 (d, *J*=5.4 Hz, 1 H), 8.61 (d, *J*=1.5 Hz, 1 H), 8.47 (d, *J*=8.8 Hz, 1 H), 8.30 (d, *J*=5.4 Hz, 1 H), 8.23 (s, 1 H), 8.14 (d, *J*=2.0 Hz, 1 H), 7.77 (dd, *J*=9.0, 2.2 Hz, 1 H), 2.44 (s, 3 H).

7-Bromo-*N*-(6-methylpyrazin-2-yl)quinolin-4-amine (12h)—Prepared via *General Procedure G*. The crude material was purified by column chromatography, eluting with 0-15% methanol/dichloromethane to obtain the title compound as a pale-yellow solid (486 mg, 75%). LCMS $[M+H]^+$ 314.9 m/z (Br^{79}), 316.9 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.77 (s, 1 H), 8.72 (d, *J*=5.4 Hz, 1 H), 8.50 (s, 1 H), 8.47 (d, *J*=9.3 Hz, 1 H), 8.43 (d, *J*=5.4 Hz, 1 H), 8.15 (d, *J*=2.0 Hz, 1 H), 8.09 (s, 1 H), 7.78 (dd, *J*=8.8, 2.4 Hz, 1 H), 2.46 (s, 3 H).

***N*-Cyclohexyl-7-(2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)quinolin-4-amine, mono-formate salt (15a)—**Prepared via *General Procedure C.* The crude material was purified by column chromatography, eluting with 1-20% methanol/dichloromethane. The material was further purified by prep HPLC (method: 95-70_6p5min). The residue was triturated with dichloromethane and the subsequent precipitate was isolated by vacuum filtration, the title compound was afforded as tan solid (7.3 mg, 11%). LCMS $[M+H]^+$ 403.3 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.88 (s, 2 H), 8.45 (d, *J*=9.3 Hz, 1 H), 8.40 (d, *J*=5.4 Hz, 1 H), 8.15 (s, 2 H), 8.00 (s, 1 H), 7.81 (d, *J*=8.8 Hz, 1 H), 7.31 - 7.40 (m, 1 H), 6.60 (d, *J*=5.4 Hz, 1 H), 3.82 (br. s., 4 H), 2.41 (t, *J*=4.6 Hz, 4 H), 2.24 (s, 3 H), 2.00 (br. s., 2 H), 1.80 (br. s., 2 H), 1.67 (d, *J*=11.7 Hz, 1 H), 1.35 - 1.48 (m, 4 H), 1.13 - 1.26 (m, 1 H). ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.87 (s, 2 H), 8.55 (d, *J*=8.8 Hz, 1 H), 8.38 (br. s., 2 H), 8.35 (d, *J*=6.8 Hz, 1 H), 7.99 (br. s., 1 H), 7.96 (d, *J*=9.3 Hz, 1 H), 6.90 (d, *J*=6.8 Hz, 1 H), 4.13 (br. s., 4 H), 3.78 - 3.90 (m, 1 H), 3.08 (br. s., 4 H), 2.74 (s, 3 H), 2.12 (d, *J*=9.8 Hz,

2 H), 1.92 (d, *J*=10.7 Hz, 2 H), 1.78 (d, *J*=12.7 Hz, 1 H), 1.46 - 1.64 (m, 4 H), 1.32 (d, *J*=10.7 Hz, 1 H).

7-(2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)-*N*-(1-methylpiperidin-4-yl)quinolin-4-amine, tri-formate salt (15b)—4-Chloro-7-(2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)quinoline (**39**) (20 mg, 0.059 mmol), 1-methylpiperidin-4-amine (14.8 μL, 0.12 mmol), tris(dibenzylideneacetone)dipalladium(0) (2.7 mg, 0.003 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (2.8 mg, 0.006 mmol), and potassium *tert*-butoxide (19.8 mg, 0.177 mmol) were added to a vial that was filled with nitrogen and evacuated three times. Dry, degassed dioxane (1 mL) was then added. The reaction mixture was heated at 90 °C overnight. LCMS analysis revealed no remaining starting material and a mass consistent with the desired product. The reaction mixture was filtered through celite and purified by column chromatography eluting with 0-20% (10% ammonium hydroxide/methanol)/dichloromethane. The material was further purified by prep HPLC (method: 99-95_6p5min) to afford the title compound as a yellow solid (11.7 mg, 47%). LCMS [M+H]$^+$ 418.1 m/z; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.82 (s, 2 H), 8.52 (d, *J*=8.8 Hz, 1 H), 8.47 (s, 3 H), 8.41 (d, *J*=6.8 Hz, 1 H), 8.01 (s, 1 H), 7.93 (d, *J*=8.8 Hz, 1 H), 6.94 (d, *J*=7.3 Hz, 1 H), 4.06 (br. s., 5 H), 3.50 (br. s., 2 H), 3.06 (br. s., 2 H), 2.95 (t, *J*=5.1 Hz, 4 H), 2.62 (s, 6 H), 2.27 (d, *J*=12.7 Hz, 2 H), 2.03 - 2.17 (m, 2 H).

***N*^{1},*N*^{1}-Dimethyl-*N*^{4}-(7-(2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)quinolin-4-yl)cyclohexane-1,4-diamine (15c)—**Prepared via *General Procedure F* with some exceptions. The reaction mixture was run in a 3:1 mixture of degassed dioxane/water (0.08 M) and the reaction was heated for 48 h at 80 °C. The crude material was purified by column chromatography eluting with 4-10% methanol (10% ammonium hydroxide)/dichloromethane to afford the title compound as an off-white solid, 28 mg (27%). LCMS [M+H]$^+$ 446.3 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.86 (s, 2 H), 8.37 (d, *J*=5.4 Hz, 1 H), 8.34 (d, *J*=8.8 Hz, 1 H), 7.99 (d, *J*=2.0 Hz, 1 H), 7.71 (dd, *J*=8.8, 2.0 Hz, 1 H), 6.79 (d, *J*=7.8 Hz, 1 H), 6.48 (d, *J*=5.4 Hz, 1 H), 3.78 - 3.83 (m, 4 H), 3.41 - 3.50 (m, 1 H), 2.39 (t, *J*=5.1 Hz, 4 H), 2.23 (s, 3 H), 2.20 (s, 7 H), 2.08 (d, *J*=10.7 Hz, 2 H), 1.87 (d, *J*=11.2 Hz, 2 H), 1.39 (m, 4 H).

7-(2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)-*N*-(2-(pyrrolidin-1-yl)ethyl)quinolin-4-amine (15d)—4-Chloro-7-(2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)quinoline (**39**) (20 mg, 0.059 mmol), 2-(pyrrolidin-1-yl)ethan-1-amine (14.8 μL, 0.12 mmol), tris(dibenzylideneacetone)dipalladium(0) (2.7 mg, 0.003 mmol), 2-dicyclohexylphosphino-2′,4′,6′-triisopropylbiphenyl (2.8 mg, 0.006 mmol), and potassium *tert*-butoxide (19.8 mg, 0.177 mmol) were added to a vial that was filled with nitrogen and evacuated three times. Dry, degassed dioxane (1.5 mL) was then added. The reaction mixture was heated at 90 °C overnight. LCMS analysis revealed no remaining starting material and a mass consistent with the desired product. The reaction mixture was filtered through celite and purified by column chromatography eluting with 2-20% (5% ammonium hydroxide/methanol)/dichloromethane to afford the title compound as a yellow solid (36 mg, 59%). LCMS [M+H]$^+$ 418.3 m/z; ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 8.87 (s, 2 H), 8.40 (d, *J*=5.9 Hz, 1 H), 8.25 (d, *J*=8.8 Hz, 1 H), 8.01 (s, 1 H), 7.75 (d, *J*=8.8 Hz, 1 H), 7.20 (br.

s., 1 H), 6.45 (d, *J*=5.1 Hz, 1 H), 3.81 (m, 1 H), 3.38 - 3.47 (m, 1 H), 2.73 (t, *J*=6.6 Hz, 2 H), 2.55 (br. s., 4 H), 2.38 (t, *J*=4.8 Hz, 4 H), 2.18 - 2.26 (m, 3 H), 1.70 (br. s., 4 H).

7-(2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)-*N*-(tetrahydro-2*H*-pyran-4-yl)quinolin-4-amine (15e)—Prepared via *General Procedure F.* The crude material was purified by column chromatography eluting with 1-20% methanol (+50% ammonium hydroxide)/dichloromethane. The resulting product was triturated with ether and the precipitate was collected by vacuum filtration to afford the title compound as a light brown solid (22 mg, 36%). LCMS $[M+H]^+$ 405.2 m/z; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.74 (s, 2 H), 8.36 (d, *J*=5.9 Hz, 1 H), 8.27 (d, *J*=8.8 Hz, 1 H), 7.92 (s, 1 H), 7.69 (dd, *J*=8.8, 1.5 Hz, 1 H), 6.65 (d, *J*=5.9 Hz, 1 H), 3.99 - 4.08 (m, 2 H), 3.85 - 3.95 (m, 5 H), 3.60 (m, 2 H), 2.52 (t, *J*=5.1 Hz, 4 H), 2.34 (s, 3 H), 2.01 - 2.11 (m, 2 H), 1.68 - 1.82 (m, 2 H).

7-(2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)-*N*-((tetrahydro-2*H*-pyran-4-yl)methyl)quinolin-4-amine (15f)—Prepared via *General Procedure F* with some exceptions. The reaction mixture was run in a 3:1 mixture of degassed dioxane/water (0.08 M) and the reaction was heated for 18 h at 80 °C. The crude material was purified by column chromatography eluting with 2-20% methanol/dichloromethane. The compound was further purified by column chromatography eluting with 1-8% methanol (+5% ammonium hydroxide)/dichloromethane to afford the title compound as a colorless solid, (26 mg, 44%). LCMS $[M+H]^+$ 419.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.86 (s, 2 H), 8.38 (d, *J*=5.4 Hz, 1 H), 8.31 (d, *J*=8.8 Hz, 1 H), 8.00 (d, *J*=1.5 Hz, 1 H), 7.72 (dd, *J*=8.8, 2.0 Hz, 1 H), 7.25 (t, *J*=5.6 Hz, 1 H), 6.46 (d, *J*=5.9 Hz, 1 H), 3.86 (dd, *J*=11.2, 2.9 Hz, 2 H), 3.77 - 3.83 (m, 4 H), 3.28 (m, 2 H), 3.18 (t, *J*=6.1 Hz, 2 H), 2.38 (t, *J*=4.9 Hz, 4 H), 2.22 (s, 3 H), 1.93 - 2.03 (m, 1 H), 1.69 (dd, *J*=12.5, 1.2 Hz, 2 H), 1.26 (qd, *J*=12.2, 3.9 Hz, 2 H).

(1*R*,2*R*)-2-((7-(2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)quinolin-4-yl)amino)cyclohexan-1-ol (15g)—Prepared via *General Procedure F* with some exceptions. The reaction mixture was run in a 3:1 mixture of degassed dioxane/water (0.08 M) and the reaction was heated for 48 h at 80 °C. The crude material was purified by column chromatography eluting with 4-8% methanol (+10% ammonium hydroxide)/ dichloromethane to afford the title compound as a light brown solid (46 mg, 38%). $[\alpha]_D^{22}$ +0.57 ± 0.28 (*c* 1.1, 1:10 DCM/CH_3OH); LCMS $[M+H]^+$ 419.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.87 (s, 2 H), 8.35 (d, *J*=2.0 Hz, 1 H), 8.34 (s, 1 H), 7.99 (d, *J*=2.0 Hz, 1 H), 7.72 (dd, *J*=8.8, 2.0 Hz, 1 H), 6.72 (d, *J*=7.8 Hz, 1 H), 6.51 (d, *J*=5.9 Hz, 1 H), 4.74 (br. s., 1 H), 3.92 (br. s., 1 H), 3.74 - 3.85 (m, 4 H), 3.52 - 3.62 (m, 1 H), 2.35 - 2.43 (m, 4 H), 2.23 (s, 3 H), 1.98 (m, 2 H), 1.68 (m, 2 H), 1.31 (m, 4 H).

N^7-(4-(4-Methylpiperazin-1-yl)phenyl)-N^4-(pyrazin-2-yl)quinoline-4,7-diamine (16b)—Compound **5** (150 mg, 0.498 mmol), 4-(4-methylpiperazin-1-yl)aniline (114 mg, 0.598 mmol), cesium carbonate (325 mg, 0.996 mmol), palladium acetate (11 mg, 0.050 mmol) and XANTPhos (58 mg, 0.100 mmol) were combined in an oven dried microwave vial with anhydrous dioxane (9 ml). The reaction mixture was degassed and placed in the CEM microwave at 140 °C for 30 mins. LCMS analysis revealed no remaining starting material and a mass consistent with the desired product. The crude material was purified by

column chromatography, eluting with 5-8% methanol/dichloromethane. The material was further purified by column chromatography, eluting first with 5-15% methanol (+1% ammonium hydroxide)/ethyl acetate followed by 15-20% methanol (+1% ammonium hydroxide)/dichloromethane. The material was again further purified eluting with 5-15% methanol (+1% ammonium hydroxide)/ethyl acetate to obtain the title compound as an orange solid (112 mg, 55%). LCMS $[M+H]^+$ 412.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.58 (s, 1 H), 8.66 (s, 1 H), 8.48 (d, *J*=5.37 Hz, 1 H), 8.31 (s, 1 H), 8.28 (dd, *J*=2.44, 1.47 Hz, 1 H), 8.24 (d, *J*=9.28 Hz, 1 H), 8.13 (d, *J*=2.44 Hz, 1 H), 7.99 (d, *J*=4.88 Hz, 1 H), 7.27 (d, *J*=2.44 Hz, 1 H), 7.22 (dd, *J*=9.28, 1.95 Hz, 1 H), 7.16 (d, *J*=8.79 Hz, 2 H), 6.98 (d, *J*=9.28 Hz, 2 H), 3.07 - 3.15 (m, 4 H), 2.44 - 2.50 (m, 4 H), 2.24 (s, 3 H).

7-(2-((4-Methylpiperazin-1-yl)amino)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (16f)—Prepared via *General Procedure C.* The crude material was purified by column chromatography, eluting with 2-5-7-10% methanol/dichloromethane before switching to 5-10% methanol (+5% ammonium hydroxide)/dichloromethane to obtain the title compound as a yellow solid (28 mg, 39%). LCMS $[M+H]^+$ 414.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.90 (br. s, 1 H), 8.93 (s, 2 H), 8.78 (s, 1 H), 8.77 (s, 1 H), 8.73 (d, *J*=5.4 Hz, 1 H), 8.61 (d, *J*=8.8 Hz, 1 H), 8.31 - 8.37 (m, 2 H), 8.24 (d, *J*=2.0 Hz, 1 H), 8.20 (d, *J*=2.4 Hz, 1 H), 7.99 (dd, *J*=8.8, 2.0 Hz, 1 H), 3.24 (br. s., 4 H), 3.11 (br. s., 3 H), 2.75 (br. s., 3 H).

7-(2-(4-Methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(3-methylpyrazin-2-yl)quinolin-4-amine (17a)—Prepared via *General Procedure C.* The crude material was purified by column chromatography, eluting with 2-5-7-10% methanol/dichloromethane before switching to 5-10-20% methanol (+5% ammonium hydroxide)/dichloromethane to obtain the title compound. Further purification was performed by prep HPLC (method: 95-50_6p5min) to obtain the title compound as a yellow, oily solid (11 mg, 8%). LCMS $[M+H]^+$ 427.3 m/z; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.83 (s, 2 H), 8.63 (d, *J*=5.4 Hz, 1 H), 8.30 (d, *J*=8.8 Hz, 1 H), 8.18 (d, *J*=2.4 Hz, 1 H), 8.14 (d, *J*=2.9 Hz, 1 H), 8.11 (d, *J*=1.0 Hz, 1 H), 7.84 (dd, *J*=8.8, 1.5 Hz, 1 H), 7.75 (d, *J*=5.4 Hz, 1 H), 4.16 (t, *J*=4.4 Hz, 2 H), 3.98 (t, *J*=6.3 Hz, 2 H), 3.34 (d, *J*=4.4 Hz, 2 H), 3.25 (t, *J*=5.4 Hz, 2 H), 2.81 (s, 3 H), 2.69 (s, 3 H), 2.24 (quin, *J*=5.4 Hz, 2 H).

7-(2-(4-Methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(5-methylpyrazin-2-yl)quinolin-4-amine (17b)—Prepared via *General Procedure D.* The crude material was purified by column chromatography, eluting with 1-10% methanol (+5% ammonium hydroxide)/dichloromethane. A second purification was necessary, and this was done by column chromatography, eluting with 5-10% methanol/dichloromethane to obtain the title compound as a yellow solid (20 mg, 29% over two steps). LCMS $[M+H]^+$ 427.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.67 (s, 1 H), 8.93 (s, 2 H), 8.68 (d, *J*=5.4 Hz, 1 H), 8.64 (d, *J*=1.5 Hz, 1 H), 8.56 (d, *J*=9.3 Hz, 1 H), 8.25 (d, *J*=5.4 Hz, 1 H), 8.23 (s, 1 H), 8.20 (d, *J*=2.0 Hz, 1 H), 7.96 (dd, *J*=8.8, 2.0 Hz, 1 H), 3.91 (t, *J*=4.4 Hz, 2 H), 3.83 (t, *J*=6.1 Hz, 2 H), 2.66 (t, *J*=4.9 Hz, 2 H), 2.52 (m, 2 H), 2.44 (s, 3 H), 2.28 (s, 3 H), 1.91 (quin, *J*=5.7 Hz, 2 H).

7-(2-(4-Methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(6-methyl-pyrazin-2-yl)quinolin-4-amine (17c)—Prepared via *General Procedure D*. The crude material was purified by column chromatography, eluting with 1-10% methanol (+5% ammonium hydroxide)/dichloromethane to obtain the title compound as a brown solid (11 mg, 7% over two steps). LCMS $[M+H]^+$ 427.1 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.70 (s, 1 H), 8.93 (s, 2 H), 8.71 (d, *J*=4.9 Hz, 1 H), 8.56 (d, *J*=8.8 Hz, 1 H), 8.53 (s, 1 H), 8.37 (d, *J*=4.9 Hz, 1 H), 8.20 (d, *J*=2.0 Hz, 1 H), 8.08 (s, 1 H), 7.96 (dd, *J*=8.8, 2.0 Hz, 1 H), 3.90 (t, *J*=4.9 Hz, 2 H), 3.82 (t, *J*=6.3 Hz, 2 H), 2.65 (t, *J*=5.4 Hz, 2 H), 2.47 (s, 3 H), 2.27 (s, 3 H), 1.91 (dt, *J*=11.7, 5.9 Hz, 2 H).

*One of the methylenes is under the DMSO signal.

***N*-Methyl-7-(2-(4-methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4-amine (18)—**Prepared via *General Procedure C.* The crude material was purified by column chromatography, eluting with 5-15% methanol (+5% ammonium hydroxide)/ethyl acetate to obtain the title compound as a brown oil (70 mg, 53%). LCMS $[M+H]^+$ 427.1 m/z; ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 9.02 (d, *J*=4.9 Hz, 1 H) 8.73 (s, 2 H) 8.32 (d, *J*=1.5 Hz, 1 H) 8.19 (dd, *J*=2.4, 1.5 Hz, 1 H) 7.96 (d, *J*=2.4 Hz, 1 H) 7.83 (d, *J*=8.8 Hz, 1 H) 7.73 (d, *J*=1.5 Hz, 1 H) 7.68 (dd, *J*=8.8, 1.5 Hz, 1 H) 7.33 (d, *J*=4.4 Hz, 1 H) 4.02 (br. s., 2 H) 3.91 (t, *J*=6.1 Hz, 2 H) 3.62 (s, 3 H) 2.76 (br. s., 2 H) 2.62 (br. s., 2 H) 2.42 (s, 3 H) 2.06 (br. s., 1 H).

(*E*)-7-Bromo-1-methyl-*N*-(pyrazin-2-yl)quinolin-4(1*H*)-imine (19a)—Prepared via *General Procedure I.* The crude material was purified by column chromatography, eluting with 2-7% methanol (+5% ammonium hydroxide)/dichloromethane (step gradient) to obtain the title product as a yellow solid (663 mg, 63%). LCMS $[M+H]^+$ 314.9 m/z (Br^{79}), 317.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.51 (d, *J*=8.8 Hz, 1 H), 8.34 (dd, *J*=2.4, 1.5 Hz, 1 H), 8.31 (s, 1 H), 8.15 (d, *J*=2.4 Hz, 1 H), 7.88 (br. s., 1 H), 7.79 (br. s., 1 H), 7.63 (d, *J*=8.3 Hz, 1 H), 6.84 - 7.05 (m, 1 H), 3.81 (s, 3 H).

(*E*)-7-Bromo-1-ethyl-*N*-(pyrazin-2-yl)quinolin-4(1*H*)-imine (19b)—Prepared via *General Procedure I.* The crude material was purified by column chromatography eluting with 1-10% methanol/dichloromethane to obtain the title compound as a dark orange oil (59 mg, 27%). LCMS $[M+H]^+$ 329.0 m/z (Br^{79}), 331.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.49 (d, *J*=8.8 Hz, 1 H), 8.32 (dd, *J*=2.4, 1.5 Hz, 1 H), 8.27 (d, *J*=1.5 Hz, 1 H), 8.13 (d, *J*=2.9 Hz, 1 H), 7.93 (d, *J*=1.5 Hz, 1 H), 7.75 (d, *J*=7.8 Hz, 1 H), 7.57 (dd, *J*=8.8, 2.0 Hz, 1 H), 6.84 (d, *J*=7.8 Hz, 1 H), 4.24 (q, *J*=7.0 Hz, 2 H), 1.31 (t, *J*=7.1 Hz, 3 H).

(*E*)-7-Bromo-1-propyl-*N*-(pyrazin-2-yl)quinolin-4(1*H*)-imine (19c)—Prepared via *General Procedure I.* The crude material was purified by column chromatography, eluting with 1-10% methanol/dichloromethane to obtain the title compound as an orange oil (91 mg, 40%). LCMS $[M+H]^+$ 343.0 m/z (Br^{79}), 345.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.52 (d, *J*=8.3 Hz, 1 H), 8.30 - 8.37 (m, 2 H), 8.16 (d, *J*=2.4 Hz, 1 H), 8.00 (br. s., 1 H), 7.83 (br. s., 1 H), 7.61 (d, *J*=7.3 Hz, 1 H), 6.97 (d, *J*=7.8 Hz, 1 H), 4.21 (t, *J*=6.8 Hz, 2 H), 1.74 (sxt, *J*=7.3 Hz, 2 H), 0.90 (t, *J*=7.3 Hz, 3 H).

(*E*)-1-Methyl-7-(2-(4-methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4(1*H*)-imine (22a)—Prepared via *General Procedure C.* The crude material was purified by column chromatography, eluting with 10-20% methanol/dichloromethane before switching to 10-15% methanol (+5% ammonium hydroxide)/dichloromethane to elute the product. The title compound was obtained as a yellow solid (242 mg, 51% over two steps). LCMS $[M+H]^+$ 427.1 m/z; ^{1}H NMR (500 MHz, METHANOL-d_4) δ ppm 8.81 (s, 2 H), 8.65 (d, *J*=8.8 Hz, 1 H), 8.31 - 8.35 (m, 2 H), 8.14 (d, *J*=2.4 Hz, 1 H), 7.76 (s, 1 H), 7.70 (dd, *J*=8.3, 1.5 Hz, 1 H), 7.65 (d, *J*=7.8 Hz, 1 H), 6.58 - 6.72 (m, 1 H), 4.00 (t, *J*=4.9 Hz, 2 H), 3.89 - 3.94 (m, 2 H), 3.91 (s, 3 H), 2.78 (t, *J*=4.9 Hz, 2 H), 2.64 (t, *J*=5.4 Hz, 2 H), 2.40 (s, 3 H), 2.05 (dt, *J*=11.8, 6.0 Hz, 2 H). ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.91 (s, 2 H), 8.60 (d, *J*=8.8 Hz, 1 H), 8.30 (dd, *J*=2.4, 1.5 Hz, 1 H), 8.25 (d, *J*=1.5 Hz, 1 H), 8.08 (d, *J*=2.9 Hz, 1 H), 7.78 (d, *J*=1.5 Hz, 1 H), 7.68 - 7.75 (m, 2 H), 6.84 (d, *J*=7.3 Hz, 1 H), 3.90 (t, *J*=4.9 Hz, 2 H), 3.85 (s, 3 H), 3.82 (t, *J*=6.1 Hz, 2 H), 2.64 (t, *J*=4.9 Hz, 2 H), 2.27 (s, 3 H), 1.90 (dt, *J*=11.6, 5.7 Hz, 2 H).

*One of the methylenes is under the DMSO signal.

(*E*)-1-Ethyl-7-(2-(4-methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-*N*-(pyrazin-2-yl)quinolin-4(1*H*)-imine (22b)—Prepared via *General Procedure C.* The crude material was purified by column chromatography, eluting with 2-5-7-10% methanol/dichloromethane first before switching to 5-10-20% methanol (+5% ammonium hydroxide)/dichloromethane to obtain the title compound as a glassy brown solid (31 mg, 39% over two steps). LCMS $[M+H]^+$ 441.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.92 (s, 2 H), 8.63 (d, *J*=8.3 Hz, 1 H), 8.31 (dd, *J*=2.4, 1.5 Hz, 1 H), 8.29 (s, 1 H), 8.11 (d, *J*=2.4 Hz, 1 H), 7.88 (s, 1 H), 7.81 (d, *J*=7.8 Hz, 1 H), 7.74 (d, *J*=8.8 Hz, 1 H), 6.93 (d, *J*=6.3 Hz, 1 H), 4.37 (q, *J*=7.0 Hz, 2 H), 3.93 (br. s., 2 H), 3.84 (t, *J*=6.3 Hz, 2 H), 2.76 (br. s., 2 H), 2.62 (br. s., 1 H), 2.35 (br. s., 3 H), 1.95 (tt, *J*=6.3, 4.9 Hz, 2 H), 1.37 (t, *J*=7.1 Hz, 3 H).

(*E*)-7-(2-(4-Methyl-1,4-diazepan-1-yl)pyrimidin-5-yl)-1-propyl-*N*-(pyrazin-2-yl)quinolin-4(1*H*)-imine (22c)—Prepared via *General Procedure C.* The crude material was purified by column chromatography, eluting with 2-5-7-10% methanol/dichloromethane first before switching to 5-10-20% methanol (+5% ammonium hydroxide)/dichloromethane to obtain the title compound as a brown glassy solid (44 mg, 36% over two steps). LCMS $[M+H]^+$ 455.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.93 (s, 2 H), 8.65 (d, *J*=8.8 Hz, 1 H), 8.29 - 8.34 (m, 2 H), 8.12 (d, *J*=2.4 Hz, 1 H), 7.89 (s, 1 H), 7.84 (d, *J*=6.8 Hz, 1 H), 7.76 (d, *J*=8.8 Hz, 1 H), 6.92 - 7.03 (m, 1 H), 4.32 (t, *J*=7.3 Hz, 2 H), 3.94 (d, *J*=6.3 Hz, 2 H), 3.84 (t, *J*=6.1 Hz, 2 H), 2.82 (br. s., 2 H), 2.68 (br. s., 2 H), 2.40 (br. s., 3 H), 1.97 (t, *J*=5.4 Hz, 2 H), 1.80 (dq, *J*=14.3, 7.4 Hz, 2 H), 0.92 (t, *J*=7.3 Hz, 3 H).

***N*-methylpyrazin-2-amine (24)—**Prepared via *General Procedure I.* The crude material was purified by column chromatography eluting with 2-5% methanol/dichloromethane to obtain the title compound as a yellow oil (140 mg, 49%). LCMS $[M+H]^+$ 109.4 m/z; ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 7.90 (d, *J*=14.7 Hz, 2 H), 7.62 (d, *J*=2.2 Hz, 1 H), 7.00 (br. s., 1 H), 2.77 (d, *J*=4.4 Hz, 3 H). ^{1}H NMR (399 MHz, DMSO-d_6 plus D_2O) δ ppm 7.91 (br. s., 1 H), 7.86 (s, 1 H), 7.61 (d, *J*=2.2 Hz, 1 H), 2.75 (s, 3 H).

7-Bromo-N-methyl-*N*-(pyrazin-2-yl)quinolin-4-amine (25)—Prepared via *General Procedure G*. The sodium hydride was quenched by addition of methanol until no further effervescence was observed. All the volatiles were removed *in vacuo* and the crude material was purified by column chromatography, eluting with 50-75% ethyl acetate/hexanes, before switching to 0-5% methanol/dichloromethane to obtain the title compound as a yellow solid (140 mg, 82%). LCMS $[M+H]^+$ 315.0 m/z (Br^{79}), 317.0 m/z (Br^{81}); ^{1}H NMR (399 MHz, DMSO-d_6) δ ppm 9.00 (d, *J*=4.4 Hz, 1 H), 8.33 (s, 1 H), 8.12 (s, 1 H), 7.99 (d, *J*=2.2 Hz, 1 H), 7.96 (s, 1 H), 7.64 - 7.72 (m, 2 H), 7.61 (d, *J*=5.1 Hz, 1 H), 3.54 (s, 3 H).

Diethyl 2-(((3-bromo-4-methylphenyl)amino)methylene)malonate (26)—3-Bromo-4-methylaniline (2.0 g, 10.75 mmol) and diethyl 2-(ethoxymethylene)malonate (2.15 mL, 10.75 mmol) were combined and stirred at ambient temperature until homogenous. The reaction mixture was then heated to 100 °C for 2.5 h. The reaction mixture was cooled and a precipitate formed which was filtered and washed with cold hexanes to obtain the title compound as a yellow solid (3.4 g, 89%). LCMS $[M+H]^+$ 355.9 m/z (Br^{79}), 358.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 10.95 (d, *J*=14.0 Hz, 1H), 8.42 (d, *J*=13.5 Hz, 1H), 7.33 (d, *J*=2.5 Hz, 1H), 7.20 (d, *J*=8.5 Hz, 1 H), 6.98 (dd, *J*=2.5, 8.0 Hz, 1H), 4.30 (q, *J*=7.0 Hz, 2H), 4.25 (q, *J*=6.5 Hz, 2H), 2.37 (s, 3H), 1.37 (t, *J*=7.5 Hz, 3H), 1.33 (t, *J*=6.5 Hz, 3H).

Ethyl 7-bromo-6-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (27)—Diethyl 2-(((3-bromo-4-methylphenyl)amino)methylene)malonate **26** (3.4 g, 9.54 mmol) was suspended in diphenylether (60 mL). The reaction mixture was heated at 250 °C for 3 h before being cooled to ambient temperature. The resulting precipitate was isolated by vacuum filtration and washed with hexanes to obtain the title compound as a beige solid (2.5 g, 84%). LCMS $[M+H]^+$ 310.0 m/z (Br^{79}), 312.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.54 (s, 1H), 8.06 (s, 1H), 7.86 (s, 1H), 4.20 (q, *J*=7.0 Hz, 2H), 2.45 (s, 3H), 1.27 (t, *J*=7.0 Hz, 3H).

7-Bromo-6-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (28)—Ethyl 7-bromo-6-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate **27** (1.0 g, 3.22 mmol) was stirred in a 2.5 M aqueous sodium hydroxide solution (2 mL) and the reaction mixture was heated at 100 °C overnight. The reaction mixture was obtained before being cooled to ambient temperature before being acidified with dilute hydrochloric acid to pH~3-4. The solid precipitate was isolated by vacuum filtration and washed with water. The solid was air dried to obtain the title compound (700 mg, 77%). LCMS $[M+H]^+$ 282.0 m/z (Br^{79}), 284.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 15.01 (bs, 1H), 13.35 (s, 1H), 8.18 (s, 1H), 8.14 (s, 1H), 7.99 (s, 1H), 2.4 (s, 3H).

7-Bromo-6-methylquinolin-4(1*H*)-one (29)—7-Bromo-6-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **28** (900 mg, 3.19 mmol) was heated in diphenylether (30 mL) at 250 °C for 4 h. The reaction mixture was cooled to ambient temperature and a solid precipitated which was isolated by vacuum filtration. The solid was washed with hexanes and air dried to obtain the title compound (699 mg, 92%). LCMS $[M+H]^+$ 238.0

m/z (Br^{79}), 240.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.0 (bs, 1H), 8.01 (s, 1H), 7.95 (d, *J*=7.6 Hz, 1H), 7.83 (s, 1H), 6.09 (d, *J*=7.2 Hz, 1H), 2.44 (s, 3H).

7-Bromo-4-chloro-6-methylquinoline (30)—7-Bromo-6-methylquinolin-4(1*H*)-one **29** (698 mg, 2.93 mmol) was refluxed in phosphorous oxychloride for 2 h. The phosphorous oxychloride was removed by distillation and the resulting residue was cooled in an iced-water bath before being diluted with water. The aqueous phase was neutralized by addition of a saturated sodium bicarbonate solution before being extracted with dichloromethane. The dichloromethane layers were washed with water, saturated sodium chloride solution and dried with sodium sulfate. All of the volatiles were removed *in vacuo* to obtain the title compound as a cream solid which was used without further purification (370 mg, 49%). LCMS $[M+H]^+$ 256.0 m/z (Br^{79}/Cl^{35}), 258.0 m/z (Br^{79}/Cl^{37}, Br^{81}/Cl^{35}), 260.0 m/z (Br^{81}/Cl^{37}); ^{1}H NMR (500 MHz CHLOROFORM-*d*) δ ppm 8.71 (d, *J*=4.5 Hz, 1H), 8.36 (s, 1H), 8.06 (s, 1H), 7.47 (d, *J*=4.5 Hz, 1H), 2.63 (d, 3H).

7-Bromo-6-methyl-*N*-(pyrazin-2-yl)quinolin-4-amine (31)—Prepared via *General Procedure G*. However, water was used to quench the reaction. The title compound was obtained as a tan solid (714 mg, 86%). LCMS $[M+H]^+$ 315.0 m/z (Br^{79}), 317.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 9.77 (s, 1H), 8.69 (d, *J*=1.5 Hz, 1H), 8.67 (d, *J*=5.5 Hz, 1H), 8.46 (s, 1H), 8.34 (d, *J*=5.5 Hz, 1H), 8.33-8.21 (m, 1H), 8.19 (s, 1H), 8.18 (d, *J*=2.5 Hz, 1H), 2.59 (s, 3H).

Diethyl 2-(((3-bromo-2-methylphenyl)amino)methylene)malonate (32)—3-Bromo2-methylaniline (1.0 g, 5.37 mmol) and diethyl 2-(ethoxymethylene)malonate (1.08 mL, 5.37 mmol) were combined and stirred at ambient temperature until homogenous. The reaction mixture was then heated to 100 °C for 2.5 h. The reaction mixture was cooled and a precipitate formed which was filtered and washed with cold hexanes to obtain the title compound as a white solid (1.3 g, 68%). LCMS $[M+H]^+$ 356.0 m/z (Br^{79}), 358.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 11.17 (d, *J*=12.5 Hz, 1H), 8.46 (d, *J*=14.0 Hz, 1H), 7.38 (d, *J*=8.0 Hz, 1H), 7.17 (d, *J*=8.5 Hz, 1H), 7.10 (t, *J*=8.0 Hz, 1H), 4.32 (q, *J*=7.0 Hz, 2H), 4.24 (q, *J*=7.0 Hz, 2H), 2.46 (s, 3H), 1.38 (t, *J*=7.0 Hz, 3H), 1.32 (t, *J*=7.0 Hz, 3H).

Ethyl 7-bromo-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate (33)—Diethyl 2-(((3-bromo-2-methylphenyl)amino)methylene)malonate **32** (1 g, 2.81 mmol) and diphenylether was heated at 205 °C for 5 h. The reaction mixture was cooled to ambient temperature and a solid precipitated which was isolated by vacuum filtration. The solid was washed with hexanes and air dried to obtain the title compound as a buff solid (501 mg, 57%). LCMS $[M+H]^+$ 310.1 m/z (Br^{79}), 312.1 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.73 (s, 1H), 8.38 (s, 1H), 7.93 (d, *J*=9.0 Hz, 1H), 7.62 (d, *J*=9.0 Hz, 1H), 4.22 (q, *J*=7.0 Hz, 2H), 2.59 (s, 3H), 1.28 (t, *J*=7.0 Hz, 3H).

7-Bromo-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (34)—Ethyl 7-bromo-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylate **33** (500 mg, 1.61 mmol) was stirred in a 2.5 M aqueous sodium hydroxide solution (4 mL) and the reaction mixture was heated at 100 °C overnight. The reaction mixture was obtained before being cooled to

ambient temperature before being acidified with dilute hydrochloric acid to pH~3-4. The solid precipitate was isolated by vacuum filtration and washed with water. The solid was air dried to obtain the title compound as white solid (420 mg, 93%). LCMS $[M+H]^+$ 282.0 m/z (Br^{79}), 284.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.80 (s, 1H), 8.62 (d, *J*=4.5 Hz, 1H), 8.05 (d, *J*=8.5 Hz, 1H), 7.80 (d, *J*=9.0 Hz, 1H), 2.67 (s, 3H).

7-Bromo-8-methylquinolin-4(1*H*)-one (35)—7-Bromo-8-methyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **34** (415 mg, 1.47 mmol) was refluxed in diphenylether (3.75 mL) for 3 h. The reaction mixture was cooled to ambient temperature and a solid precipitated which was isolated by vacuum filtration. The solid was washed with hexanes and air dried to obtain the title compound as a white solid (308 mg, 88%). LCMS $[M+H]^+$ 238.0 m/z (Br^{79}), 240.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 11.24 (d, *J*=4.5 Hz, 1H), 7.87-7.83 (m, 2H), 7.51 (d, *J*=8.5 Hz, 1H), 6.08 (d, *J*=8.5 Hz, 1H), 2.56 (s, 3H).

7-Bromo-4-chloro-8-methylquinoline (36)—7-Bromo-8-methylquinolin-4(1*H*)-one **35** (241 mg, 1.01 mmol) was refluxed in phosphorous oxychloride for 2 h. The phosphorous oxychloride was removed by distillation and the resulting residue was cooled in an iced-water bath before being diluted with water. The aqueous phase was neutralized by addition of a saturated sodium bicarbonate solution before being extracted with dichloromethane. The dichloromethane layers were washed with water, saturated sodium chloride solution and dried with sodium sulfate. All of the volatiles were removed *in vacuo* and the crude material was purified by column chromatography using 5-50% ethyl acetate/hexanes to obtain the title compound as an off-white powder (213 mg, 82%). LCMS $[M+H]^+$ 256.0 m/z (Br^{79}/Cl^{35}), 258.0 m/z (Br^{79}/Cl^{37}, Br^{81}/Cl^{35}), 260.0 m/z (Br^{81}/Cl^{37}); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.82 (d, *J*=4.5 Hz, 1H), 7.98 (d, *J*=9.0 Hz, 1H), 7.79 (d, *J*=9.0 Hz, 1H), 7.53 (dd, *J*=1.0, 4.5 Hz, 1H), 2.95 (s, 3H).

7-Bromo-8-methyl-*N*-(pyrazin-2-yl)quinolin-4-amine (37)—Prepared via *General Procedure G*. The title compound was obtained as a brown solid (132 mg, 93%). LCMS $[M+H]^+$ 315.0 m/z (Br^{79}), 317.0 m/z (Br^{81}); ^{1}H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.83 (d, *J*=5.0 Hz, 1H), 8.52 (s, 1H), 8.30-8.29 (m, 1H), 8.21 (d, *J*=3.0 Hz, 1H), 8.01 (d, *J*=5.0 Hz, 1H), 7.73-7.68 (m, 2H), 2.95 (s, 3H).

7-(2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)quinolin-4(1*H*)-one (38)—Prepared via *General Procedure C.* A precipitate was isolated by vacuum filtration and washed with water to afford the title compound as a tan solid, (411 mg, 85%). LCMS $[M+H]^+$ 322.2 m/z; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.77 (s, 1 H), 8.08 - 8.15 (m, 1 H), 7.87 - 7.92 (m, 1 H), 7.68 - 7.72 (m, 1 H), 7.57 - 7.63 (m, 1 H), 6.31 (s, 2 H), 5.99 - 6.05 (m, 1 H), 3.76 - 3.85 (m, 4 H), 2.34 - 2.42 (m, 4 H), 2.22 (s, 3 H).

4-Chloro-7-(2-(4-methylpiperazin-1-yl)pyrimidin-5-yl)quinoline (39)—7-(2-(4-Methylpiperazin-1-yl)pyrimidin-5-yl)quinolin-4(1*H*)-one (**38**) (103 mg, 0.32 mmol) was suspended in neat phosphorus oxychloride (excess, 1.0 mL). The reaction mixture was refluxed overnight. Phosphorus oxychloride was then removed by vacuum distillation. The resulting residue was put in an ice bath, diluted with dichloromethane, and quenched using a

saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted 3 times with dichloromethane. The combined organic layers were washed with water and a saturated sodium chloride solution, before being dried with sodium sulfate. The volatiles were removed *in vacuo* to obtain the title compound as a light tan solid (77.2 mg, 71%). LCMS $[M+H]^+$ 340.1 m/z (Cl^{35}), 342.2 m/z (Cl^{37}); ^1H NMR (500 MHz, CHLOROFORM-*d*) δ ppm 8.80 (d, *J*=4.9 Hz, 1 H), 8.73 (s, 2 H), 8.31 (d, *J*=8.8 Hz, 1 H), 8.23 (d, *J*=1.5 Hz, 1 H), 7.78 - 7.83 (m, 1 H), 7.48 (d, *J*=4.9 Hz, 1 H), 3.91 - 3.98 (m, 4 H), 2.51 (t, *J*=4.9 Hz, 4 H), 2.37 (s, 3 H).

Supplementary Material

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Acknowledgements

This work was supported by National Institutes of Health grants (R01AI082577 (M.P.P), R56AI099476 (K.M.-W. and M.P.P), R01AI124046 (M.P.P and K.M.-W.), R21AI127594 (M.P.P)). We are grateful to AstraZeneca for performing the *in vitro* ADME experiments presented throughout. We thank Dr. Bo Li at the X-ray crystallography center at Boston College for obtaining the crystal structure of compound **19a**. Finally, we would like to thank Biogen for training K.B. in the art of salt formation and characterization.

Abbreviations used

Clint	intrinsic clearance
DND*i*	Drugs for Neglected Diseases *initiative*
HAT	human African trypanosomiasis
HLM	human liver microsomes
T.b. brucei	*Trypanosoma brucei brucei*
TPP	target-product profile

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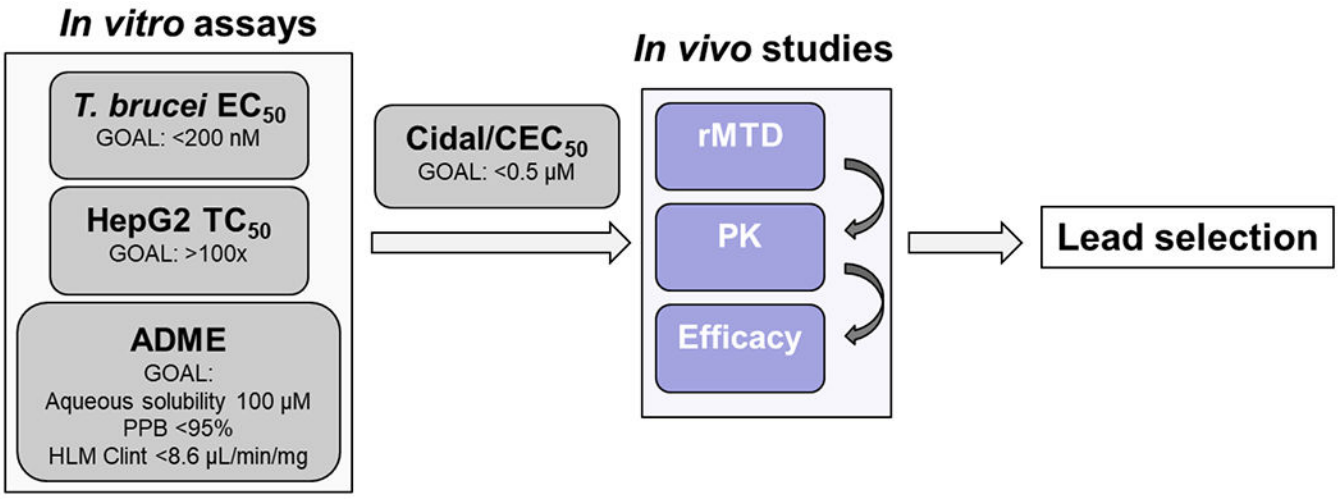
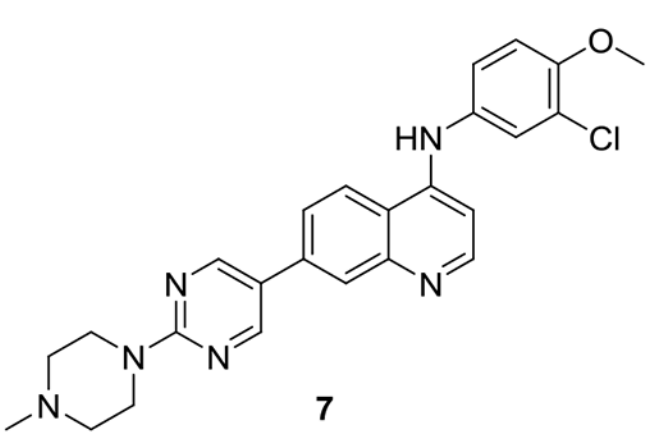


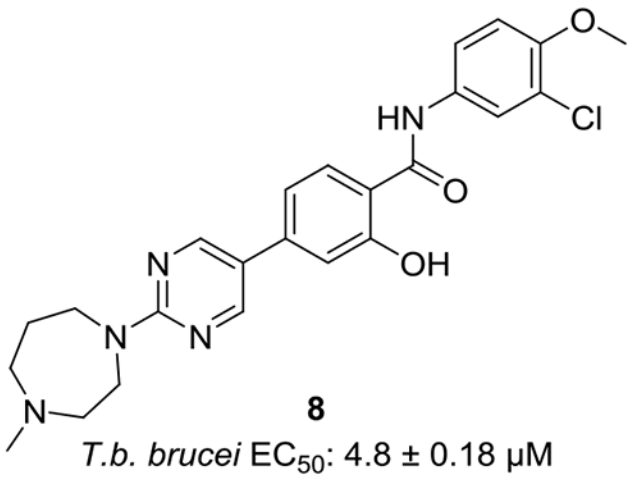
Figure 1.

Hit-to-lead screening cascade employed in this project, along with the goal values used for the various parameters, as determined when considering the TPP for HAT.



7

T.b. brucei EC_{50}: 0.91 ± 0.050 μM
Aq. sol. (μM): <1
PPB: 98%
HLM Clint (μL/min/mg): 170
HepG2 TC_{50}: >3 μM*
*compound dissolved at 0.5 mg/mL



8

T.b. brucei EC_{50}: 4.8 ± 0.18 μM
Aq. sol. (μM): 6
PPB: 100%
HLM Clint (μL/min/mg): 24
HepG2 TC_{50}: >6.5 μM*
*compound dissolved at 1 mg/mL

Figure 2.
Pseudo-ring analogs of the previously reported compound **7**.[20]

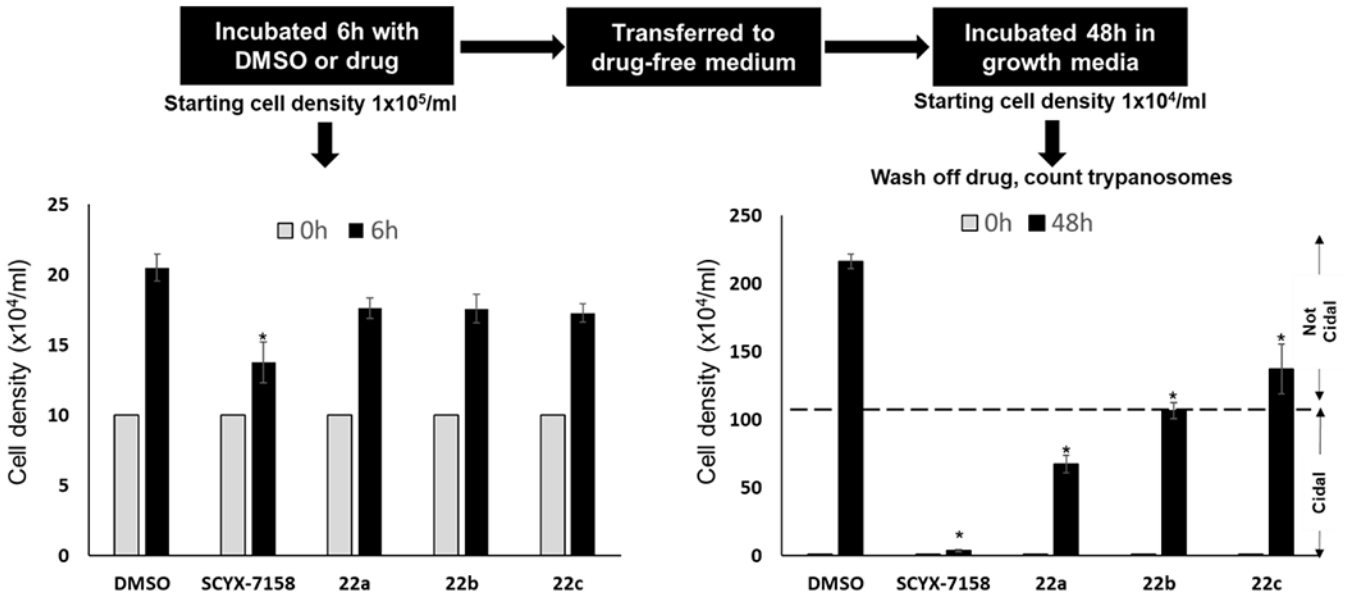


Figure 3. Trypanocidal effect of test compounds.

Log phase *T.b. brucei* (1×10^5 /mL) were incubated with the hit (at $25\times EC_{50}$ concentration) for 6 h and transferred into drug free HMI-9 medium for 48 h at a starting cell density of 1×10^4 cells/mL. Trypanosomes were enumerated with a hemocytometer after 48 h (*indicates student's T-test $p<0.01$; error bars indicate standard deviation). The dashed line corresponds to expected density of cells if 50% were killed in the earlier 6 h exposure to a hit.

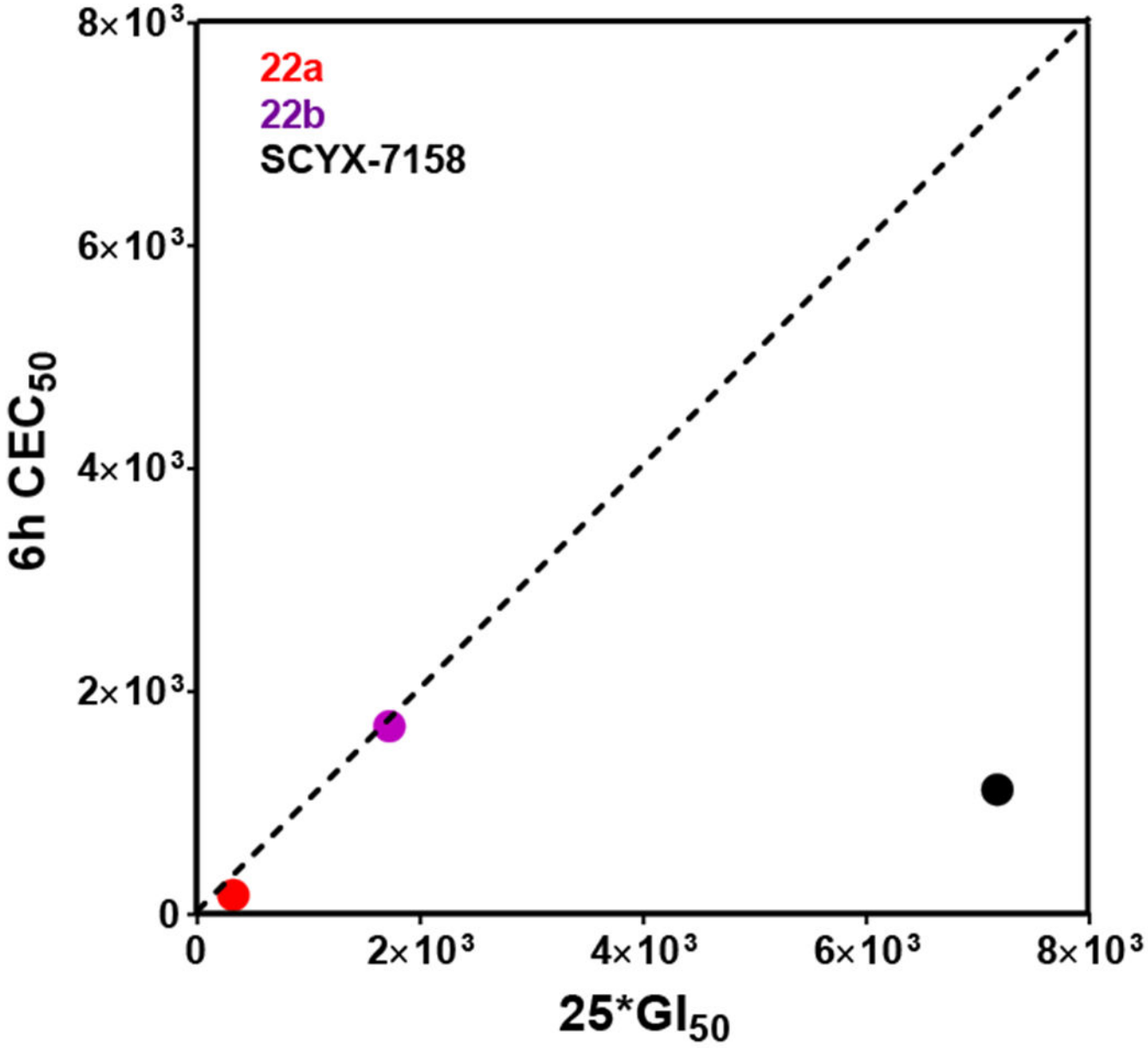


Figure 4.
Plot of 25×T. brucei EC_{50} *against* CEC_{50} *of hits.*

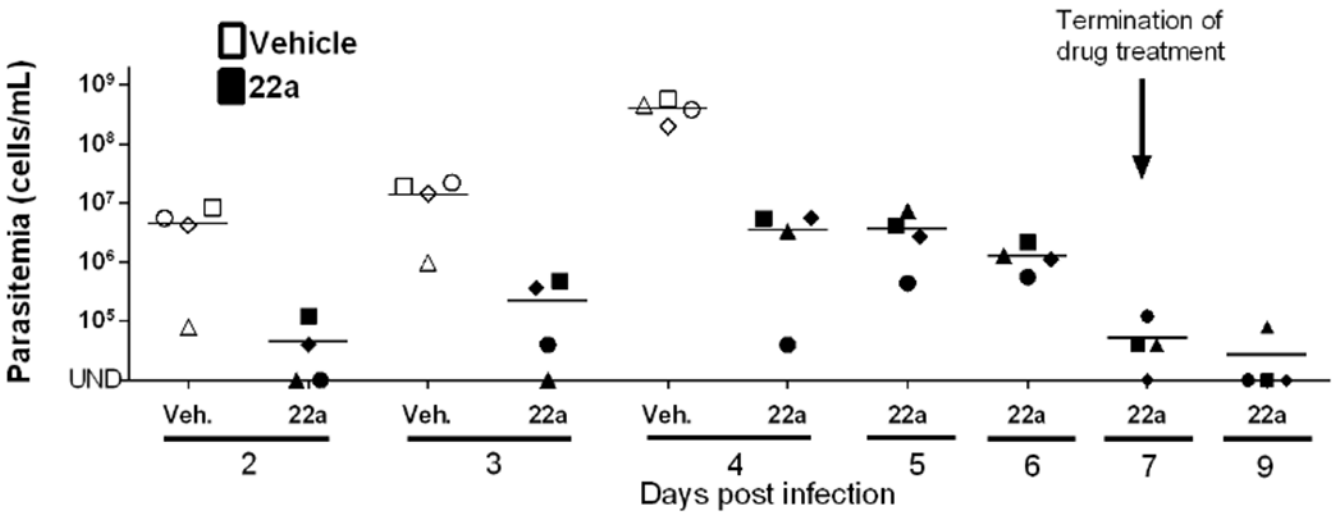
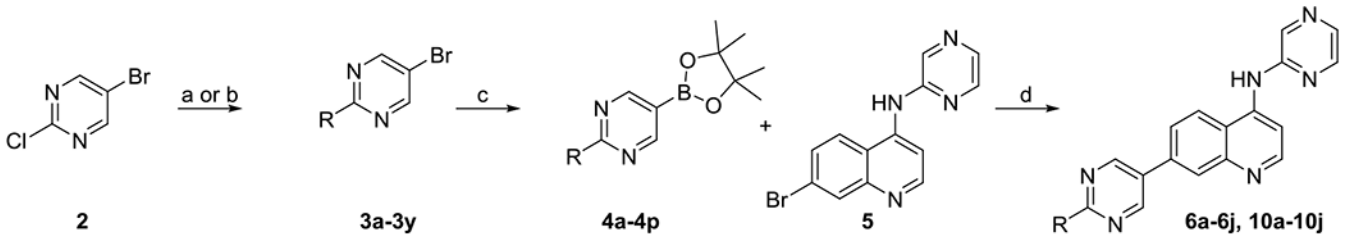


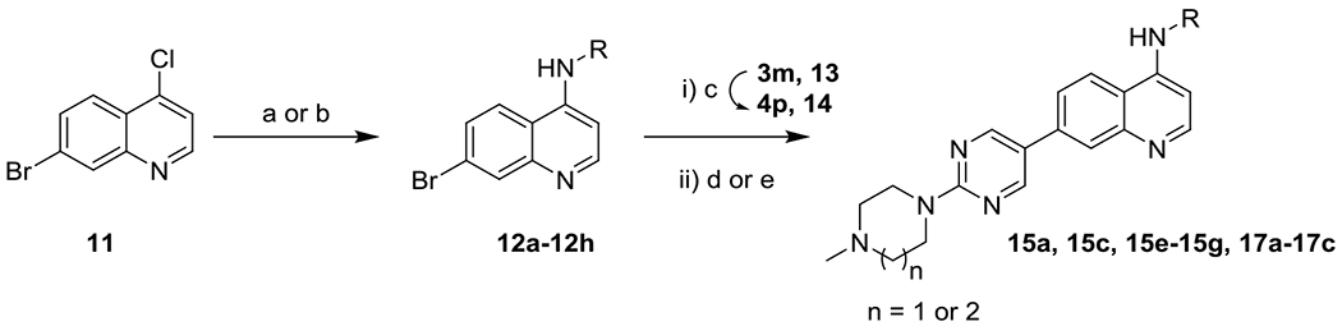
Figure 5. Effect of 22a on trypanosomes in a mouse model of HAT.

Mice (four per group) were each infected with 10^5 *T. brucei brucei* (day 0). Compound **22a** (100 mg/kg) was administered p.o. for six days, (an additional 50 mg/kg was dosed on day six). Control mice received vehicle. Different shapes represent individual mice, and horizontal lines in each group indicate mean parasitemia. Parasitemia was determined with a hemocytometer. UND = undetectable.



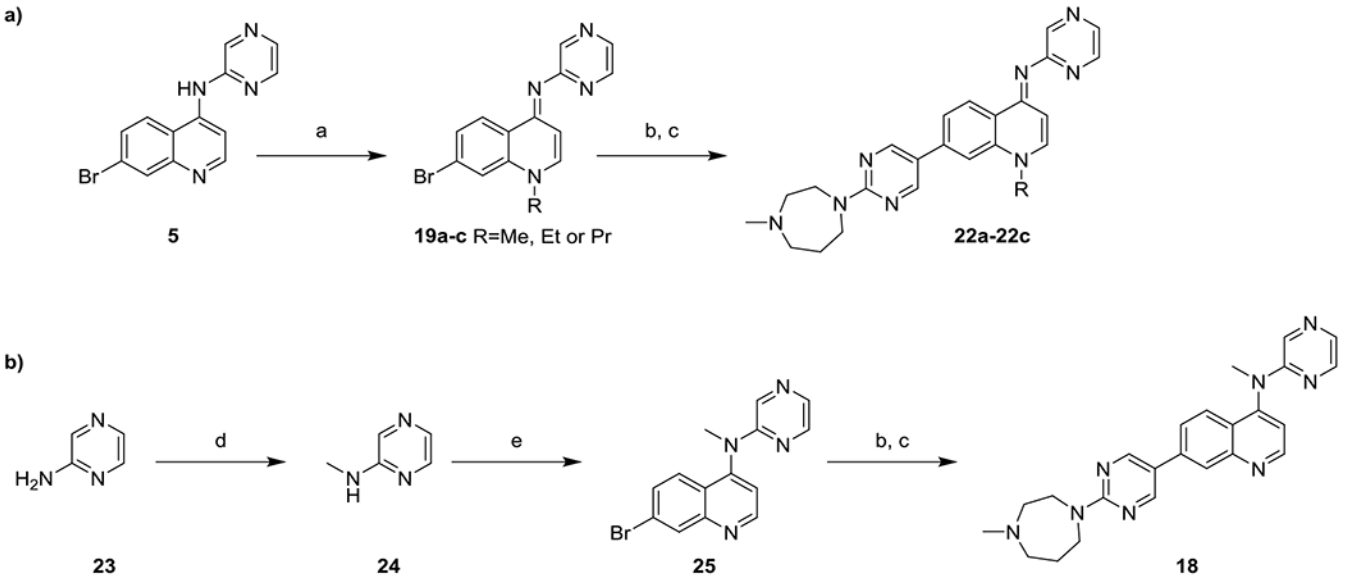
Scheme 1. General synthetic scheme for addition of ionizable groups or increased sp3 carbon content as new tail groups for analogs of 1.

Reagents and conditions: a) various substituted amines, Et_3N, EtOH, ambient temperature, 18 h (32-72%); b) various substituted amines, DIPEA, *tert*-butanol, 150 °C, microwave, 30 mins (57-97%); c) B_2pin_2, KOAc, $PdCl_2(dppf)\cdot CH_2Cl_2$, dioxane, 145 °C, 1 h (49%-assumed quant.); d) K_2CO_3, $PdCl_2(dppf)\cdot CH_2Cl_2$, 3:1 dioxane/$H_2O$, 130 °C, microwave, 30 mins (21-99%).



Scheme 2. Synthetic route for various head group analogs.

Reagents and conditions: a) saturated primary amine, DIPEA, *n*-butanol, 200 °C, microwave, 3 h (22-98%); b) aryl primary amine, NaH, DMF, 60 °C (38-75%); c) B_2pin_2, KOAc, $PdCl_2(dppf)\cdot CH_2Cl_2$, dioxane, 145 °C, microwave, 1 h (assumed quantitative); d) Cs_2CO_3, $Pd(PPh_3)_4$, 3:1 dioxane/H_2O, 80 °C, 48 h (11-44%); e) K_2CO_3, $PdCl_2(dppf)\cdot CH_2Cl_2$, 3:1 dioxane/$H_2O$, 130 °C, microwave, 30 mins (7-29%).

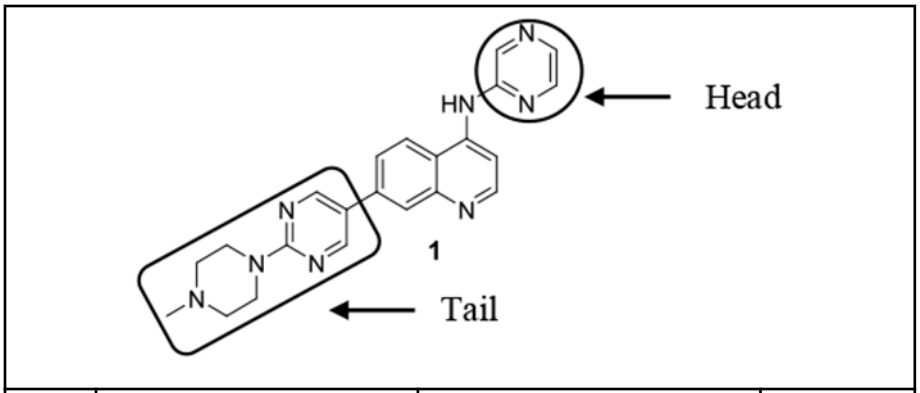


Scheme 3. General synthetic route to quinolinimine core analogs, and 18.

Reagents and conditions: a) NaH, R-I, THF, 0 °C-ambient temperature (27-63%); b) **3m**, B_2pin_2, KOAc, $PdCl_2(dppf)\cdot CH_2Cl_2$, dioxane, 145 °C, microwave, 1 h; c) K_2CO_3, $PdCl_2(dppf)\cdot CH_2Cl_2$, 3:1 dioxane/$H_2O$, 130 °C, microwave, 30 mins (36-53% over two steps); d) 2-aminopyrazine, NaH, CH_3I, THF, 0 °C-ambient temperature (49%); e) **11**, NaH, DMF, ambient temperature (82%).

Table 1.

Criteria used to select an advanced lead compound.



	Properties	Desired values	1 (NEU-1953)
In vitro	***T.b. brucei* EC_{50}**	<0.03 μM, cidal	0.43 μM
	HepG2 TC_{50}	>100× EC_{50}	>30 μM
	Human liver microsome clearance	Cl_{int} <8.6 μL/min/mg	180 μL/min/mg
	Plasma protein binding (PPB)	<95%	87%
	Thermodynamic solubility (pH=7)	>100 μM	44 μM
In vivo	**Pharmacokinetics**	>10× EC_{50} >6 h	nd
	CNS exposure	>10× EC_{50} >6 h	nd
	Mouse efficacy, bloodstream HAT	<50 mg/kg p.o. × 10 days; >90% cure	Failed
	Mouse efficacy, CNS HAT model	<100 mg/kg p.o. × 10 days; >90% cure	nd

nd = not determined

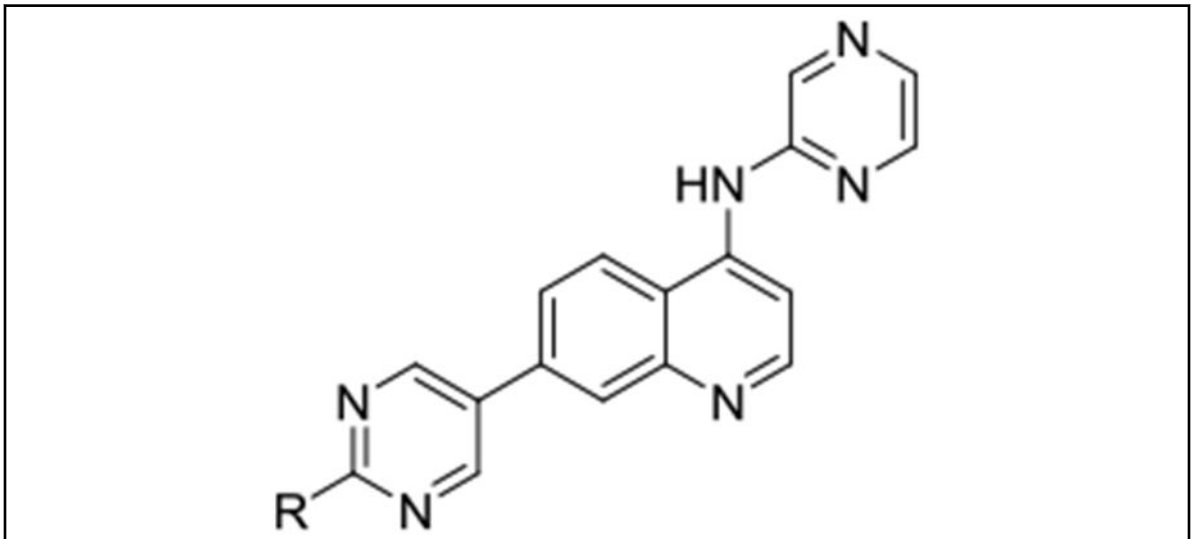
Table 2.

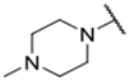
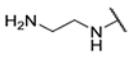
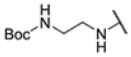
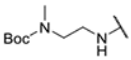
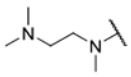
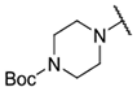
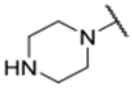
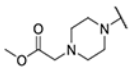
*Salts of **1**: Potency against* T. brucei, *thermodynamic and kinetic solubility, and metabolic stability.*

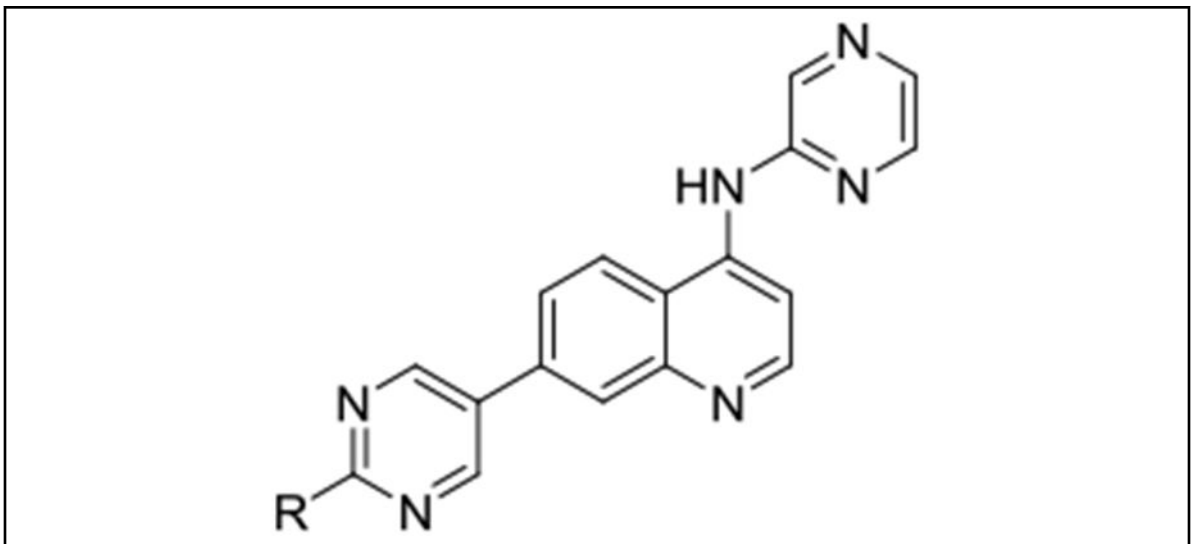
Salt form	**Salt ratio**	***T. brucei* EC_{50} (μM) ± SEM**	**Thermodynamic solubility (μM)**	**Kinetic solubility (μM)**	**HLM Cl_{int} (μL/min/mg)**
Free base	-	0.43 ± 0.015	44	25	180
Hydrochloride (**1a**)	4:1	0.33 ± 0.025	42	25	160
Sulfate (**1b**)	1:1	0.45 ± 0.024	39	25	170
Citrate (**1c**)	2:1	0.51 ± 0.027	61	50	190
Methanesulfonate (**1d**)	2:1	0.18 ± 0.023	16	100	170

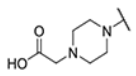
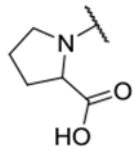
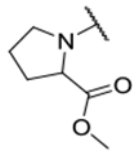
Table 3.

*Effect of incorporation of ionizable groups on **1** potency against* T. brucei, *metabolic stability, and aqueous solubility.*



ID	R	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM)	LLE
1		0.43 ± 0.015	44	87	180	>30	4.3
6a		21 ± 0.22	18	100	86	nd[d]	3.9
6b		18 ± 1.6	0.2	96	>300	>30	2.5
6c		>10	3.0	95	>300	8.0	-
6d		1.8 ± 0.076	630	67	45	>30	3.5
6e		3.3 ± 0.10	0.1	99	nd	>30	2.5
6f		2.6 ± 0.10	39	nd	30	>30	3.9
6g		7.7 ± 0.88	16	nd[b]	nd[c]	>30	3.4



ID	R	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM)	LLE
6h		>10	17	78	53	>30	-
6i		38 ± 1.6	42	95	<3	>30	3.6
6j		>50	0.3	93	220	>30	-

nd = no data

[a] **SCYX-7158** was used as a control, EC_{50}: 0.29 μM

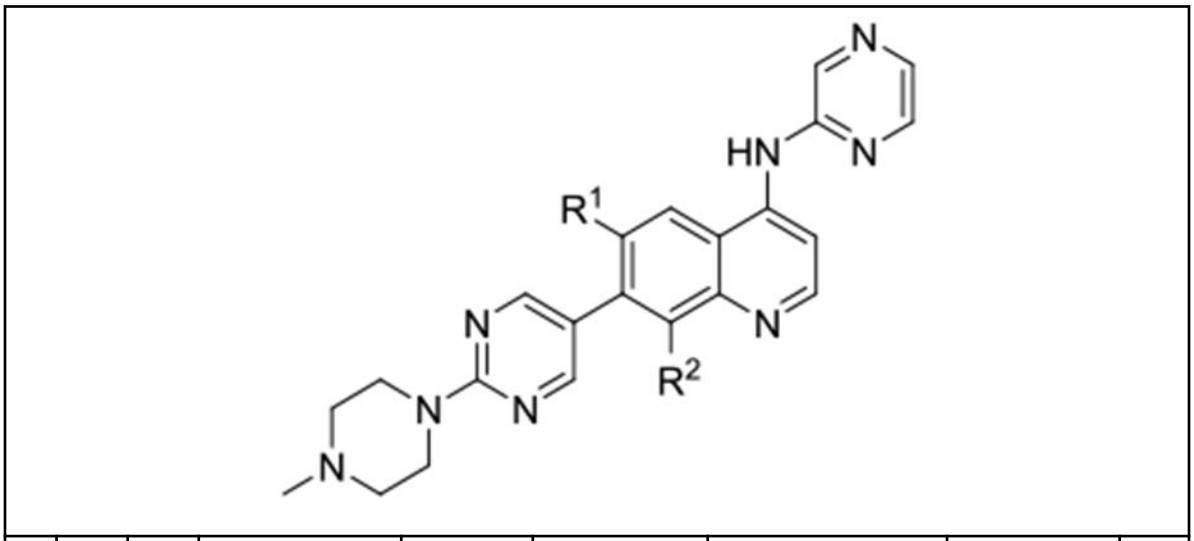
[b] compound lost, likely due to unspecific binding

[c] compound detected in only the first sample

[d] not tested initially and a combination of poor properties and potency meant that the compound was not considered for progression

Table 4.

Methylation of the quinoline reduces anti-trypanosome potency and increases toxicity of analogs of **1**.



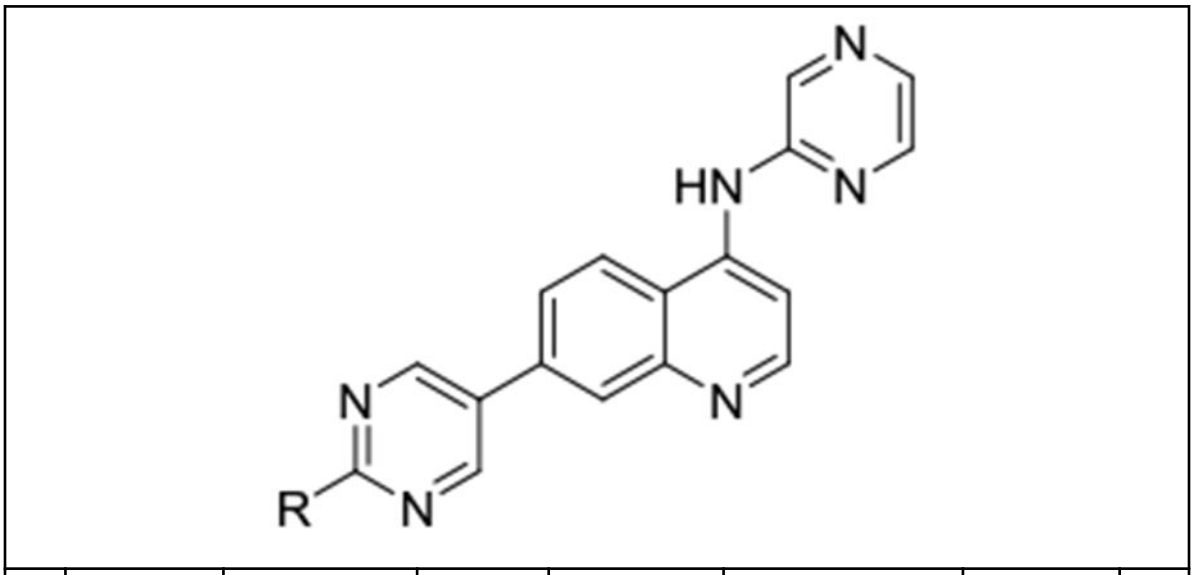
ID	R^1	R^2	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM) (R^2)	LLE
1	-H	-H	0.43 ± 0.015	44	87	180	>30	4.3
9a	$-CH_3$	-H	2.5 ± 0.30	<0.02	97	130	5.9 (0.98)	3.0
9b	-H	$-CH_3$	15 ± 1.4	5.0	97	120	9.6 (0.98)	2.2

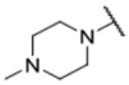
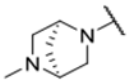
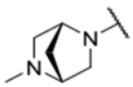
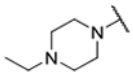
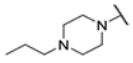
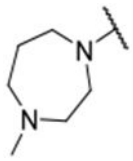
nd = no data

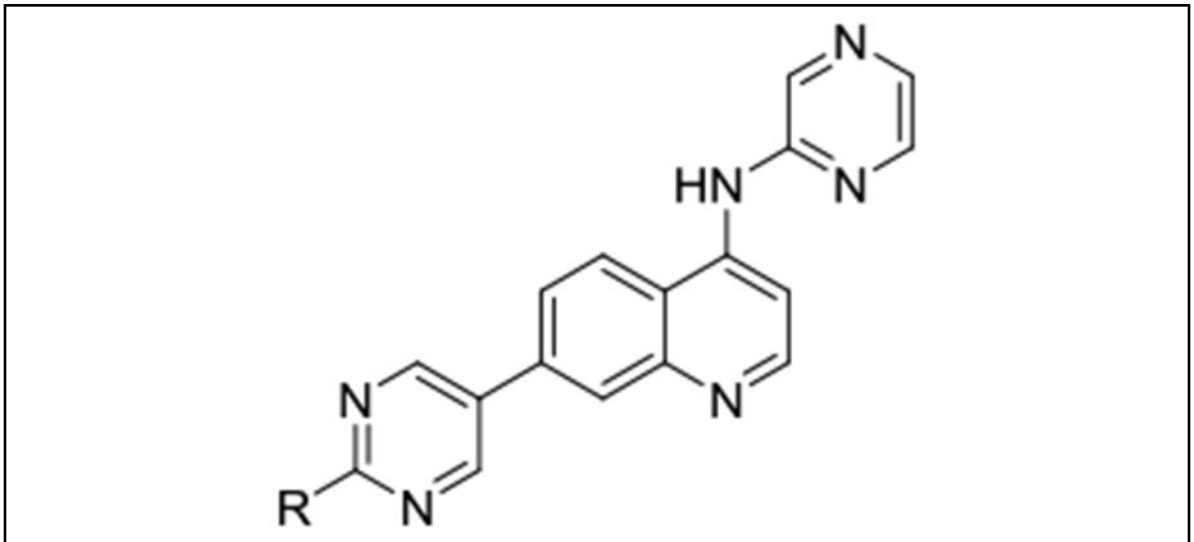
[a] **SCYX-7158** was used as a control, EC_{50}: 0.29 μM

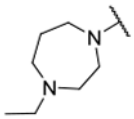
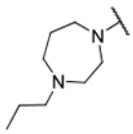
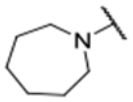
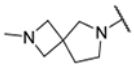
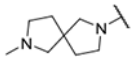
Table 5.

Increased sp^3 carbon content in the tail region can improve metabolic stability and aqueous solubility of **1**.



ID	R	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM) (R^2)	LLE
1		0.43 ± 0.015	44	87	180	>30	4.3
10a		0.20 ± 0.020	910	51	37	>30	4.6
10b		0.15 ± 0.020	960	58	41	>30	4.7
10c		0.070 ± 0.030	14	94	>300	25 (0.93)	4.7
10d		0.38 ± 0.030	44	95	nd[b]	>30	3.4
10e		0.19 ± 0.0080	990	75	77	>30	4.6



ID	R	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM) (R^2)	LLE
10f		0.25 ± 0.050	800	80	200	>30	4.1
10g		0.10 ± 0.014	78	90	300	>10	4.0
10h		25 ± 4.2	2.0	99	>300	3.4 (0.90)	1.1
10i		0.25 ± 0.010	690	48	43	>24	4.6
10j		0.15 ± 0.010	700	70	77	16 (0.96)	4.5

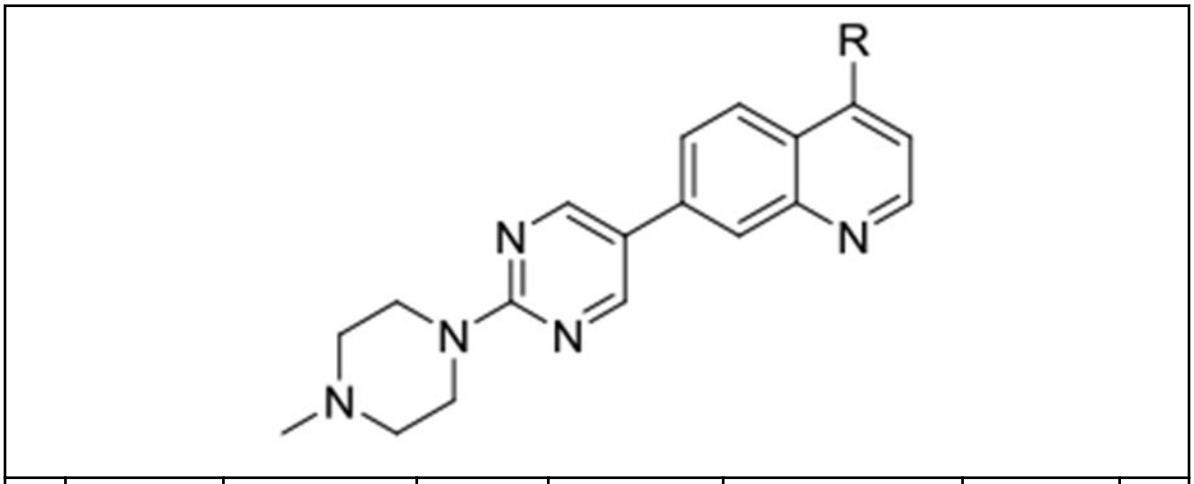
nd = no data

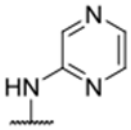
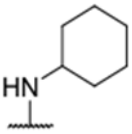
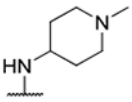
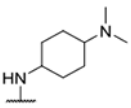
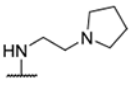
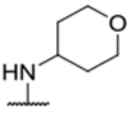
[a]**SCYX-7158** was used as a control, EC_{50}: 0.29 μM

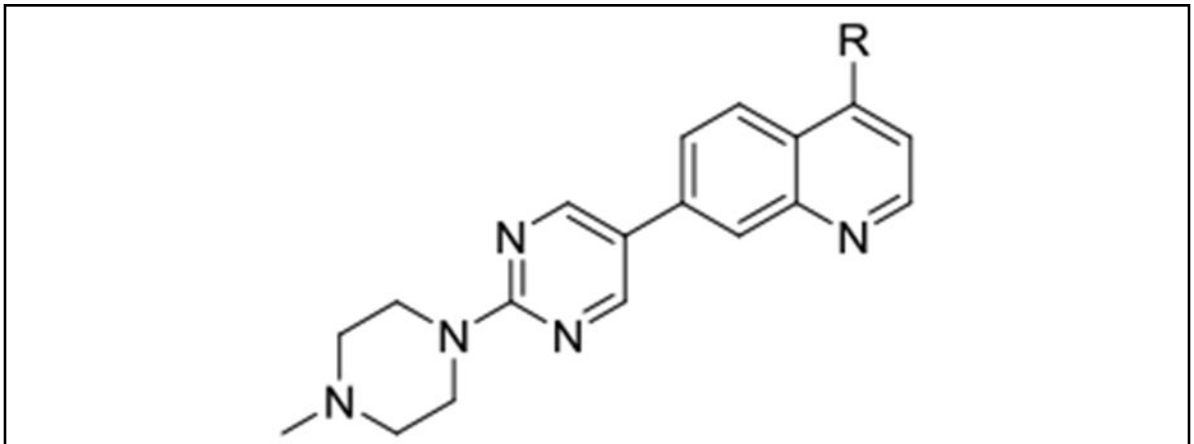
[b]compound detected in only the first sample

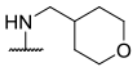
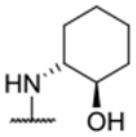
Table 6.

Increase in sp^3 carbon content of the head group region of **1**: Effect of anti-trypanosome potency, toxicity to HepG2, aqueous solubility, and metabolic stability.



ID	R	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM) (R^2)	LLE
1		0.43 ± 0.015	44	87	180	>30	4.3
15a		0.29 ± 0.010	98	85	300	nt[f]	2.8
15b		0.053 ± 0.00	>1000	nd[b]	nd[c]	nt[f]	5.3
15c		0.17 ± 0.010	660	nd[d]	nd[d]	2.5 (0.95)	3.7
15d		0.022 ± 0.001	590	100	nd[e]	3.0 (0.90)	5.3
15e		0.38 ± 0.040	760	50	94	15 (0.96)	4.5



ID	R	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM) (R^2)	LLE
15f		0.36 ± 0.030	760	47	170	16 (0.98)	4.0
15g		1.9 ± 0.26	980	63	160	>3.6	3.0

nt = not tested

nd = not determined

[a]**SCYX-7158** was used as a control, EC_{50}: 0.29 μM

[b]compound lost, likely due to unspecific binding

[c]assay failed due to poor curve fitting

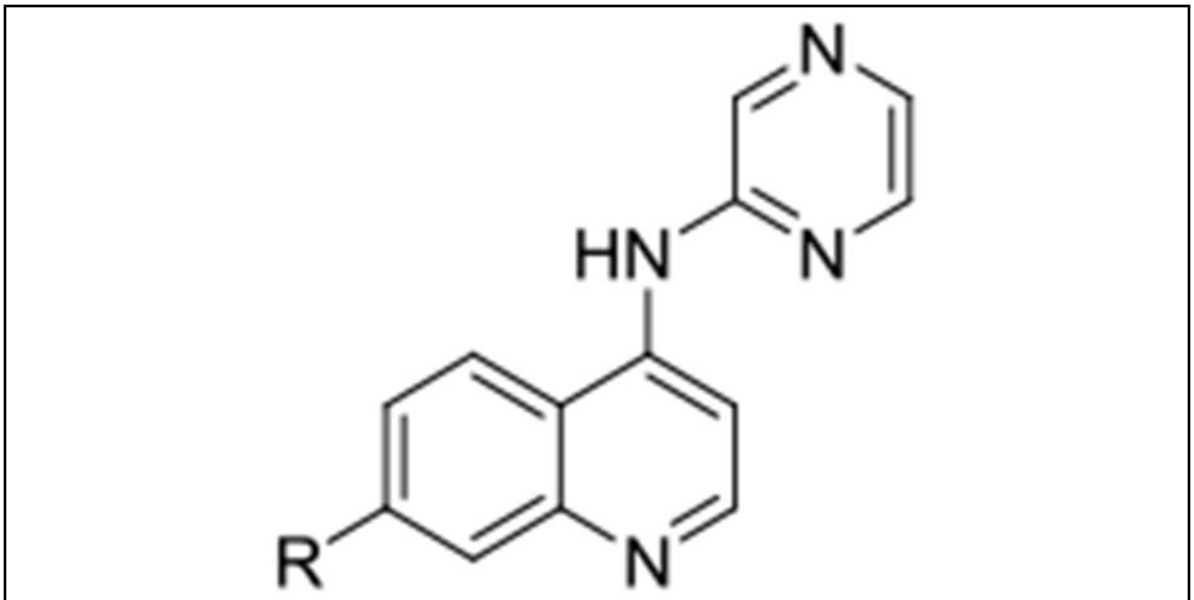
[d]poor MS response

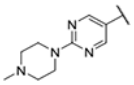
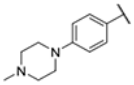
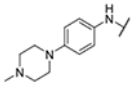
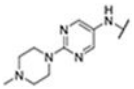
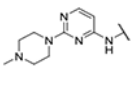
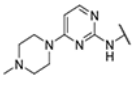
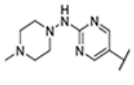
[e]low recovery

[f]not tested initially and compounds with a superior profile overall had already been identified

Table 7.

Influence of adding -NH linkers to analogs on metabolic stability, solubility, and potency against T. brucei.



ID	R	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM) (R^2)	LLE
1		0.43 ± 0.015	44	87	180	>30	4.3
16a[19]		0.91 ± 0.027	1.7	91	61	>30	2.7
16b		0.91 ± 0.092	810	71	nd[b]	17 (0.98)	2.9
16c[19]		5.1 ± 0.20	130	88	46	24 (0.96)	3.4
16d[19]		0.81 ± 0.023	2	nt[c]	nt[c]	21 (0.98)	3.6
16e[19]		0.38 ± 0.029	26	nd[b]	nd	22 (0.98)	3.9
16f		2.3 ± 0.070	>1000	51	16	nd[c]	4.1

nd = no data

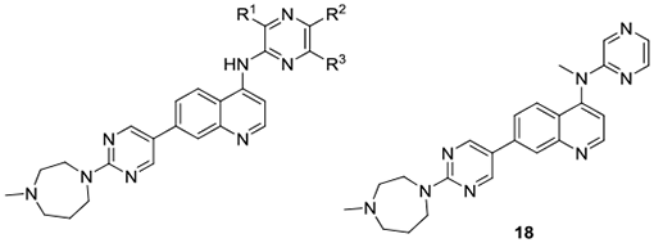
[a] **SCYX-7158** was used as a control, EC_{50}: 0.29 μM

[b] low MS/MS signal

[c] not tested initially and compounds with a superior profile overall had already been identified

Table 8.

Headgroup modifications of **10e**: Effect on anti-trypanosome potency, metabolic stability, aqueous solubility, and plasma protein binding.

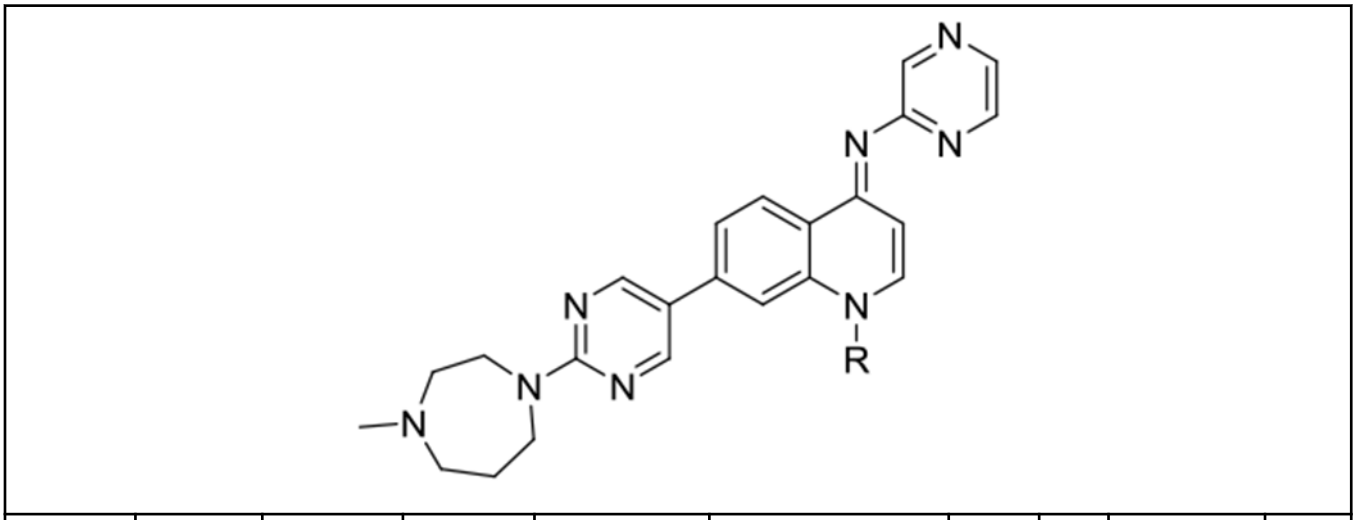


ID	R^1	R^2	R^3	*T. brucei* EC_{50} (μM) ± SEM[a]	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM) (R^2)	LLE
10e	H	H	H	0.19 ± 0.0080	990	75	77	>30	4.6
17a	$-CH_3$	H	H	0.31 ± 0.009	830	58	79	>20	4.2
17b	H	$-CH_3$	H	0.46 ± 0.049	650	78	100	>30	4.1
17c	H	H	$-CH_3$	0.13 ± 0.027	>1000	77	72	18 (0.90)	4.6
18				2.7 ± 0.070	810	67	170	>30	3.2

[a]**SCYX-7158** was used as a control, EC_{50}: 0.29 μM

Table 9.

*Quinolinimine derivatives of **10e**: potency against* T. brucei, *metabolic stability, aqueous solubility and plasma protein binding.*



ID	R	*T. brucei* EC_{50} (μM) ± SEM	Aq sol (μM)	Human PPB (%)	HLM Clint (μL/min/mg)	HepG2 TC_{50} (μM) (R^2)	LLE	25× EC_{50} (μM)	6 h CEC_{50} (μM)
10e		0.19 ± 0.0080	990	75	77	>30	4.6	nt	nt
22a	$-CH_3$	0.013 ± 0.001	880	15	22	>30	5.4	0.33	180
22b	$-CH_2CH_3$	0.069 ± 0.010	1000	41	38	>30	4.3	1.7	1700
22c	$-(CH_2)_2CH_3$	0.15 ± 0.00	890	41	51	>20	3.4	3.9	nd[a]
SCYX-7158		0.29						7.2	1100

nt = not tested

[a] compound did not meet the threshold of 50% cidality in either of 6 h or 12 h (data not shown) assays using 25×EC_{50}

Table 10.

Summary table showing progress 1 and **22a**.

	Properties	Targeted lead values	1	22a
in vitro	***T. brucei* EC_{50}**	<0.03 μM, cidal	0.43 μM	0.013 μM, cidal
	HepG2 TC_{50}	>100× EC_{50}	>30 μM	>30 μM
	HLM clearance	Cl_{int} <8.6 μL/min/mg	180	22
	Plasma protein binding	<95%	87%	15%
	Solubility (pH=7)	>100 μM	44 μM	880 μM
in vivo	**Pharmacokinetics**	>10× EC_{50} for >6 h	nd	Pass at 100 mg/kg, p.o.
	Mouse efficacy, bloodstream stage	<50 mg/kg p.o. × 5 days; >90% cure	Failed	100 mg/kg, p.o., 7 days, 75% UND parasitemia

nd = no data