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J. Med. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.jmedchem.9b01741 • Publication Date (Web): 17 Dec 2019

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Selectivity and Physicochemical Optimization of Repurposed Pyrazolo[1,5-*b*]pyridazines for the Treatment of Human African Trypanosomiasis

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Keywords. Lead repurposing; human African trypanosomiasis; neglected tropical diseases; GSK-3 β ; CDK-2; CDK-4.

Abstract

From a high-throughput screen of 42,444 known human kinases inhibitors, a pyrazolo[1,5-*b*]pyridazine scaffold was identified to begin optimization for the treatment of human African trypanosomiasis. Previously reported data for analogous compounds against human kinases GSK-3 β , CDK-

2 and CDK-4 were leveraged to try to improve the selectivity of the series, resulting in **23a** which showed selectivity for *T. b. brucei* over these three human enzymes. In parallel, properties known to influence the absorption, distribution, metabolism, and excretion (ADME) profile of the series were optimized resulting in **20g** being progressed into an efficacy study in mice. Though **20g** showed toxicity in mice it also demonstrated CNS penetration in a PK study, and significant reduction of parasitemia in four out of the six mice.

Introduction

Human African trypanosomiasis (HAT), or sleeping sickness as it is commonly referred to, is a parasitic disease caused by two subspecies of *Trypanosoma brucei*.¹ Despite sharing a life cycle and causing similar symptoms in patients these two subspecies, , progress at vastly different rates. *T. b. gambiense*, is more common (98% of cases) and infection takes an average of three years to run its course (ending in fatality), while *T. b. rhodesiense*, is more virulent and infections can cause death within weeks to months of infection.²⁻⁴ There were 1,446 documented cases of HAT in 2017, with an estimated 13 million people living in areas of moderate to high risk of infection.¹⁻² Fatal if left untreated, HAT has two stages; in the first stage *T. brucei* resides and multiplies in the blood and lymphatic systems. Infections often are not diagnosed due to nondescript symptoms, commonly associated with the flu.^{1, 3} In the second stage, the parasite crosses the blood-brain barrier (BBB) to infect the central nervous system (CNS), and those infected present more readily diagnosable symptoms such as disruptions to sleeping patterns, cognitive dysfunction, and can become comatose.¹

There are currently two approved therapies for stage 1 HAT, pentamidine for *T. b. gambiense* infections and suramin for *T. b. rhodesiense* infections, both are ineffective against stage 2 of the disease.² In the past, the two principal treatments for stage 2 of the disease were eflornithine for *T. b. gambiense* and melarsoprol for *T. b. rhodesiense*: the mechanisms and drawbacks of each is discussed in a thorough review by Barret *et al.*⁵ In 2009, a nifurtimox-eflornithine combination therapy (NECT) was approved for treatment of *T. b. gambiense*, infections.⁶ NECT requires 14 IV infusions of eflornithine (400 mg/kg/day) over 7 days

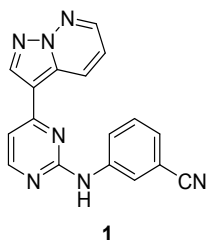
as well as oral nifurtimox 3 times a day for 10 days.^{1-2, 7-8} Fexinidazole, a nitroaromatic compound which recently concluded a Phase II/III clinical trial for treatment of *T. b. gambiense* infections demonstrated a 91% cure rate after 10 days of treatment, compared to 97% for NECT therapies.⁹ This lower cure rate was deemed acceptable as fexinidazole has a more manageable dosing regimen due to it being orally bioavailable, thus fexinidazole was approved for distribution in 2019 by the European Medicines Agency, becoming the first oral therapy for HAT.^{3, 10-11} Notably, *T. b. brucei* cell lines which were resistant to nifurtimox (also a nitroaromatic compound) were found to be resistant to fexinidazole.¹² This cross-resistance could imply similar mechanisms of action, meaning *T. b. gambiense* strains resistant to NECT could also be resistant to fexinidazole.¹²⁻¹³ Acoziborole (SCYX-7158), which recently had a successful Phase I clinical trial could prove to be the first, single-dose, oral therapy for HAT, however, it is not expected to conclude Phase II/III trials until 2020.¹⁴ While promising, it should be noted that treatments for infectious diseases fail at a higher rate in the clinic than other drug discovery programs.¹⁵ Should resistance to NECT/fexinidazole emerge, or acoziborole fail in clinical trials, there would be few remaining treatment options for HAT.

Despite the need for treatment options, there has been little investment from the pharmaceutical industry due, in-part, to a lack of financial incentive, which led to the designation of HAT as a neglected tropical disease by the World Health Organization.¹⁶⁻¹⁷ With the lack of investment there has been an increasing effort in academic settings, and in industrial-academic partnerships to aid the drug discovery process.¹⁸ Traditional drug discovery programs can be time consuming and costly, often starting from a high-throughput screen (HTS), and going through target validation, hit-to-lead optimization and *in vivo* testing before advancing to clinical trials.¹⁷ We hypothesize that employing one of a variety of drug repurposing strategies can help to shorten the timelines associated with this process.¹⁷

T. b. brucei expresses 176 kinases (156 which are described as eukaryotic protein kinases). Many of these are crucial to survival of the parasite; and several have human orthologs which have been pursued in drug discovery.¹⁹⁻²² With this in mind, we undertook a lead repurposing study against *T. b. brucei* using a biased library of known human kinase inhibitors.¹⁸ Starting from a library of 42,444 kinase inhibitors we

identified 797 compounds which showed sub-micromolar activity against *T. b. brucei*, as well as > 100-fold selectivity over human HepG2 cells (used as a toxicity counter screen). Compounds were clustered based on structural similarity and scored based on a variety of properties: their activity against *T. b. brucei*, rate and reversibility of action in parasite growth inhibition, and predicted CNS penetration as predicted by the multiparameter optimization (MPO) score; these properties were folded into a composite score to help prioritize starting points for medicinal chemistry optimization.^{18, 23} One of these clusters contained a pyrazolo[1,5-*b*]pyridazine: a representative compound is shown in **Table 1**. Its properties are listed, including those known to influence drug absorption, distribution, metabolism, and excretion (ADME), such as human liver microsome (HLM) and rat hepatocyte (RH) intrinsic clearance (Cl_{int}), thermodynamic aqueous solubility (aq. sol.) and human plasma protein binding (PPB).

Table 1. Representative hit from the *T. b. brucei* HTS.¹⁸



	Targeted Properties	1
<i>T. b. brucei</i> pEC ₅₀	> 7.5	8.2 ± 0.02
MRC5 pTC ₅₀	< 5	< 4.3 ± 0.00
HepG2 pTC ₅₀	< 5	< 4.0 ± 0.00
Aq. Sol. (μM)	> 10	< 0.1
MW	≤ 360	310
cLogP	≤ 3	2.8
cLogD	≤ 2	3.6
MPO Score	≥ 4	5.1
RH Cl_{int} *	≤ 9	66
HLM Cl_{int} **	≤ 5	210
PPB (%)	≤ 95	98

Aq. Sol. = Aqueous solubility.

MW = Molecular weight.

MPO = Multiparameter optimization.

RH = Rat hepatocytes.

HLM = Human liver microsomes.

PPB = Plasma protein binding.

* = ($\mu\text{L}/\text{min}/10^6$ cells).

** = ($\mu\text{L}/\text{min}/\text{mg}$ protein).

Based on the properties of **1**, this class of pyrazolo[1,5-*b*]pyridazines was considered a promising starting point for treatment of both stage 1 and 2 of HAT according to the published target product profile.²⁴⁻

²⁵ This scaffold had already been investigated for activity against serine/threonine kinase (Stk1) in *Staphylococcus aureus* and for three human kinases: glycogen synthase kinase 3 beta (GSK-3 β), and cyclin dependent kinases (CDK) 2 and 4.²⁶⁻²⁸ This was unsurprising as the compound screening set used in the HTS had been selected from a library of known kinase inhibitors, and homologs of both GSK-3 and CDK have been identified in *T. brucei* as potential targets for drug discovery.^{21, 29}

With this information, we set out to answer the following questions. First, would we be able to discern a difference between the series activity against *T. b. brucei* cells and human enzymes GSK-3 β , CDK-2 and CDK-4 by utilizing available data for compounds in this class? Second, would understanding this difference enable us to design compounds with improved activity against *T. b. brucei* and reduced potency against these human kinases? Third, would compounds from this class demonstrate efficacy in mouse models of trypanosome infection? While we used known human kinase inhibitor data to help guide our molecular design, we did not infer anything about the parasitic target from this information, as previous work optimizing for activity against *T. brucei* GSK-3 has demonstrated anti-parasitic activity to have arisen from a multi-target effect.³⁰

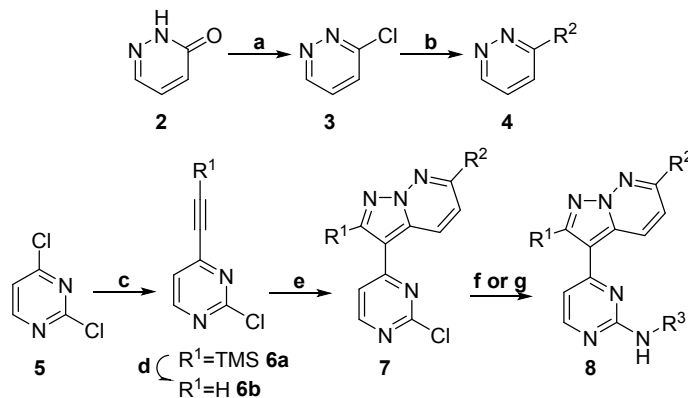
Results

Selectivity

In order to assess the difference in compound potency between *T. b. brucei* and GSK-3 β , CDK-2 and CDK-4, we evaluated previously reported compounds from the original HTS (and synthesized a set of previously reported analogs) with potency data against the three human kinases, to see if we could find any

difference in the selectivity profiles.^{26, 31} A synthetic strategy was devised (**Scheme 1**) that would allow for late stage derivatization at the three positions of principal interest (R^1 - R^3 , as shown in **8**). Starting from 2,4-dichloropyrimidine (**5**), a Sonogashira coupling was employed with trimethylsilyl acetylene. Subsequent silyl deprotection with potassium hydroxide yielded 2-chloro-4-ethynylpyrimidine **6b**. Pyridazine **4** was reacted with hydroxylamine-*O*-sulfonic acid (HOSA) to generate a 1-aminopyridizinium ion *in situ*, which was then reacted with **6b** in a [3+2] cycloaddition reaction to form the 3-(2-chloropyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazine intermediate **7**. Subsequent nucleophilic aromatic substitution or palladium-mediated cross-coupling provided the corresponding product **8**.³²

Scheme 1. Synthesis of pyrazolo[1,5-*b*]pyridazine analogs.

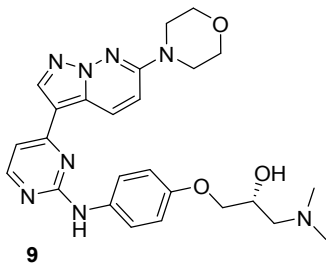


Reagents and conditions: a) POCl_3 , 80 °C, 1 h, 70%; b) NaOtBu , MeOH or morpholine, *n*-BuOH, 80 °C, 12 h, 60-100%; c) HCCR^1 , CuI, $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$, TEA, THF, 50 °C, 12 h, 19-61%; d) KOH, MeOH, RT, 30 min, 94%; e) **4**, hydroxylamine-*O*-sulfonic acid, KOH, NaHCO_3 , H_2O , DCM, 70 °C→RT, 12 h, 40-84%; f) NH_2R^3 , *n*-BuOH, 150 °C, 2 h, 11-90%; g) NH_2R^3 , $\text{Pd}(\text{OAc})_2$, BINAP or Xantphos, Cs_2CO_3 , dioxane, 160 °C, 1 h, 6-70%.

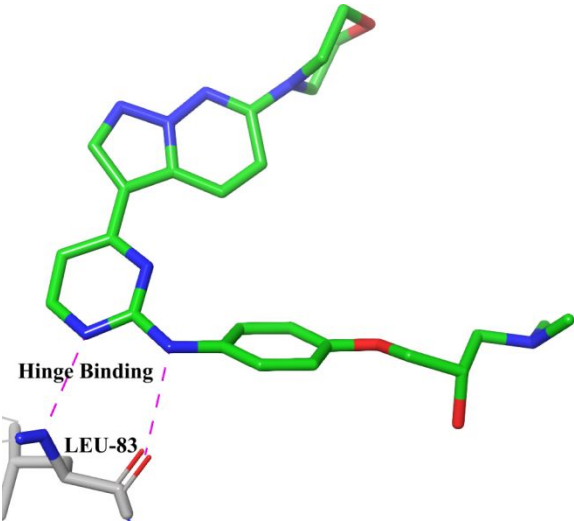
Figure 1. Previously reported crystal structure of pyrazolo[1,5-*b*]pyridazine compound, (*R*)-1-(dimethylamino)-3-(4-((4-(6-morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-

yl)amino)phenoxy)propan-2-ol, **9** (green, stick representation), bound in human CDK-2 (grey, surface/wire representation) (PDB: 3EID) displaying;³¹ (a) structure of **9**, (b) binding mode of **9** within the binding pocket, highlighting key hinge binding interactions with LEU-83, (c) binding position of **9** showing ligand-surface interactions within 5Å, displaying position of R¹ inside the binding pocket.

(a)

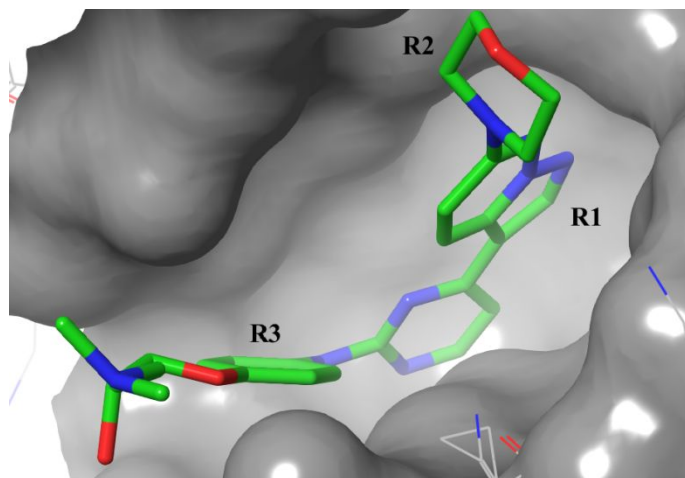


(b)



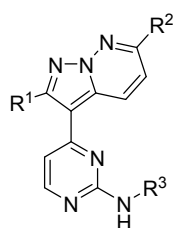
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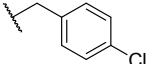
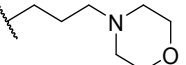

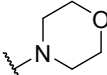
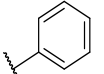
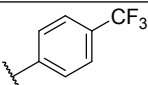
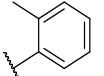
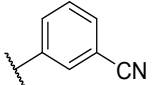
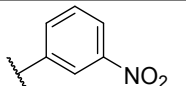
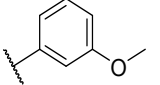

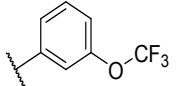
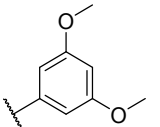
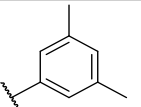
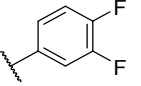

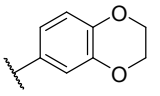
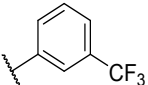
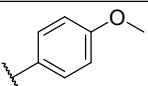
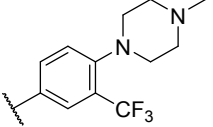


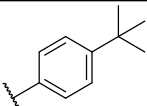

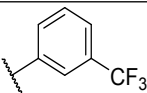
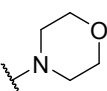
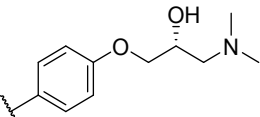


A previously reported crystal structure of a pyrazolo[1,5-*b*]pyridazine, **9** (**Figure 1a**), bound into CDK-2 (PDB: 3EID) (**Figure 1b** and **1c**), indicated that substitution at the R¹ and R² positions gave the best chance to improve selectivity, due to the increased interaction with the binding pocket at these positions, while the R³ position was solvent exposed, and the binding pocket would be less sterically restrictive of the molecule.³¹ Previously-reported docking of a pyrazolo[1,5-*b*]pyridazine into a GSK-3 β crystal structure (PDB: 1GNG) predicted a similar binding mode.^{26, 31} Available IC₅₀ data against all three enzymes indicated that substitution at any of these three positions could cause significant shifts in potency, but the largest decreases in potency came with substitution at the R¹ and R² positions. Armed with the published human kinase selectivity data, we compared compounds from the HTS and resynthesized a selected set of analogs from the literature. We sought to identify which substitutions could provide analogs with divergent activity for *T. b. brucei* over GSK-3 β , CDK-2 and CDK-4 (**Table 2**).

Table 2. Pyrazolo[1,5-*b*]pyridazine analogs with published human kinase data, compared with whole cell *T. b. brucei* potency data.



ID	R ¹	R ²	R ³	<i>T. b. brucei</i> pEC ₅₀ [*]	GSK-3β pIC ₅₀	CDK-2 pIC ₅₀	CDK-4 pIC ₅₀
10a	-H	-H		5.2	5.0 ³¹	6.0 ³¹	6.0 ³¹
10b	-H	-H		4.8	4.5 ³¹	--	5.5 ³¹
10c	-H	-H		6.4	5.8 ³¹	6.9 ³¹	7.1 ³¹
10d	-H			6.4	4.6 ³¹	--	6.4 ³¹
10e	-H	-H		7.4	7.7 ²⁶	8.3 ²⁶	6.8 ²⁶
10f		-H		5.3	< 4.5 ²⁶	< 4.7 ²⁶	--
10g	-H	-H		5.7	5.2 ²⁶	--	--
1	-H	-H		8.2	8.0 ³¹	8.7 ³¹	8.4 ³¹
10h	-H	-H		8.2	9.5 ³¹	9.5 ³¹	8.7 ³¹
10i	-H	-H		7.6	8.0 ²⁶	8.7 ²⁶	7.2 ²⁶
10j	-H			5.7	7.3 ²⁶	6.7 ²⁶	< 4.7 ²⁶
10k	-H	-H		7.2	7.9 ²⁶	7.8 ²⁶	7.7 ²⁶
10l	-H	-H		6.1	9.0 ²⁶	8.5 ²⁶	5.8 ²⁶
10m	-H	-H		6.8	8.0 ²⁶	8.7 ²⁶	--
10n		-H		6.1	4.6 ²⁶	< 4.7 ²⁶	--
10o	-H	-H		7.2	9.0 ²⁶	8.2 ²⁶	6.5 ²⁶
10p		-H		5.8	7.3 ²⁶	< 4.7 ²⁶	--
10q		-H		6.0	5.3 ²⁶	< 4.7 ²⁶	--
10r	-H	-H		7.8	7.8 ³¹	8.4 ³¹	8.2 ³¹

10s	-H	-H		6.2	7.3 ²⁶	6.8 ²⁶	5.7 ²⁶
10t		-CH ₃		5.6	7.4 ²⁶	< 4.7 ²⁶	--
9	-H			6.7	6.2 ³¹	6.4 ³¹	7.4 ³¹

- Compounds without published data against a human kinase data were left blank.

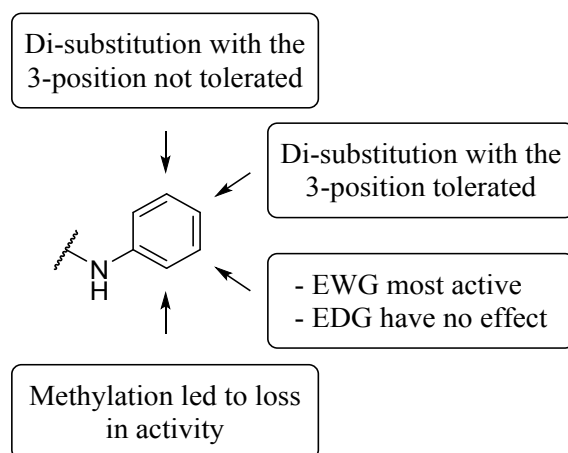
* standard error of mean (SEM) within 0.17.

Substitution at the R¹ position caused varying shifts in potency between *T. b. brucei* and the human kinases of interest. In general, substitution at this position led to a significant loss in potency against CDK-2, an observation that is consistent with the literature.²⁶ While substituting R¹ with 4-(trifluoromethyl)phenyl (**10f**) caused a 100-fold decrease in potency against *T. b. brucei* (compared with **10e**) there was a much greater impact on the potency against GSK-3 β and CDK-2, which both exhibited a > 1,000-fold decrease. Similarly, substitution with 4-methoxyphenyl (**10q**) caused a 10-fold decrease in the potency against *T. b. brucei* (compare with **10o**), however the potency against GSK-3 β and CDK-2 was decreased by > 10,000-fold. Replacement of the phenyl ring with a (smaller) cyclopropyl group (**10n**) also resulted in a slight decrease in the potency against *T. b. brucei* relative to the unsubstituted analog (**10m**). This loss in potency was again much less dramatic than observed against GSK-3 β and CDK-2 (between 3.5- and 4-log units). This indicated that while small substituents were tolerated, there was a sharp decrease in activity against *T. b. brucei* as R¹ became larger. In sum, we were able to demonstrate the potential of compounds with *T. b. brucei* activity that is divergent from human GSK-3 β and CDK-2 activity particularly when substituted at R¹ or R².

Substitution at the R² position with a morpholine group (**10d**) led to an equipotent compound against *T. b. brucei* relative to the unsubstituted analog (**10c**), with a concomitant and significant decrease in activity against both GSK-3 β and CDK-4 (~10 \times against both). The presence of a methoxy at the R²

position (**10j**) led to a dramatic decrease in activity of the series against *T. b. brucei*; it also contributed to a complete loss of activity against CDK-4.

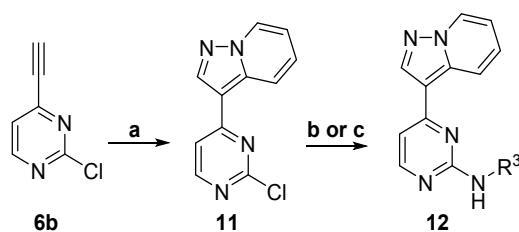
Figure 2. Preliminary structure-activity relationship (SAR) trends for substitution of aromatic rings at R³ position.



Substitution at R³ caused a significant shift in the potency of the series against *T. b. brucei*. Analogs with an alkyl attachment at the R³ position (**10a**, **10b**, **10c**, **10d**) showed moderate inhibition of *T. b. brucei*, though aryl substituents (summarized in **Figure 2**) caused a noticeable increase in potency against *T. b. brucei* (typified by **10e**). While these changes caused significant shifts in the activity of the compounds, they did not provide any tangible selectivity improvement against the three human kinases. Substitution of the aryl ring at the 3-position with electron withdrawing groups (EWG) yielded the most potent compounds against both *T. b. brucei* and the human enzymes (**1**, **10h**). Electron donating groups (EDG) at the 3-position (**10i**) did not cause any significant shift in the potency, though di-substitution at the 3- and 5-positions (**10k**, **10l**), caused a noticeable decrease in activity against *T. b. brucei*. Di-substitution at the 3- and 4-positions of the phenyl ring (**10m**, **10r**) did not seem to cause this shift in potency, this trend implied there were less steric restraints on the 4-position of the R³ ring for activity against both *T. b. brucei* cells and the human

enzymes. This observation is consistent with the crystal structures for human GSK-3 β , and CDK-2, wherein the 4-position of the ring points out of the binding pocket.^{26, 31}

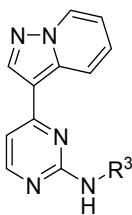
Scheme 2. Synthesis of pyrazolo[1,5-*a*]pyridine analogs.



Reagents and Conditions. a) pyridine, hydroxylamine-*O*-sulfonic acid, KOH, NaHCO₃, H₂O, DCM, 70 °C→RT, 12 h, 51%; b) NH₂R³, K₃PO₄, XPhos, Pd₂(dba)₃, toluene, 110 °C, 12 h, 22%; c) NH₂R³, *n*-BuOH, 110 °C, 12 h, 40-88%.

Curious to the role of the pyrazolo[1,5-*b*]pyridazine head group to the activity of the cluster, a series of analogs was prepared to elucidate the impact of the nitrogen atom positioning within the head group upon *T. b. brucei* activity. The pyrazolo[1,5-*a*]pyridine head was synthesized via Sonogashira reaction using trimethylsilylacetylene (**Scheme 2**). Following desilylation, a [3+2] dipolar cycloaddition was effected using a 1-aminopyridinium ion generated *in situ*, to give 3-(2-chloropyrimidin-4-yl)pyrazolo[1,5-*a*]pyridine (**11**) in moderate yield. Subsequent nucleophilic aromatic substitution gave the desired products.

Table 3. Effect of pyrazolo[1,5-*a*]pyridine head on *T. b. brucei* potency ADME properties.



ID	R ³	<i>T. b. brucei</i> pEC ₅₀ [*]	Aq. Sol. (μM)	LogD 7.4	RH Cl _{int} ^{**}	HLM Cl _{int} ^{***}	PPB (%)	MRC5 pTC ₅₀
12a		6.0	64	2.9	160	75	91	< 4.3
12b		6.6	2	4	76	> 300	99	< 4.3
12c		6.6	18	4.3	140	220	> 99	< 4.3
12d		7.0	< 0.9	> 3.9	63	nd ^a	99	< 4.3

* = SEM within 0.05.

** = (μL/min/10⁶ cells).

*** = (μL/min/mg protein).

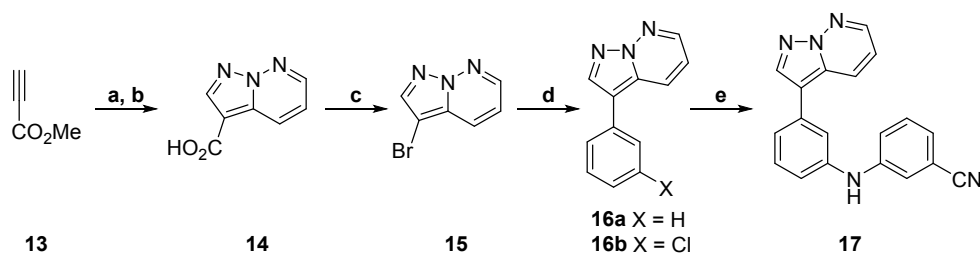
nd = not determined.

^acompound detected only in first sample.

As **Table 3** shows, replacement with the pyrazolo[1,5-*a*]pyridine head caused a decrease in potency for all four analogs compared to their matched pyrazolo[1,5-*b*]pyridazine counterparts. Compound **12a** showed the smallest decrease in potency compared to **10c** but was still 2-fold less potent. The aromatic substituents at the R³ position were a minimum of 7-fold less active against *T. b. brucei* (**12b** compared to **10e**), all the way up to 15-fold less active (**12d** compared to **1**). Due to this decrease in activity, and no-tangible gain in the ADME profile of the series, we turned our attention to replacing the nitrogen atoms in the pyrimidine ring.

The crystal structure and docking study for both GSK-3β and CDK-2 indicated that the key hinge binding interaction (highlighted in **Figure 1b**) with the series occurred between the pyrimidine nitrogen, and amine at the R³ position.^{26, 31} If altering or removing this interaction did not cause a significant loss in potency against *T. b. brucei*, it would likely result in a large selectivity improvement over the human

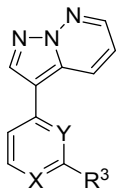
Scheme 3. Synthesis of pyrimidine replacement analogs containing phenyl ring.



Reagents and conditions. a) pyridazine, hydroxylamine-*O*-sulfonic acid, KOH, NaHCO₃, H₂O, DCM, 70 °C→RT, 12 h, 71%; b) NaOH, H₂O, 70 °C, 1 h, 37%; c) NBS, DMF, 25 °C, 3 h, 79%; d) R(BOH)₂, Pd(dppf)Cl₂·DCM, K₂CO₃, 3:1 dioxane/H₂O, 130 °C, 1 h, 15-72%; e) 3-aminobenzonitrile, Pd(OAc)₂, Xantphos, Cs₂CO₃, dioxane, 160 °C, 1 h, 47%.

Both pyrimidine nitrogen atoms were deemed crucial for potent activity (**Table 4**), as removal of either (**18d**, **18b**) resulted in a > 10-fold potency loss compared to the original hit (**1**). Removal of both nitrogen atoms (**17**), resulted in an additional decrease in potency. Removal of the hydrogen bond donor (HBD) and R³ ring (**16a**, **18a**, **18c**) resulted in a total loss of activity against *T. b. brucei*, though it also corresponded with a substantial increase in the solubility of all three compounds.

Table 4. *T. b. brucei* activity of the pyrimidine replacement analogs, and the associated ADME data.



ID	R ³	X	Y	<i>T. b. brucei</i> pEC ₅₀ [*]	Aq. Sol. (μM)	LogD 7.4	RH Cl _{int} ^{**}	HLM Cl _{int} ^{***}	PPB (%)	MRC5 pTC ₅₀ ^{****}
16a	-H	CH	CH	< 4.4	850	2.8	66	> 300	91	< 4.3
17		CH	CH	5.4	0.7	4.2	82	300	99	4.7
18a	-H	CH	N	< 4.4	> 1000	1.9	57	170	61	< 4.3
18b		CH	N	6.2	0.6	4.1	84	280	99	< 4.3
18c	-H	N	CH	< 4.4	990	1.2	5	37	86	< 4.3
18d		N	CH	6.6	0.3	3.7	87	> 300	99	< 4.3

* = SEM within 0.08.

** = (μL/min/10⁶ cells).

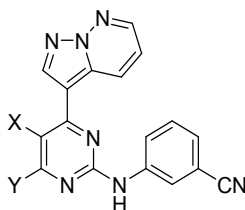
*** = (μL/min/mg protein).

**** = SEM within 0.02.

Given the crystal structures of GSK-3β and CDK-2, as well as the selectivity gained from the presence of a cyclopropyl group at the R¹ position in **10n**, we performed a methyl scan around the pyrimidine ring to assess potency changes against *T. b. brucei*. The 5 and 6 positions on the pyrimidine ring were positioned in a similar part of the binding pocket in the GSK-3β crystal structure as the cyclopropyl group of **10n**. Since substitution pointing into the binding pocket of the human enzymes had given selectivity for **10n**, we hoped that substitution off of the pyrimidine ring would give us a similar selectivity advantage. Using the same chemistry shown in **Scheme 1**, two analogs were prepared from the starting pyrimidines with methyl groups at the desired positions. As shown in **Table 5**, substitution at the 5-position of the pyrimidine ring (**19b**), lowered the activity of the series by 10-fold compared to the original hit (**1**)

whereas substitution at the 6-position (**19a**), resulted in a complete loss of activity. In the published crystal structures and docking models against GSK-3 β and CDK-2, these two positions on the pyrimidine ring had pointed into the same part of the binding pocket as R¹.^{26, 31} While substitution at this position may have resulted in increased selectivity against the human enzymes, the loss in activity against *T. b. brucei* indicated that there was no room for substitution on this portion of the scaffold.

Table 5. Analogs for methyl scan of pyrimidine.



ID	X	Y	<i>T. b. brucei</i> pEC ₅₀ *	Aq. Sol. (μ M)	LogD 7.4	RH Cl _{int} **	HLM Cl _{int} ***	PPB (%)	MRC5 pTC ₅₀
19a	-H	-CH ₃	< 4.4	0.8	4	17	56	97	< 4.3
19b	-CH ₃	-H	7.0	0.2	4	80	170	99	< 4.3

* = SEM within 0.09.

** = (μ L/min/10⁶ cells).

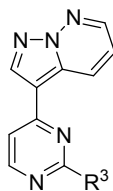
*** = (μ L/min/mg protein).

ADME Optimization

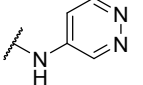
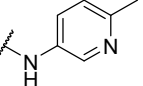
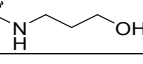
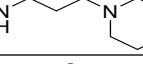
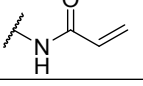
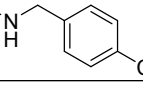
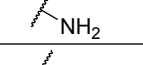
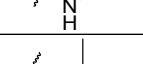
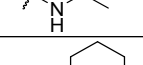
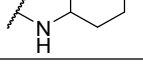
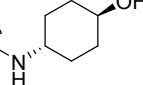
Armed with the knowledge of the substitutions that were tolerated at the R¹ and R² positions, our attention turned to the exploration of the SAR around the R³ position of our hit compound, **1**, with the view to determining which R³ substituents would provide the most favorable combination of potency and ADME properties (**Table 6**). Compound **20a**, and **20b** demonstrated the importance of the HBD for potency against *T. b. brucei*. Removal of the nitrile (**10e**) did not cause any significant change in the ADME properties of the series, though replacement with a trifluoromethyl (**20c**) improved the clearance of the series in both RH and HLM, though it also resulted in a 4.4-fold decrease in potency against *T. b. brucei*. *Ortho*-methyl

substitution of the phenyl ring (**10g**) provided a substantial improvement in the aqueous solubility; comparison of **10g**'s melting point (mp = 177-178 °C) to the melting point of **10e** (mp = 207-209 °C) suggested that this increase in solubility was due to the disruption of the crystal packing of the compound. However, **10g** showed a decrease in the activity against *T. b. brucei*. To further explore whether *ortho*-substitution was a viable route to improve the ADME properties while maintaining potency, we investigated compounds with a fluorine atom at either the 2- or 6- position of the ring (**20d**, **20e**). These analogs did not show any significant improvement in the solubility of the series (compared with **1**). Next, a number of heterocyclic replacements for the R³ phenyl group were prepared, including pyridines (**20f**, **20g**, **20h**), pyrimidines (**20i**) and pyridazines (**20j**). The 3-pyridyl analog (**20g**) showed a 3.5-fold reduction in the potency compared to **1** but showed improvements in the aqueous solubility and RH Cl_{int} (though HLM Cl_{int} was still high). As blocking the 4-position of the ring (**10s**) had previously shown reduced HLM clearance, **20k** was prepared, though it demonstrated increased HLM and RH clearance over **20g**. The pyrimidine analog (**20i**) displayed further improvement in solubility and HLM clearance, though this was at a 4.9-fold cost to potency when compared to **20g**.

In addition to aryl substituents, a variety of alkyl substituents were explored at the R³ position, as increasing the sp³ carbon content has been previously shown to improve the aqueous solubility of a chemical series.³⁴⁻³⁵ As the steric bulk and lipophilicity of the compounds increased, an increase in potency against *T. b. brucei* was observed, however this increase in potency typically came with a significant reduction in solubility. Compound **20q** was the most active of the compounds against *T. b. brucei* but demonstrated low solubility (2 μM) and high intrinsic clearance against both RH and HLM. Introduction of a polar group at the 4-position was trialed as this region was known to be solvent exposed in human enzymes and we had observed a similar SAR relationship between *T. b. brucei* and these human targets at the R³ position.^{26, 31} The introduction of a *trans*-alcohol (**20r**) greatly improved the ADME profile whilst simultaneously maintaining the activity against *T. b. brucei* (pEC₅₀: 6.8) as seen with **20q**.

Table 6. *T. b. brucei* activity and ADME properties of analogs with substituents at the R³ position.

ID	R ³	<i>T. b. brucei</i> pEC ₅₀ [*]	Aq. Sol. (μM)	LogD 7.4	RH Cl _{int} ^{**}	HLM Cl _{int} ^{***}	PPB (%)	MRC5 pTC ₅₀ ^{****}
1		8.2	< 0.1	3.6	66	210	98	< 4.3
20a		< 4.7	2	2.4	41	13	90	< 4.3
20b		5.9	4	3.0	240	200	96	< 4.6
10e		7.4	0.8	3.1	62	> 300	96	< 4.3
10g		5.7	38	3.4	43	250	96	< 4.3
20c		7.6	3	> 4.3	30	76	100	< 4.3
20d		7.2	0.4	3	50	180	nt	< 4.3
20e		7.3	1.4	3.1	57	240	nt	< 4.3
10s		6.2	0.4	5	70	8.8	100	5.7
20f		6.0	7	2.4	43	300	93	< 4.3
20g		7.7	6	2.5	5	69	89	< 4.3
20h		5.3	350	-0.6	3.5	3	41	< 4.4
20i		7.0	26	2	35	32	84	< 4.6

20j		5.1	840	-0.6	1.3	3	44	< 4.3
20k		7.7	5	2.9	38	180	92	< 4.3
20l		5.9	170	1.3	190	25	59	< 4.3
10b		4.8	950	1.3	38	12	40	< 4.3
20m		5.4	1	> 3.8	> 300	110	nd ^a	< 4.3
10a		5.2	2	4.1	94	> 300	99	< 4.3
20n		4.8	360	1	31	34	58	< 4.3
20o		5.3	270	1.7	56	220	86	< 4.3
20p		6.3	88	2.6	98	> 300	85	< 4.3
20q		6.8	2	3.7	140	240	97	< 4.3
20r		6.8	54	2	8.7	14	68	< 4.3

* = SEM within 0.14.

** = (μL/min/10⁶ cells).

*** = (μL/min/mg protein).

**** = SEM within 0.17.

nt = not tested.

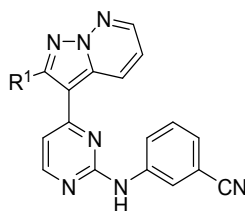
nd = not determined.

^alow recovery/low stability.

Comparison of the initial human kinase and *T. b. brucei* data indicated that substitution at the R¹ position provided a promising opportunity to obtain selectivity. We also wanted to evaluate the impact that substitution at this position would have on the ADME properties of the series. Compound **21d** demonstrated sub-micromolar activity against *T. b. brucei* and an improved ADME profile (Table 7), and the corresponding methyl ether (**21e**) showed that the presence of the HBD did not have a significant effect on the potency. The secondary alcohol (**21f**) and the isopropyl (**21g**) derivatives were equipotent, though a slight boost in potency was observed with the cyclopropyl (**21h**), suggesting that there are steric limitations. Compound **21h** also demonstrated an improvement in the solubility and clearance compared to **1**.

Additionally, the cyclopropyl group at the R¹ position was previously shown to cause a total loss of activity against GSK-3 β and CDK-2 (**Table 2; 10n**).

Table 7. *T. b. brucei* activity of analogs with a substituent at the R¹ position.



ID	R ¹	<i>T. b. brucei</i> pEC ₅₀ [*]	Aq. Sol. (μ M)	LogD 7.4	RH Cl _{int} ^{**}	HLM Cl _{int} ^{***}	PPB (%)	MRC5 pTC ₅₀ ^{****}
21a		5.9	nt	nt	nt	nt	nt	< 4.9
21b		6.2	0.2	4.8	37	16	97	< 4.3
21c		4.9	12	2.8	25	46	95	< 4.3
21d		6.5	20	2.5	63	240	93	< 4.3
21e		6.2	0.9	3.3	53	> 300	99	< 4.3
21f		6.1	2	2.9	27	150	nt	< 4.3
21g		6.2	28	4.4	83	100	nt	4.5
21h		6.7	11	> 4.2	18	80	> 99	< 4.3

* = SEM within 0.08.

** = (μ L/min/10⁶ cells).

*** = (μ L/min/mg protein).

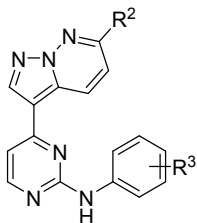
**** = SEM within 0.03.

nt = not tested.

Substitution at the R² position with a morpholine or methoxy had previously shown a reduction in activity versus human CDK-2 and CDK-4, as such, we wanted to assess whether these substituents could

also help improve the ADME properties of the series (**Table 8**). The morpholine was found to have no effect on the potency of the series, (**22c** vs. **1**) though small improvements in the HLM clearance were observed. The methoxy group was found to lower potency by 1-log unit (**22d** vs. **1**) against *T. b. brucei* but had previously been reported to lead to a total loss in activity against CDK-4 (**Table 2; 10j**).

Table 8. *T. b. brucei* activity, and associated ADME profile of analogs with a substituent at the R² position.

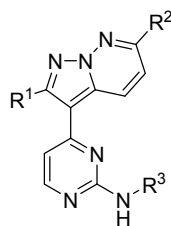


ID	R ²	R ³	<i>T. b. brucei</i> pEC ₅₀ [*]	Aq. Sol. (μM)	Log D 7.4	RH Cl _{int} ^{**}	HLM Cl _{int} ^{***}	PPB (%)	MRC5 pTC ₅₀
22a		-H	7.5	< 0.9	4	37	74	98	< 4.3
22b		-3-OMe	7.7	< 1	4.1	47	72	99	< 4.3
22c		-3-CN	8.3	< 19	> 3.7	66	100	nd ^a	< 4.6
22d		-3-CN	7.1	nt	nt	nt	nt	nt	< 4.3

* = SEM within 0.13.
** = (μL/min/10⁶ cells).
*** = (μL/min/mg protein).
nd = not determined.
nt = not tested.
^alow MS/MS signal.

Given the observed trends in selectivity, activity, and ADME we set out to make a series of combination analogs (**Table 9**). For the R¹ position we examined the cyclopropyl group and primary alcohol as these groups were small enough to not significantly impact the potency of the molecule and could potentially provide a selectivity advantage. We selected three different R³ groups: 3-aminobenzonitrile, 3-aminopyridine, and *trans*-4-aminocyclohexanol based on the observed potency and ADME properties they displayed.

Table 9. *T. b. brucei* activity and ADME properties for a series of combination analogs, combining various R^1 , R^2 and R^3 substituents.



ID	R^1	R^2	R^3	<i>T. b. brucei</i> pEC ₅₀ [*]	Aq. Sol. (μ M)	Log D 7.4	RH Cl _{int} ^{**}	HLM Cl _{int} ^{***}	PPB (%)	MRC5 pTC ₅₀
23a				7.2	0.4	3.6	170	45	99	< 4.3
23b				6.8	< 0.3	3.3	74	24	100	< 4.3
23c				7.1	0.4	nd ^a	nt	49	100	< 4.3
23d		-H		6.8	20	3.3	16	60	98	< 4.3
23e	-H			7.2	nd ^b	3.4	15	120	97	< 4.3
23f				6.6	13	2.5	70	22	94	< 4.3
23g				7.2	nd ^b	3.6	110	230	100	< 4.3
23h				6.8	2	3.8	45	110	99	< 4.3
23i	-H			7.9	0.8	3.1	13	53	94	< 4.3
23j	-H			6.8	7	2.6	14	7.3	88	< 4.3
23k	-H			6.3	8.2	2.6	90	52	85	< 4.3
23l				6.3	nd ^b	3.4	50	100	95	< 4.3

23m				5.3	<i>nt</i>	<i>nt</i>	<i>nt</i>	<i>nt</i>	<i>nt</i>	< 4.3
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* = SEM within 0.13.

** = ($\mu\text{L}/\text{min}/10^6$ cells).

*** = ($\mu\text{L}/\text{min}/\text{mg}$ protein).

nd = not determined.

nt = not tested.

^apoor MS response.

^bno compound detected.

The series of matched analogs detailed in **Table 9** gave further insight into the SAR of the cluster. At the R¹ position, analogs with a cyclopropyl group (**23c**, **23g**, **23l**) were more potent than the matched pair analogs containing a primary alcohol (**23b**, **23f**, **23m**, respectively). In addition, comparison of **23g** and **23l** to their matched pair analogs with no substitution at the R¹ position (**23e** and **23k** respectively) showed no decrease in potency. Interestingly this trend was not observed with compounds containing a morpholine group at the R² position, as **23h** was over 10-fold less active than its unsubstituted matched pair **23i**. It should also be noted that the unsubstituted analogs at R¹ showed the lowest clearance against both RH and HLM, indicating that R¹ substituents may be a site of metabolism.

At the R² position, analogs with a methoxy group **23a** and **23g** were more potent than their morpholine matched pair analogs **23b** and **23h**, respectively. The reverse of this trend was seen with **23k** compared to **23j** (and **22c** vs. **22d** previously). This reversal in trends seems to further indicate that substitution at the R¹ position could have an impact on the compound-target interactions at the R² position. Analogs with substitution at R¹ appear to be more potent with a methoxy group at R², while analogs without substitution at R¹ were more potent with the morpholine. Compound **23g**, with a methoxy group at R², showed an increased potency in comparison to the analog with no substitution, **23d**. Compound **23d** was equipotent against *T. b. brucei* with the morpholine derivative, **23h**. This increase in potency from the introduction of a methoxy group can also be observed comparing **21d** (**Table 7**) to **23a**, where **23a** was 4-fold more active. Overall the morpholine group was better for controlling the clearance of the series with matched analogs (**23h** and **23j**) containing the morpholine showing lower RH and HLM clearance than the

methoxy counterparts (**23g** and **23k**), though all R² analogs showed higher clearance and were less soluble than the unsubstituted analogs (**23d** and **20r**).

A subset of these combination molecules was sent for single point inhibition assays against all three human kinases (**Table 10**). Analogs were prioritized based on their predicted selectivity. As expected, **23i** and **23e** displayed the highest percent inhibition of all three human enzymes, reaffirming the need for substitution at R¹ to provide selectivity. This was also evident when examining the selectivity trend for CDK-4, as any substitution at R¹ caused a significant decrease in the activity against the enzyme. At R¹ the primary alcohol (**23a**, **23b**) was generally less active than the cyclopropyl (**23d**, **23g**, **23h**) against CDK-2. The morpholine group did appear to decrease affinity for CDK-2 provided there was substitution at R¹ (**23h**, **23b**). For GSK-3 β the cyclopropyl group at R¹ provided the greatest decrease in percent inhibition (**23d**, **23h**), however the morpholine at R² seemed to increase potency against the human enzyme. The most selective of these compounds, **23a**, displayed less than 50% inhibition against all three targets, however due to a poor ADME profile it was not considered for further evaluation.

Table 10. Selected analogs % inhibition at 1 μ M against human kinases CDK-2, CDK-4, and GSK-3 β .

Cpd	<i>T. b. brucei</i> pEC ₅₀ *	CDK-2 cyclin A (%)	CDK-2 cyclin E (%)	CDK-4 cyclin D3 (%)	GSK- 3 β (%)
23a	7.2	47	29	1	49
23b	6.8	46	54	32	75
23d	6.8	76	70	21	21
23e	7.2	84	84	69	89
23g	7.2	84	77	25	65
23h	6.8	67	49	26	34
23i	7.9	89	87	90	81

* = SEM within 0.13.

With evidence of what was required to obtain selectivity in the series, we set out to determine whether the pyrazolo[1,5-*b*]pyridazine head would be efficacious in an animal model of infection. Based

on the observed SAR and ADME data, summarized in **Figure 3**, we prioritized **20r**, and **20g**, for further investigation. Further studies showed that both **20r** and **20g** were stable in mouse plasma (**Table S3, Supplemental Information**), were not substrates of the cytochrome P450 isozyme 3A4, and did not induce testosterone metabolism in CYP450 3A4 (**Table S4, Supplemental Information**). Both compounds were also found to be permeable in Caco-2 cells and were not actively effluxed (**Table S5, Supplemental Information**).

A small number of compounds were assessed to see if they were still fast acting and parasitocidal, including **20r** and **20g** (**Table S6, Supplemental Information**). Using a set of criteria previously disclosed,¹⁸ inhibitory properties of both compounds were assessed at set time points (**Figure 4**); we defined fast-acting compounds as having a $pEC_{50} \geq 6$ after 18 h of incubation. Given this definition, **20g** was fast-acting and **20r** was slow acting. In parallel, washout assays were performed to assess whether the compounds were cidal or static. The compounds were incubated for 18 h with *T. b. brucei* then washed out and the parasite was incubated for 72 h before the wells were assayed to determine pEC_{50} values. Compound **20g** was deemed cidal as it demonstrated a $pEC_{99} \geq 6$ following the washout assay, whereas **20r** was not cidal.

Figure 3. Summary of SAR and SPR relationships for the pyrazolo[1,5-*b*]pyridazines.

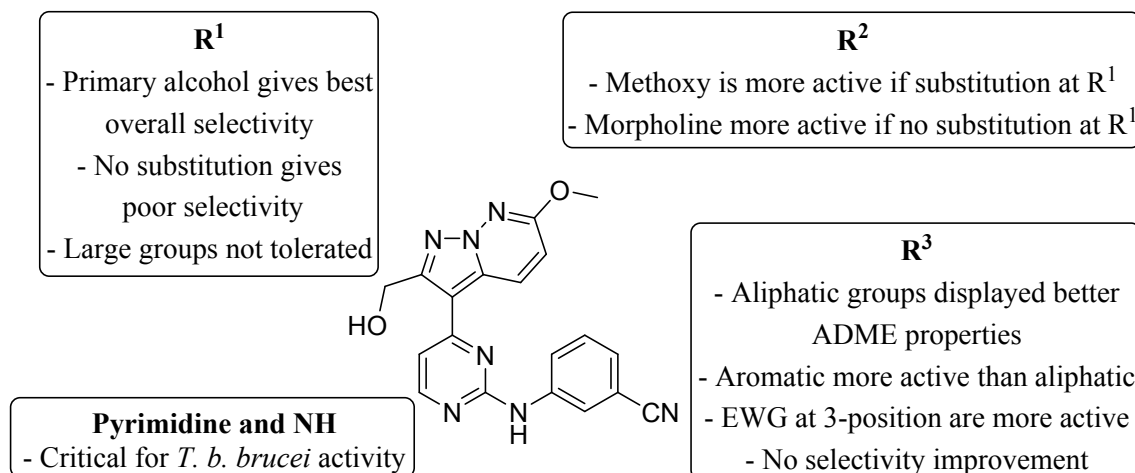
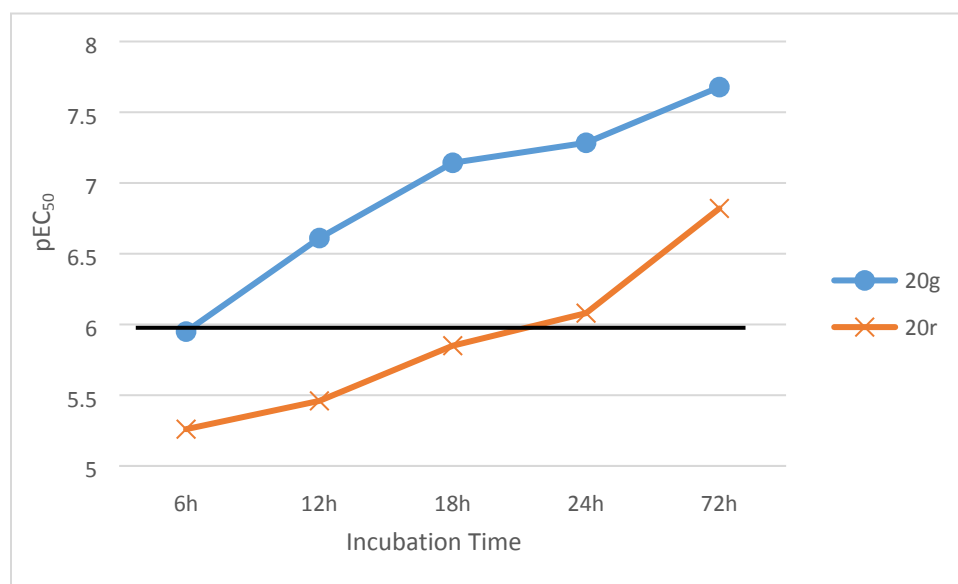


Figure 4. Rate of action of **20g** and **20r**, comparing pEC_{50} values against *T. b. brucei* following defined periods of incubation with the compound. Compounds with a $pEC_{50} \geq 6$ at 18 h were deemed fast acting (shown with a darkened line).



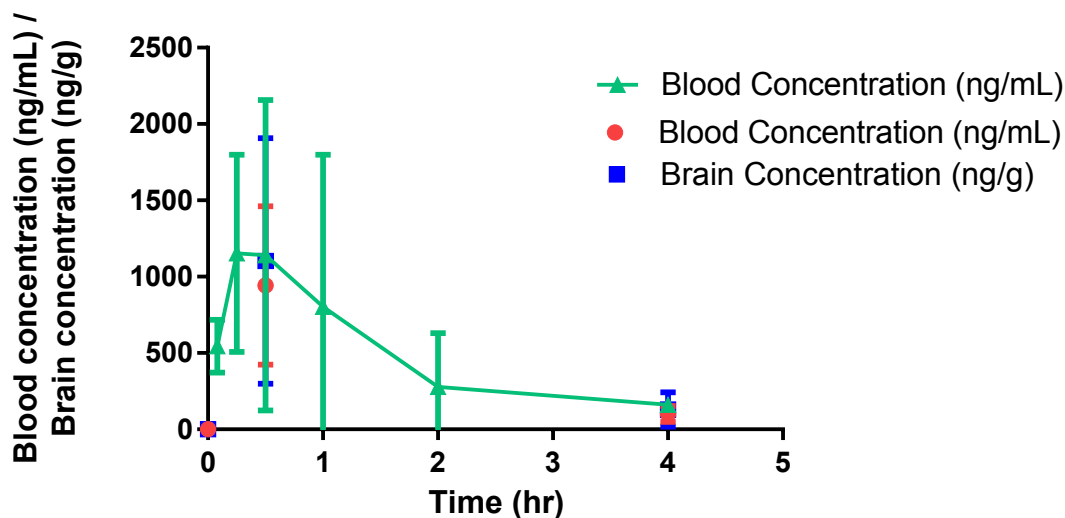
Both **20g** and **20r** were advanced to pharmacokinetic (PK) studies in mice, **20r** for its superior ADME profile, and **20g** for its rate of action, cidal activity, and high potency. Although neither compound contained structural features that were indicative of selectivity over human GSK-3 β , CDK-2 and CDK-4, we theorized that their ADME profiles and activity gave the highest probability of providing proof of concept efficacy. With the knowledge that inhibitors of GSK-3 β have been shown to have no negative effect in *in vivo* studies, and the lack of activity observed against human MRC5 cells for either **20g** and **20r**, we believed that there may be an acceptable therapeutic window.^{19, 36}

Following a 10 mg/kg intraperitoneal dose (IP) into female NMRI mice, **20r** (Figure S2, Supplemental Information) showed low whole blood exposure, with levels above the EC_{50} value (152 nM

or 47 ng/mL) for < 4 h following IP administration. In addition, the compound levels were below the lower limit of quantitation in the CNS after 4 h. We therefore deprioritized **20r** for efficacy studies.

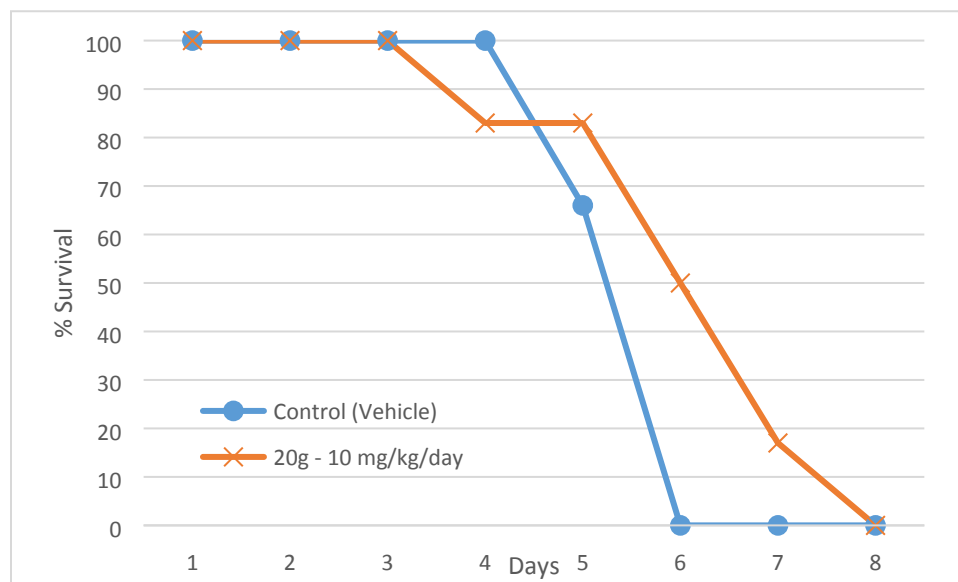
Compound **20g** demonstrated much better BBB penetration with similar concentrations in both the blood and brain (**Figure 5**). In addition, although the compound displayed a short half-life ($t_{1/2} = 0.68$ h), blood and brain concentrations were $> 10 \times EC_{50}$ value (20 nM or 6.1 ng/mL) for the entire duration of the study. Given the window for activity, as well as BBB penetration **20g** was taken forward into an efficacy study.

Figure 5. Peripheral blood levels of **20g** after IP administration to female NMRI mice (n=3) at a target dose of 10 mg/kg in 1% DMSO, 20% Captisol® in water. Individual values for each time point are represented in the plot.



For the efficacy study, mice were infected with *T. b. brucei* STIB795 and three days post-infection **20g** was administered at 10 mg/kg/day IP for five consecutive days with daily monitoring of parasitemia levels. A significant reduction was observed in parasitemia for four of six mice, resulting in parasite concentrations dropping below detectable levels by day 4 of treatment (**Table S10, Supplemental Information**). Despite the reduction of parasitemia, **20g** showed a negligible improvement in the survival rate of the mice compared with the control group, likely due to the toxicity of the compound, as mice presented with conjunctivitis, lacrimation, facial inflammation, in addition to an inflamed liver and kidneys (**Figure 6**).

Figure 6. Effect of **20g** on trypanosomes in a mouse efficacy model of HAT. Percent (%) survival rate of mice dosed IP at 10 mg/kg/day, compared to the control vehicle.

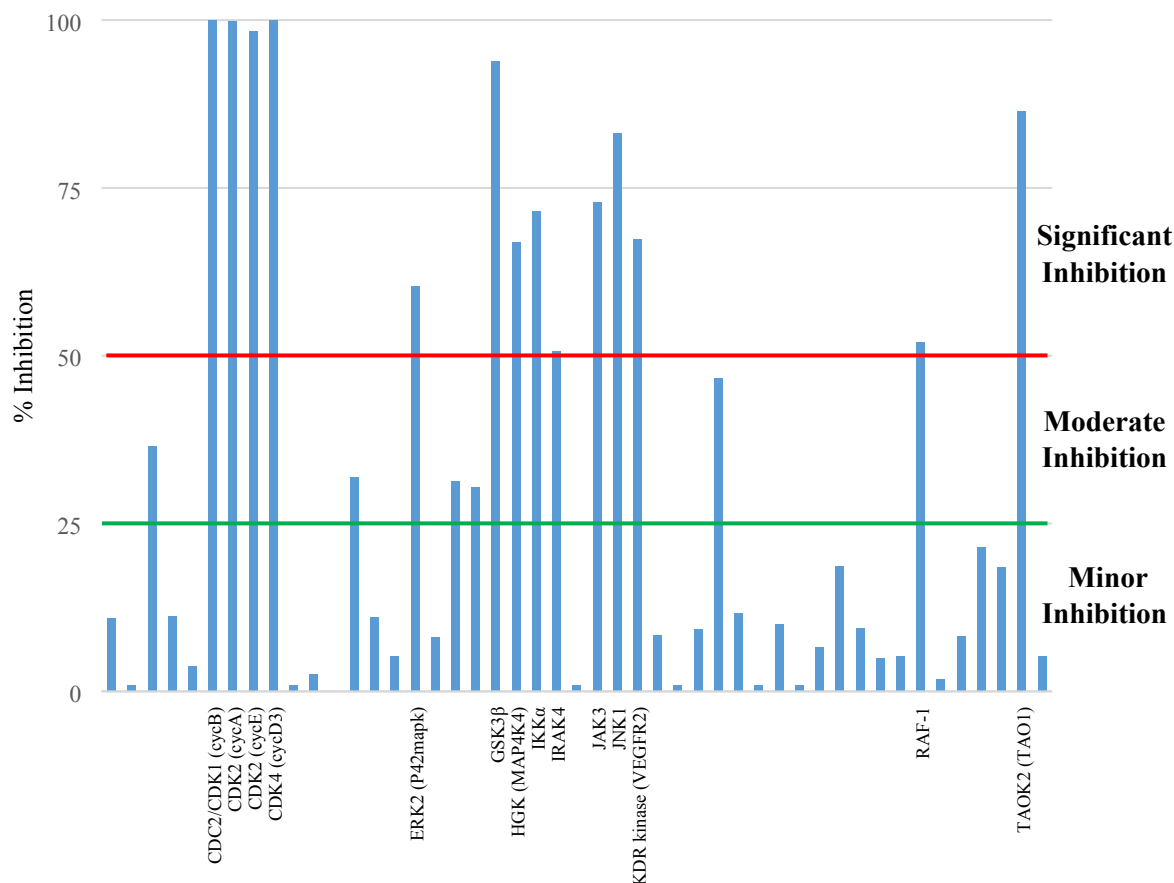


Due to the toxicity observed from the efficacy study we deemed it pertinent to examine the wider kinase selectivity of **20g**, profiling it against a panel of human kinases (**Figure 7**). Of the 47 kinases **20g** was profiled against, fourteen showed greater than 50% inhibition at 1 μ M. Unsurprisingly, given the lack of substitution around R¹ and R², **20g** still showed potent inhibition of GSK-3 β , CDK-2 and CDK-4. Further efforts in the optimization of this series would need to address this toxicity. Given the improvement in the selectivity of **23a** a more thorough investigation of substituents at R¹ and R² is warranted. The difference in toxicity, shown in **Table S11, Supplemental Information**, against L6 (**20g** pTC₅₀ > 6.2, **23a** pTC₅₀ < 4.3) and THP-1 (**20g** pTC₅₀ > 6.2, **23a** pTC₅₀ < 4.3) indicate that these may be better cell lines to use when optimizing for toxicity.

Figure 7. Percent (%) inhibition at 1 μ M of **20g** against human kinases. Line drawn to show 50% inhibition of enzymes. Kinases showing significant inhibition (above 50%) are listed on the X-axis. We define

significant inhibition is defined as > 50% inhibition at 1 μ M (red line), weak to moderate inhibition to be between 25 (green line) and 50%, and < 25% to be insignificant inhibition. For all values of inhibition see

Table S9, Supplemental Information.



A selection of compounds from this cluster was also screened against *T. cruzi* (Table S11, Supplemental Information), *Leishmania donovani* (Table S11, Supplemental Information), and adults of the flatworm pathogen, *Schistosoma mansoni* (Table S12, Supplemental Information). Compound **10e** was the most active compound against *T. cruzi*, although this was accompanied by poor selectivity versus L6 cells. Whilst **S1a** was the most active against *L. donovani* and had a good selectivity profile. Further optimization to understand the SAR versus *T. cruzi* and *L. donovani* is warranted. Against *S. mansoni*, most compounds were inactive, although **10s** and **S1k** showed similar activities characterized by a marked decrease in movement and an inability by the parasite to adhere to the bottom of floor of the assay well

with its oral and ventral suckers. Apart from these two compounds, this cluster does not warrant further investigation as a source of anti-schistosomal agents.

Conclusions

Starting from a screen of 42,444 known kinase inhibitors we set out to optimize a pyrazolo[1,5-*b*]pyridazine scaffold for the treatment of HAT. Using published SAR data against known human kinases GSK-3 β , CDK-2 and CDK-4, we were able to design an analog, **23a**, which showed selectivity against all three human targets. As **23a** did not have an ADME profile we deemed acceptable **20g** was taken forward into a mouse model to determine whether the series as a whole had potential to be effective *in vivo*. Compound **20g** was selected because it was fast acting, parasitocidal, had desirable ADME properties close to our targeted values and showed good BBB penetration in the PK study. Compound **20g** demonstrated acceptable brain concentrations and levels in excess of 10 \times EC₅₀ for 4 h in the PK study, and demonstrated a reduction in parasitemia in four out of the six mice tested. Although **20g**'s toxicity *in vivo* did not warrant further investigation, it highlights the promise of this series as anti-HAT agents.

Experimental

Strains and media

Bloodstream *Trypanosoma brucei brucei* Lister 427 was cultured in Hirumi's modified Iscove's medium (HMI-9), supplemented with 10% heat-inactivated FBS, at 37 °C and 5% CO₂ in T-25 vented flask (Corning®).

MRC5-SV2 cell line (SV40-transformed human lung fibroblast cell line) was cultured in DMEM medium supplemented with 10% FBS at 37 °C and 5% CO₂ in T-75 vented flask (Corning®).

The *T. cruzi* Tulahuen C4 strain, expressing the β -galactosidase gene (LacZ) and L6 rat skeletal muscle cells, used as host cells, were cultured in RPMI-1640 supplemented with 10% iFBS, 2 mM L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin at 37 °C and 5% CO₂.

Leishmania donovani MHOM/ET/67/HU3 cells with the luciferase gene integrated into the parasite genome were grown at 28 °C in RPMI 1640-modified medium (Invitrogen) supplemented with 20% FBS with 100 mg/ml of hygromycin B.³⁷

The Human myelomonocytic cell line THP-1 was grown at 37 °C and 5% CO₂ in RPMI-1640 supplemented with 10% iFBS, 2 mM glutamate, 100 U/mL penicillin and 100 mg/mL streptomycin. 3 x 10⁴ THP-1 cells per well in 96-well plates were differentiated to macrophages with 20 ng/mL of PMA treatment for 48 h followed by 24 h of culture in fresh medium.

Maintenance of the *Schistosoma mansoni* life cycle (NMRI isolate), the preparation of adult worms (\geq 42-days-old) and phenotypic screens with test compounds were as described.³⁸⁻⁴⁰

Preparation of compound plates

For dose-response experiments, compound plates were prepared for each analogue by serial 3-fold dilutions in 100% DMSO. Five concentration points (mammalian cytotoxicity) or ten concentration points (parasite growth inhibition), were made in 96-well transparent Nunclon plate, Internal control (pentamidine) is included randomly for *Trypanosoma brucei* assay plates.

Trypanosoma brucei growth inhibition assay

In order to determine the *T. b. brucei* EC₅₀ values, 4 μ L per well from compound master plates were dispensed into a new plate and 96 μ L of HMI-9 per well were added to generate a 4% DMSO intermediate plate. Mid-log phase growth *T. b. brucei* was diluted to a working cell density of 2,750 cells/mL and 90 μ L/well dispensed into 96-well flat-bottom transparent assay plates (Nunc®) where 10 μ L/well from intermediate plates were added. Final top concentration of compounds was 40 μ M in 0.4% DMSO per well.

Assay plates were incubated for 72 h at 37 °C and 5% CO₂. Four hours prior to the end of the incubation, 20 μ L of a 440 μ M resazurin solution in prewarmed HMI-9 was added to each well and incubated for another 4 h. Fluorescence was then measured in an Infinite F200 plate reader (Tecan®) at

550 nm (excitation filter) and 590 nm (emission filter). A 4-parameter equation was employed to fit the dose-response curves and determine of EC₅₀ using the SigmaPlot® 13.0 software. Assays were performed in duplicate at least twice, to achieve a minimal n=3 per dose response.

Rate of Action assays

Mid-log *T. brucei brucei* cultures were diluted to the required cell density, according to the different incubation time points described. 90 µL per well were dispensed in final assay plates Nunclon 96-well flat bottom Solid White and 10 µL of intermediate plates were added to each well, as described before. Four sets of assay plates were arranged to assay in order to be sequentially stopped at each indicated time point. Top and bottom rows were dismissed for compound assay, to reduce evaporation effects.

Plates were incubated at 37 °C and 5% CO₂ for the indicated time points; incubation was stopped by addition of 10 µL of prewarmed Cell Titer Glo reagent (Promega®), and after shaking the plates were incubated at room temperature for 10 min, to allow the signal to settle. Plate luminescence was read on an Infinite F200 plate reader (Tecan), and raw data were processed and analyzed as previously described.

Reversibility assays

Mid-log *T. brucei brucei* cultures were adjusted to a working density of 3,500 cell/mL. 90 µL per well were dispensed in final assay plates 96-well transparent Nunclon plates, and 10 µL of intermediate plates were added to each well, as described before. Top and bottom rows were dismissed for compound assay, to reduce evaporation effects.

Plates were incubated at 37 °C and 5% CO₂ for 18 h; once drug exposure was finished, plates were spun for 5 min at 500 rpm and room temperature to allow cells to settle at the well bottoms. 95 µL of media was replaced with fresh prewarmed media, and the process was repeated 3 times. Finally, 10 µL of each well were seeded in a new microtiter plate with 90 µL of fresh prewarmed media, on duplicate. Plates were incubated for 72 h, and viability was determined by resazurin reduction as previously described.

Cytotoxicity assay in MRC5 cells

Intermediate plates were made as described, adding 95 μL of DMEM complete media to 5 μL of compound per well setting a 5% DMSO amount.

Log-phase MRC5 cells were removed from a T-75 TC flask using TrypLE® Express (Thermo®) and dispersed by gentle pipetting. Cell density was adjusted to working concentration in prewarmed DMEM medium: 25,000 cells in 90 μL of culture were plated in 96-well transparent Nunclon plates and let to settle for 24 h at 37 °C and 5% CO_2 . After settling incubation, 10 μL of fresh made intermediate plate were added per well: final maximal concentration for compounds was 50 μM in 0.5% DMSO per well. Plates were incubated for 48 h at 37 °C and 5% CO_2 . 4 h prior to fluorescence measurement, 20 μL of 500 μM resazurin solution was added. Fluorescence was read in an Infinite F200 plate reader (Tecan®) at 550 nm (excitation filter) and 590 nm (emission filter).

A 4-parameter equation was used to fit the dose-response curves and determination of EC_{50} by SigmaPlot® 13.0 software. Assays were performed in duplicate at least twice for positive compounds, to achieve a minimal $n=3$ per dose response.

β -D-Galactosidase Transgenic *T. cruzi* Assay

A Thermo Scientific Multidrop Combi dispenser (MTX Lab Systems, Vienna, VA) was used to dispense 90 μL of *T. cruzi* amastigote-infected L6 cell culture (4×10^3 infected L6 cells per well) into 96-well Corning assay plates (Corning Inc., Corning, NY) already containing 10 μL of the compounds to be screened and controls. The plates were incubated at 37 °C for 96 h. Then, 30 μL of 100 μM CPRG and 0.1% NP40 diluted with PBS were added to each well, and the plates were incubated for 4 h at 37 °C in the dark. Absorbance at 585 nm was measured in a Vmax kinetic microplate reader (Molecular Probes). Compound activities were normalized using the in-plate negative (benznidazole at 10 $\mu\text{g/mL}$) and positive (0.2% DMSO) growth controls.

Resazurin-Based L6 Assay

One hundred microliters (100 μ L) per well of culture medium containing the compounds and controls were added to L6 cells previously cultured (4×10^3 L6 cells per well). After 72 h at 37 °C the medium was exchanged and the viable cell number was determined by resazurin (Sigma–Aldrich) reduction. 20 μ L of resazurin (1.1 mg/ml) was added to each well and incubated in the dark for 2 h at 37 °C. Cell viability was estimated by measuring the final fluorescence at 570-590 nm in an Infinite F200 plate reader (Tecan).

Cytotoxicity assay in THP-1

Cellular toxicity of all compounds was determined using the colorimetric MTT-based assay after incubation at 37 °C for 72 h in the presence of increasing concentrations of compounds (final maximal concentration was 50 μ M in 0.5% DMSO per well).⁴¹ The results are expressed as EC₅₀ values, the concentration of compound that reduces cell growth by 50% versus untreated control cells. Assays were performed in duplicate at least twice to achieve a minimal n=3 per dose response.

Determination of EC₅₀ in *L. donovani*

Macrophage-differentiated THP-1 cells were infected at a macrophage/parasite ratio of 1/10 with stationary *L. donovani* promastigotes for 24 h at 35 °C and 5% CO₂, and extracellular parasites were removed by washing with PBS. Infected cell cultures were then incubated with different compounds concentrations at 37 °C for 72 h. Luminescence was measured using the Promega kit luciferase assay system (Promega, Madison, WI). Assays were performed in duplicate at least twice, to achieve a minimal n=3 per dose response.

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.⁴² 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 $\mu\text{L}/\text{min}/\text{mg}$ protein.

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) $\mu\text{L}/\text{min}/1$ million cells.

Calculated LogP and LogD values. Both LogP and LogD predictions are based on a modified version of the method⁴³ where the predicted partition coefficients are composed of the molecules' atomic increments.

Matrix stability. Stability in mouse matrix, CD-1 is determined at 37 °C. The assay is performed by adding 500 μ M compound solution to the matrix to achieve a final concentration of 2 μ M. Following incubation for 0, 0.25, 0.5, 1, 2, and 4 h, the samples are quenched and analyzed using LC/MS to determine the percentage of drug remaining.

Caco-2 permeability. The Caco-2 permeability of test compounds is measured using an established protocol at Charles River Labs. Concentration of test compounds is determined by measuring the peak area ratios at T=0 and 2 h on the apical and basolateral sides of the basal plate.

CYP induction. CYP induction is measured using established protocols at Charles River Labs. The assay is performed by adding the test compound to the positive or negative controls, incubating for 3 h at 37 °C, following determination of the fold-induction relative to the vehicle control by LC/MS/MS analysis.

CYP inhibition. CYP inhibition is measured using established protocols at Charles River Labs. A 50 mM stock solution was prepared and serially diluted (1.5-fold and 4-fold), this was then combined with a buffer/cofactor/substrate solution before combining with the human liver microsomes. The resulting mixture was incubated at 37 °C for 30 mins with gentle shaking before the percentage inhibition was calculated relative to zero inhibition by LC/MS/MS analysis.

For all experiments, compounds were marked as not tested if they were not run in an assay, and marked as not determined if they were run in an assay, but the result was inconclusive.

Pharmacokinetics of 20r and 20g following ip administration to female NMRI. Estimation of brain penetration

Compounds **20r** and **20g** were dosed to two groups of NMRI female mice animals (Group 1 and 2 n=3; Group 3 and 4 n=6) by intraperitoneal (IP) route: The dosing volume was 10 mL/kg for a total dose of 10 mg/kg and dosing formulation was prepared in 1% (v/v) Dimethyl sulfoxide (DMSO):99% (v/v) 20%

(w/v) sulfobutyl ether-beta-cyclodextrin (SBE- β -CD) (Captisol®) in water. Food and tap water was available *ad libitum*.

For Group 1 and 2 and following ip dosing of each compound, blood samples were collected from the tail vein into capillary tubes containing K2EDTA at the following time-points: 0.0833, 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post dose.

In order to obtain simultaneous blood and brain samples, mice included in group 3 and 4 were placed under terminal anaesthetic (isoflurane) and blood samples (0.3 mL) collected from the retro-orbital sinus into K2EDTA tubes at 0.5 h (n= 3) and 4 h (n=3) post intraperitoneal administration of **20r** (Group 3) and **20g** (Group 4). Immediately following blood sample collection death was confirmed by cervical dislocation and the brain removed.

Aliquots of each blood sample were diluted with an equal volume of water. Mouse brain samples were weighed, water was added at a 1/2, w/v ratio (brain/water), and then homogenized. Both, blood and brain samples were stored in a freezer set to maintain a temperature of -80 °C until analysis. Diluted blood and brain homogenates were processed under standard liquid-liquid extraction procedures using acetonitrile containing internal standard and analyzed by LC-MS/MS.

Non-compartmental analysis was performed using Phoenix pharmacokinetic software version 1.4 (Certara) and the main pharmacokinetic parameters were estimated.

Mouse efficacy study of acute HAT.

Six female NMRI mice (35-40 g weight from Charles River Laboratories) per group were infected by intraperitoneal (IP) injection with 10^4 bloodstream forms of *T. b. brucei* (STIB795) strain in 0.2 ml TDB glucose on Day 0. After three days, the infected mice were divided into two groups: control (infected mice treated with vehicle) and **20g** (infected mice treated with 10 mg/kg/day). On day 3, all mice received a 0.2 ml IP injection with 10 mg/kg/day **20g** or 5 % DMSO, 20 % Captisol for control group. This treatment was administered for five consecutive days. Parasitemia (measured as parasites per milliliter) was individually

checked by direct microscopic counting of parasites in a Neubauer chamber using 2 μ L of blood from mouse tail, suspending in 100 μ L of TDB glucose. Mortality was checked daily up to 30 days post infection and expressed as a percentage of cumulative mortality.

Chemistry Experimental

General methods

All starting materials were commercially obtained 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). NMR spectra were obtained on Varian NMR systems, operating at 400 or 500 MHz for ^1H acquisitions. LCMS analysis was performed using a Waters AllianceHT e2795 reverse phase HPLC (Waters SunFire C18 4.6×50 mm, $3.5 \mu\text{m}$ column), with a Waters 2996 photodiode array detector scanning from 210 nm to 600 nm, and Waters Micromass ZQ detector (electrospray ionization). Where required, final compounds were purified by preparative reverse phase HPLC (columns Waters Symmetry RP8 30×50 mm, $5 \mu\text{m}$ column, or OBD RP18 30×50 mm, $5 \mu\text{m}$), with a single wavelength UV–visible detector and Waters Micromass ZQ (electrospray ionization). All final compounds have purities greater than 95% based upon LCMS analysis. Melting points were obtained on a Thermo Fisher Mel-Temp apparatus. ^1H NMR spectra were obtained with Varian NMR systems, operating at either 400 or 500 MHz at room temperature, using solvents from Cambridge Isotope Laboratories. Chemical shifts (δ , ppm) are reported relative to the solvent peak (CDCl_3 : 7.26 [^1H]; $\text{DMSO-}d_6$: 2.50 [^1H]; Acetone- d_6 : 2.05; or CD_3OD : 3.31 [^1H]). Data for ^1H NMR spectra are reported as follows: chemical shift (ppm), multiplicity (s for singlet, d for doublet, t for triplet, dd for doublet of doublet, m for multiplet), coupling constant (Hz), and integration.

Chemical synthesis and characterization

3-((4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (1). In a vial **7a** (20 mg, 86 μmol), K_3PO_4 (26 mg, 121 μmol), XPhos (6 mg, 13 μmol), bis(dibenzylideneacetone)palladium(0) (4 mg, 4.3 μmol) and 3-aminobenzonitrile (20 mg, 173 μmol) were combined. The vial was sealed evacuated and back filled with N_2 three times. Degassed and anhydrous toluene (1 mL, dried over molecular sieves and stored under N_2) was added to a sealed vial. The reaction was left stirring overnight at 101 $^\circ\text{C}$. The reaction mixture which was heterogeneous was red at RT, turned brown and almost clear upon heating. After 16 h, the reaction mixture was evaporated to dryness to get a slurry which was extracted with DCM and washed with water and then dried over sodium sulfate, decanted and concentrated to get crude product which was purified by column chromatography using EtOAc: hex 40-100% 12 CV to afford the title compound as a colorless solid (15 mg, 55%). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ ppm 9.96 (s, 1 H) 9.11 - 9.18 (m, 1 H) 8.93 (s, 1 H) 8.64 (dd, $J=4.4, 2.0$ Hz, 1 H) 8.55 (d, $J=5.4$ Hz, 1 H) 8.38 (br. s, 1 H) 7.93 - 7.98 (m, 1 H) 7.55 (t, $J=8.1$ Hz, 1 H) 7.46 - 7.51 (m, 2 H) 7.43 (d, $J=7.8$ Hz, 1 H). LC-MS (m/z): 314.1 $[\text{M}+\text{H}]^+$.

3-Chloropyridazine (3). Compound **2** (470 mg, 4.7 mmol) and POCl_3 (4.5 mL, 49 mmol) were heated at 80 $^\circ\text{C}$ for 2 h. The heterogeneous mixture slowly turned clear and then a dark red/brown color. The reaction mixture was then cooled to room temperature, poured into water, neutralized slowly with sat. NaHCO_3 and extracted with DCM. The organic layers were washed with water, brine, dried over sodium sulfate, decanted and concentrated to afford the crude compound as a brown solid (360 mg, 64%). ^1H NMR (500 MHz, methanol- d_4) δ ppm 9.16 (dd, $J=4.9, 1.5$ Hz, 1 H) 7.84 (dd, $J=8.8, 1.5$ Hz, 1 H) 7.74 (dd, $J=8.8, 4.9$ Hz, 1 H). LC-MS (m/z): 114.4 (^{35}Cl), 116.5 (^{37}Cl) $[\text{M}+\text{H}]^+$.

4-(Pyridazin-3-yl)morpholine (4a). To a solution of **3** (360 mg, 3.1 mmol) in *n*-BuOH (5 mL) was added morpholine (0.62 mL, 7.8 mmol). The reaction mixture was heated to 80 $^\circ\text{C}$ for 16 h, then cooled to RT. Ethyl acetate was added to the reaction mixture and a solid precipitated from solution. The precipitate was

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3 filtered and filtrate was concentrated *in vacuo*, then purified by column chromatography using MeOH:
4 EtOAc, 0-20%, 12 CV to afford the product as a pale-yellow solid (430 mg, 90%). ¹H NMR (500 MHz,
5 DMSO-*d*₆) δ ppm 8.57 (dd, *J*=4.4, 1.0 Hz, 1 H) 7.39 (dd, *J*=9.3, 4.4 Hz, 1 H) 7.25 (dd, *J*=9.3, 1.0 Hz, 1 H)
6 3.71 (t, *J*=4.9 Hz, 4 H) 3.52 (t, *J*=4.9 Hz, 4 H). LC-MS (*m/z*): 165.8 [M+H]⁺.
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13 **3-Methoxypyridazine (4b).** Compound **3** (2.4 g, 21 mmol) was dissolved in MeOH (150 mL), sodium *t*-
14 butoxide (2.01 g, 21 mmol) was added and the reaction was heated to 65 °C for 16 h. All volatiles were
15 removed *in vacuo* and then the reaction mixture was taken up in EtOAc and MeOH, and filtered to give the
16 product in the filtrate. All volatiles were removed *in vacuo* to obtain the desired compound as a red oil (2.30
17 g, 99%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.87 (dd, *J*=4.4, 1.5 Hz, 1 H) 7.59 (dd, *J*=8.8, 4.4 Hz, 1 H)
18 7.19 (dd, *J*=8.8, 1.5 Hz, 1 H) 4.00 (s, 3 H). LC-MS (*m/z*): 110.5 [M+H]⁺.
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28 **2-Chloro-4-((trimethylsilyl)ethynyl)pyrimidine (6a).** In a vial **5** (500 mg, 3.36 mmol),
29 dichlorobistriphenylphosphine palladium (II) PdCl₂(PPh₃)₂ (47 mg, 67 μmol), and copper (I) iodide (19 mg,
30 100 μmol) were added. The vial was evacuated and backfilled with N₂ three times. To this a degassed
31 mixture of triethylamine (1.40 mL, 10.1 mmol) and THF (20 mL) was added followed by
32 ethynyltrimethylsilane (0.525 mL, 3.69 mmol). The resultant solution was stirred at 50 °C overnight. The
33 reaction mixture turned from yellow/orange in color to dark brown after addition of TMS-acetylene. Upon
34 completion, reaction mixture was concentrated to give a dark slurry which was extracted with DCM and
35 washed with water (3 x 50 mL) followed by brine, the organic layer was dried using Na₂SO₄, decanted and
36 concentrated to give the crude product. Column purification was performed using EtOAc: hex, 5% to 50%
37 12 CV, 50-100% 5 CV to afford the product as a white solid (425 mg, 61%). ¹H NMR (500 MHz,
38 chloroform-*d*) δ ppm 8.55 (d, *J*=4.9 Hz, 1 H) 7.28 (d, *J*=4.9 Hz, 1 H) 0.23 (s, 9 H). LC-MS (*m/z*): 210.9
39 (³⁵Cl), 212.9 (³⁷Cl) [M+H]⁺.
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2-Chloro-4-ethynylpyrimidine (6b). A solution of **6a** (390 mg, 1.85 mmol) in methanol (10 mL) was treated with half of a stock solution of KOH (0.519 mg, 9.25 μ mol) in 1 mL MeOH. After 30 min, another half of KOH solution was added and the mixture was stirred at RT, total consumption of the reactant was observed. The mixture was concentrated to get crude product as buff colored solid. Column purification done using EtOAc: Hexane: 10-100%, 15 CV to yield the desired compound as a buff solid (200 mg, 78%). ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.64 (d, $J=4.9$ Hz, 1 H) 7.37 (d, $J=4.9$ Hz, 2 H) 3.48 (s, 1 H). LC-MS (*m/z*): 138.6 (^{35}Cl), 140.6 (^{37}Cl) [$\text{M}+\text{H}$] $^+$.

2-Chloro-4-((4-(trifluoromethyl)phenyl)ethynyl)pyrimidine (6c). In a vial **5** (200 mg, 1.34 mmol), dichlorobis(triphenylphosphine) palladium (II) $\text{PdCl}_2(\text{PPh}_3)_2$ (19 mg, 27 μ mol), triethylamine (0.6 mL, 4.03 mmol) and copper (I) iodide (8 mg, 40 μ mol) were added followed by 1-ethynyl-4-(trifluoromethyl)benzene (0.244 mL, 1.48 mmol). A degassed mixture of triethylamine (0.6 mL, 4.03 mmol) and THF (5 mL) was then added. The resultant solution was stirred at 50 $^\circ\text{C}$ overnight. After completion of reaction column purification was done using EtOAc: hex, 5% to 50% 12 CV, 50-100% 5 CV to isolate the desired compound as an orange solid (191 mg, 50%). ^1H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.89 (d, $J=5.4$ Hz, 1 H) 7.85 - 7.95 (m, 5 H). LC-MS (*m/z*): 283.0 (^{35}Cl), 285.0 (^{37}Cl) [$\text{M}+\text{H}$] $^+$.

2-Chloro-4-(cyclopropylethynyl)pyrimidine (6d). In a reaction vial **5** (1.0 g, 6.7 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (94 mg, 134 μ mol), and CuI (38 mg, 201 μ mol) were added. A degassed solution of triethylamine (2.81 mL, 20.1 mmol) and THF (12 mL) was added under N_2 to the reaction vial, followed by ethynylcyclopropane (625 μL , 7.38 mmol). The reaction mixture was heated to 50 $^\circ\text{C}$ for 16 h. The reaction mixture was then filtered through celite and washed with EtOAc. The reaction mixture was concentrated *in vacuo* then subjected to column purification EtOAc: hexanes 0-25%, to afford the desired compound as an orange solid (1.03 g, 86%). ^1H NMR (400 MHz, chloroform-*d*) δ ppm 8.52 (d, $J=5.1$ Hz, 1 H) 7.20 (d, $J=5.1$ Hz, 1 H) 1.47 - 1.56 (m, 1 H) 0.92 - 1.06 (m, 4 H). LC-MS (*m/z*): 178.9 (^{35}Cl), 180.8 (^{37}Cl) [$\text{M}+\text{H}$] $^+$.

2-Chloro-4-((4-methoxyphenyl)ethynyl)pyrimidine (6e). In a vial, **5** (0.3 g, 2.01 mmol), dichlorobis(triphenylphosphine) palladium (II) $\text{PdCl}_2(\text{PPh}_3)_2$ (28 mg, 40 μmol), and copper (I) iodide (11.5 mg, 60 μmol) were added, followed by a degassed mixture of triethylamine (0.84 mL) and THF (5 mL) and then 1-ethynyl-4-methoxybenzene (293 μL , 2.2 mmol) was added. The resultant solution was stirred at 50 °C overnight. The yellow/orange reaction mixture turned dark brown after addition of TMS-acetylene and on heating. The mixture was evaporated to dryness and then slurry obtained was dissolved in DCM. The organic layer was washed with water and brine then dried over sodium sulfate, decanted and concentrated to get crude product which was purified by column chromatography using EtOAc: hex, 5% to 50% 12 CV, 50-100% 5 CV to afford the title compound as a brown solid (0.4 g, 86%). ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.59 (d, $J=5.4$ Hz, 1 H) 7.56 - 7.60 (m, 2 H) 7.36 (d, $J=5.4$ Hz, 1 H) 6.91 - 6.95 (m, 2 H) 3.86 (s, 3 H). LC-MS (m/z): 245.0 (^{35}Cl), 247.0 (^{37}Cl) $[\text{M}+\text{H}]^+$.

4-(2-Chloropyrimidin-4-yl)-2-methylbut-3-yn-2-ol (6f). In a reaction vial **5** (2 g, 13.36 mmol), 2-methylbut-3-yn-2-ol (1.56 mL, 16 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (94 mg, 134 μmol) and CuI (51 mg, 269 μmol) were added. A degassed solution of TEA (9.4 mL) and THF (50 mL) was added under N_2 to the reaction vial. The reaction mixture was heated to 60 °C for 16 h. The reaction was then poured into water, and extracted with DCM. The organic layer was washed with water and brine then dried over sodium sulfate, decanted, concentrated to get crude product, which was purified by column chromatography using EtOAc: hex: 30-100% 15 CV affording the desired compound as a yellow solid (1.60 g, 60%). ^1H NMR (500 MHz, DMSO- d_6) δ ppm 8.78 (d, $J=5.4$ Hz, 1 H) 7.61 (d, $J=5.4$ Hz, 1 H) 5.79 (s, 1 H) 1.48 (s, 6 H). LC-MS (m/z): 196.9 (^{35}Cl), 198.9 (^{37}Cl) $[\text{M}+\text{H}]^+$.

3-(2-Chloropyrimidin-4-yl)prop-2-yn-1-ol (6g). In a reaction vial **5** (1.0 g, 6.7 mmol), $\text{Pd}(\text{PPh}_3)_2\text{Cl}_2$ (94 mg, 134 μmol), and CuI (38 mg, 201 μmol) were added. A degassed solution of triethylamine (3.74 mL, 26.9 mmol) and THF (20 mL) was added under N_2 to the reaction vial, followed by propargyl alcohol (464 μL , 8.06 mmol). The reaction mixture was heated to 50 °C for 16 h. The reaction was then filtered through

celite and washed with methanol. The reaction mixture was concentrated *in vacuo* then subjected to column purification MeOH: DCM 0-5%, to yield the desired compound as a brown solid (420 mg, 37%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.79 (d, *J*=5.4 Hz, 1 H) 7.64 (d, *J*=5.4 Hz, 1 H) 5.60 (d, *J*=5.9 Hz, 1 H) 4.39 (t, *J*=5.9 Hz, 2 H). LC-MS (*m/z*): 168.8 (³⁵Cl), 170.7 (³⁷Cl) [M+H]⁺.

2-Chloro-4-(3-methoxyprop-1-yn-1-yl)pyrimidine (6h). In a reaction vial **5** (500 mg, 3.36 mmol), Pd(PPh₃)₂Cl₂ (47 mg, 67 μmol), and CuI (19 mg, 101 μmol) were added. A degassed solution of triethylamine (1.87 mL, 13.4 mmol) and THF (10 mL) was added under N₂ to the reaction vial, followed by 3-methoxyprop-1-yne (340 μL, 4.03 mmol). The reaction mixture was heated to 50 °C for 16 h. The reaction mixture was then filtered through celite and concentrated *in vacuo* and subjected to column purification EtOAc: hexanes 10-50%, to afford the title compound as a yellow solid (183 mg, 30%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.62 (d, *J*=5.4 Hz, 1 H) 7.33 (d, *J*=5.4 Hz, 1 H) 4.37 (s, 2 H) 3.47 (s, 3 H). LC-MS (*m/z*): 182.8 (³⁵Cl), 184.8 (³⁷Cl) [M+H]⁺.

4-(2-Chloropyrimidin-4-yl)but-3-yn-2-ol (6i). In a reaction vial **5** (500 mg, 3.4 mmol), Pd(PPh₃)₂Cl₂ (47 mg, 67 μmol), and CuI (19 mg, 100 μmol) were added. A degassed solution of triethylamine (1.9 mL, 13 mmol) and THF (10 mL) was added under N₂ to the reaction vial, followed by but-3-yn-2-ol (320 μL, 4.0 mmol). The reaction mixture was heated to 50 °C for 16 h. The reaction mixture was then filtered through celite and washed with MeOH, then concentrated *in vacuo* and subjected to column purification EtOAc: hexanes 20-50%, to yield the desired compound as a red oil (292 mg, 48%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.61 (d, *J*=4.9 Hz, 1 H) 7.31 (d, *J*=4.9 Hz, 1 H) 4.76 - 4.85 (m, 1 H) 1.59 (d, *J*=6.4 Hz, 3 H). LC-MS (*m/z*): 182.9 (³⁵Cl), 184.8 (³⁷Cl) [M+H]⁺.

2-Chloro-4-(3-methylbut-1-yn-1-yl)pyrimidine (6j). In a reaction vial **5** (500 mg, 3.36 mmol), Pd(PPh₃)₂Cl₂ (47 mg, 67 μmol), and CuI (19 mg, 101 μmol) were added. A degassed solution of triethylamine (1.87 mL, 13.4 mmol) and THF (10 mL) was added under N₂ to the reaction vial, followed

by 3-methylbut-1-yne (411 μ L, 4.03 mmol). The reaction mixture was heated to 50 $^{\circ}$ C for 16 h. The reaction mixture was then filtered through celite and washed with EtOAc and methanol, then concentrated *in vacuo* and subjected to column purification EtOAc: hexanes 0-25%, to afford the title compound as a yellow solid (487.1 mg, 80%). ^1H NMR (400 MHz, chloroform-*d*) δ ppm 8.55 (d, J =5.1 Hz, 1 H) 7.25 (d, J =5.1 Hz, 1 H) 2.76 - 2.94 (m, 1 H) 1.30 (d, J =7.3 Hz, 6 H). LC-MS (m/z): 180.9 (^{35}Cl), 182.8 (^{37}Cl) [$\text{M}+\text{H}$] $^{+}$.

3-(2-Chloropyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazine (7a). (Aminooxy) sulfonic acid (906 mg, 8.01 mmol) was dissolved in water (5 mL), to the clear colorless solution 2.5 M sodium bicarbonate was added until effervescence ceased (pH \sim 5). The clear colorless solution was heated at 70 $^{\circ}$ C and pyridazine (0.385 mL, 5.34 mmol) was added then reaction was stirred at 70 $^{\circ}$ C for 2 h. The reaction mixture then cooled to RT, neutralized with 2.5 M sodium bicarbonate (until effervescence ceased) and then solution of **6b** (185 mg, 1.34 mmol) in DCM (10 mL) was added followed by KOH (22 mg, 3.9 mmol). Reaction mixture was stirred overnight at RT. After completion the reaction mixture was extracted in DCM, washed with water, and brine then dried over sodium sulfate, decanted and concentrated to get crude product. Column purification was performed using EtOAc: hex 30-100% 15 CV to afford the compound as a red solid (260 mg, 84%). ^1H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.04 (s, 1 H) 8.90 (dd, J =8.8, 2.0 Hz, 1 H) 8.73 (d, J =5.4 Hz, 1 H) 8.69 (dd, J =4.4, 2.0 Hz, 1 H) 8.07 (d, J =5.4 Hz, 1 H) 7.58 (dd, J =8.8, 4.4 Hz, 1 H). LC-MS (m/z): 231.9 (^{35}Cl), 234.0 (^{37}Cl) [$\text{M}+\text{H}$] $^{+}$.

4-(3-(2-Chloropyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazin-6-yl)morpholine (7b). (Aminooxy) sulfonic acid (421 mg, 3.72 mmol) was dissolved in 3 mL water, to the clear solution added 2.5 M sodium bicarbonate (376 mg, 4.47 mmol) until effervescence ceased (pH \sim 5). The clear colorless solution was heated at 70 $^{\circ}$ C and **4a** (462 mg, 2.79 mmol) was added and the reaction stirred at 70 $^{\circ}$ C for 2 h. The reaction mixture was then cooled to RT, neutralized with 2.5 M sodium bicarbonate (until effervescence ceased). A solution of **6b** (129 mg, 931 μ mol) in DCM (10 mL) was added followed by KOH (104 mg, 1.9 mmol) and

the reaction mixture was stirred at RT for 16 h. DCM layer turned dark pink. Reaction mixture was then extracted in into DCM, and the organic layer was washed with water, and brine then dried over sodium sulfate, decanted and concentrated to get crude product, which was purified by column chromatography using EtOAc: hex, 20-100% 15 CV, to obtain the desired compound as a white solid (160 mg, 5%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.77 (d, *J*=9.8 Hz, 1 H) 8.51 (d, *J*=5.4 Hz, 1 H) 8.29 (s, 1 H) 7.45 (d, *J*=5.4 Hz, 1 H) 7.03 (d, *J*=9.8 Hz, 1 H) 3.88 (t, *J*=4.9 Hz, 4 H) 3.62 (t, *J*=4.9 Hz, 4 H). LC-MS (m/z): 317.0 (Cl³⁵), 318.9 (Cl³⁷) [M+H]⁺.

3-(2-Chloropyrimidin-4-yl)-6-methoxypyrazolo[1,5-*b*]pyridazine (7c). (Aminooxy) sulfonic acid (1.90 g, 16.8 mmol) was dissolved in 4 mL water, to the clear solution added saturated sodium bicarbonate till effervescence ceased (pH ~ 6). The clear colorless solution was heated at 70 °C and **4b** (1.24 g, 11.2 mmol) was added in 20 mL of water and the reaction mixture was stirred at 70 °C for 2 h. The reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then solution of **6b** (622 mg, 4.49 mmol) in DCM (80 mL) and KOH (252 mg, 4.49 mmol) were added and the reaction mixture was stirred at RT for 16 h. The reaction mixture was diluted with DCM and water and extracted. The organic layer was washed with water, and brine then dried over sodium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 20-50% to provide the desired product as a brown solid (496 mg, 42%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.82 (s, 1 H) 8.73 (d, *J*=9.3 Hz, 1 H) 8.70 (d, *J*=5.4 Hz, 1 H) 8.01 (d, *J*=5.4 Hz, 1 H) 7.27 (d, *J*=9.3 Hz, 1 H) 4.02 (s, 3 H). LC-MS (m/z): 262.0 (³⁵Cl), 264.1 (³⁷Cl) [M+H]⁺.

3-(2-Chloropyrimidin-4-yl)-2-(4-(trifluoromethyl)phenyl)pyrazolo[1,5-*b*]pyridazine (7d). (Aminooxy) sulfonic acid (559 mg, 4.95 mmol) was dissolved in 5 mL water, to the clear solution added 2.5 M sodium bicarbonate until effervescence ceased (pH ~ 5). The clear colorless solution was heated to 70 °C and pyridazine (0.24 mL, 3.30 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. Reaction mixture was cooled to RT, neutralized with 2.5 M sodium bicarbonate (until effervescence

ceased) then solution of **6c** (223 mg, 824 μ mol) in DCM (4 mL) and KOH (138 mg, 2.46 mmol) was added and the reaction mixture was then stirred overnight at RT. After 16 h reaction mixture was extracted in DCM, washed the DCM layer with water, brine and dried over sodium sulfate, decanted and concentrated to get crude product which was purified by column chromatography using EtOAc: hex, 10-100% 15 CV to afford the title compound as a bronze solid (150 mg, 47%). ^1H NMR (500 MHz, $\text{DMSO}-d_6$) δ ppm 8.78 (dd, $J=9.3$, 2.0 Hz, 1 H) 8.74 (dd, $J=4.4$, 2.0 Hz, 1 H) 8.62 (d, $J=5.4$ Hz, 1 H) 7.91 (s, 4 H) 7.60 (dd, $J=9.3$, 4.4 Hz, 1 H) 7.23 (d, $J=5.4$ Hz, 1 H). LC-MS (m/z): 314.9 (^{35}Cl), 317.0 (^{37}Cl) $[\text{M}+\text{H}]^+$.

3-(2-Chloropyrimidin-4-yl)-2-cyclopropylpyrazolo[1,5-*b*]pyridazine (7e). (Aminooxy) sulfonic acid (1.63 g, 14.4 mmol) was dissolved in 4 mL water, saturated sodium bicarbonate was added to the clear solution until effervescence ceased (pH \sim 6). The clear colorless solution was heated to 70 $^\circ\text{C}$ and pyridazine (693 μL , 9.60 mmol) was added and the reaction mixture was stirred at 70 $^\circ\text{C}$ for 2 h. The reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased), then solution of **6d** (429 mg, 2.4 mmol) in DCM (7 mL) was added followed by KOH (135 mg, 2.4 mmol) and the reaction was stirred for 16 h at RT, partial conversion to product was observed so additional KOH (270 mg, 4.8 mmol) was added and the reaction was stirred for another 8 h. After 8 h another 2 eq of KOH (270 mg, 4.8 mmol) was added and the reaction continued stirring for an additional 16 h. The reaction mixture was diluted with DCM and washed with water and brine then dried over magnesium sulfate, filtered and evaporated to get the crude product which was purified by column chromatography using EtOAc: hex 20-50% and providing the desired compound as a pale-yellow solid (472.5 mg, 72%). ^1H NMR (500 MHz, $\text{chloroform}-d$) δ ppm 8.92 (dd, $J=8.8$, 2.0 Hz, 1 H) 8.61 (d, $J=5.4$ Hz, 1 H) 8.38 (dd, $J=4.4$, 2.0 Hz, 1 H) 7.87 (d, $J=5.4$ Hz, 1 H) 7.24 (dd, $J=8.8$, 4.4 Hz, 1 H) 2.23 - 2.31 (m, 1 H) 1.29 - 1.35 (m, 2 H) 1.17 - 1.23 (m, 2 H). LC-MS (m/z): 272.1 (^{35}Cl), 274.1 (^{37}Cl) $[\text{M}+\text{H}]^+$.

3-(2-Chloropyrimidin-4-yl)-2-(4-methoxyphenyl)pyrazolo[1,5-*b*]pyridazine (7f). (Aminooxy) sulfonic acid (1.11 g, 9.81 mmol) was dissolved in 5 mL water, to the clear solution 2.5 M sodium bicarbonate was

added until effervescence ceased (pH ~ 5). The clear colorless solution was heated to 70 °C and pyridazine (0.471 mL, 6.54 mmol) was added, then the reaction mixture was stirred at 70 °C for 2 h. The reaction cooled to RT, neutralized with 2.5 M sodium bicarbonate (until effervescence ceased) and then solution of **6e** (0.4 g, 1.63 mmol) in DCM (20 mL) was added followed by KOH (275 mg, 4.9 mmol). The reaction mixture was stirred at RT for 16 h then diluted with DCM, washed with water and brine then dried over sodium sulfate, decanted and concentrated to get crude product, which was purified by column chromatography using EtOAc: hex, 10-100% 15 CV to provide the title compound as an off-white solid (35 mg, 46% based on recovered starting material). Recovered 345 mg of unreacted starting material. ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.96 (dd, *J*=8.8, 2.0 Hz, 1 H) 8.46 (dd, *J*=4.9, 2.0 Hz, 1 H) 8.34 (d, *J*=5.4 Hz, 1 H) 7.58 - 7.61 (m, 2 H) 7.13 (d, *J*=5.4 Hz, 1 H) 7.02 - 7.05 (m, 2 H) 6.89 - 6.95 (m, 1 H) 3.91 (s, 3 H). LC-MS (*m/z*): 338.0 (³⁵Cl), 339.9 (³⁷Cl) [M+H]⁺.

2-(3-(2-Chloropyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazin-2-yl)propan-2-ol (7g). (Aminooxy) sulfonic acid (280 mg, 2.47 mmol) was dissolved in 5 mL water, to the clear solution 2.5 M sodium bicarbonate was added until effervescence ceased (pH ~ 5). The clear colorless solution was heated to 70 °C and pyridazine (119 μL, 1.65 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction was then cooled to RT, neutralized with 2.5 M sodium bicarbonate (until effervescence ceased) and then solution of **6f** (81 mg, 412 μmol) in DCM (10 mL) was added followed by KOH (69 mg, 1.2 mmol) and the reaction mixture was stirred at RT. After 16 h the reaction mixture was diluted with DCM, and washed with water and brine then dried over sodium sulfate, decanted and concentrated to get crude product which was purified by column chromatography using EtOAc: hex 30-100% 15 CV to afford the title compound as a brown solid (47 mg, 40%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.77 (d, *J*=5.4 Hz, 1 H) 8.73 (dd, *J*=9.3, 1.5 Hz, 1 H) 8.62 (dd, *J*=4.4, 1.5 Hz, 1 H) 8.54 (d, *J*=5.4 Hz, 1 H) 7.49 (dd, *J*=9.3, 4.4 Hz, 1 H) 5.95 (s, 1 H) 1.64 (s, 6 H). LC-MS (*m/z*): 290.0 (³⁵Cl), 292.1 (³⁷Cl) [M+H]⁺.

(3-(2-chloropyrimidin-4-yl)pyrazolo[1,5-b]pyridazin-2-yl)methanol (7h). (Aminooxy) sulfonic acid (450 mg, 4.0 mmol) was dissolved in 3 mL water, to the clear solution 2.5 M sodium bicarbonate was added until effervescence ceased (pH ~ 5). The clear colorless solution was heated to 70 °C and pyridazine (190 µL, 2.7 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction was then cooled to RT, neutralized with 2.5 M sodium bicarbonate (until effervescence ceased) and then solution **6g** (110 mg, 670 µmol) in DCM (5 mL) was added followed by KOH (40 mg, 670 µmol) and the reaction mixture was stirred at RT. After 16 h the reaction mixture was diluted with DCM, and washed with water and brine then dried over sodium sulfate. The reaction was filtered and evaporated *in vacuo* then subjected to column purification using MeOH: DCM 2% to afford the title compound as an orange solid (34 mg, 20%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.87 (dd, *J*=8.8, 2.0 Hz, 1 H) 8.78 (d, *J*=5.4 Hz, 1 H) 8.67 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.05 (d, *J*=5.4 Hz, 1 H) 7.56 (dd, *J*=8.8, 4.4 Hz, 1 H) 5.76 (t, *J*=5.4 Hz, 1 H) 4.89 (d, *J*=5.4 Hz, 1 H). LC-MS (*m/z*): 262.0 (³⁵Cl), 264.0 (³⁷Cl) [M+H]⁺.

3-(2-Chloropyrimidin-4-yl)-2-(methoxymethyl)pyrazolo[1,5-b]pyridazine (7i). (Aminooxy) sulfonic acid (680 mg, 6.01 mmol) was dissolved in 5 mL water, saturated sodium bicarbonate was added to the clear solution until effervescence ceased (pH ~ 6). The clear colorless solution was heated at 70 °C and pyridazine (289 µL, 4.01 mmol) was added, and the reaction was stirred at 70 °C for 2 h. Reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then a solution of **6h** (183 mg, 1.0 mmol) in DCM (10 mL) was added followed by KOH (112 mg, 2.0 mmol) and the reaction was stirred for 16 h at RT. The reaction mixture was extracted in DCM washed with water and brine then dried over magnesium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 20-50% to yield the title compound as a white solid (229 mg, 83%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.86 (dd, *J*=9.3, 2.0 Hz, 1 H) 8.79 (d, *J*=5.4 Hz, 1 H) 8.69 (dd, *J*=4.4, 2.0 Hz, 1 H) 7.85 (d, *J*=5.4 Hz, 1 H) 7.57 (dd, *J*=9.3, 4.4 Hz, 1 H) 4.86 (s, 2 H) 3.37 (s, 3 H). LC-MS (*m/z*): 276.0 (³⁵Cl), 278.0 (³⁷Cl) [M+H]⁺.

1-(3-(2-Chloropyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazin-2-yl)ethan-1-ol (7j). (Aminooxy) sulfonic acid (1.1 g, 9.6 mmol) was dissolved in 5 mL water, saturated sodium bicarbonate was added to the clear solution until effervescence ceased (pH ~ 6). The clear colorless solution was heated at 70 °C and pyridazine (462 μL, 6.4 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. Reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then a solution of **6i** (292 mg, 1.6 mmol) in DCM (15 mL) was added followed by KOH (180 mg, 3.2 mmol) and the reaction was stirred for 16 h at RT. The reaction mixture was diluted with DCM, and washed with water and brine then dried over magnesium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 45-80% to provide the title compound as a yellow solid (290 mg, 66%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.81 (d, *J*=9.3 Hz, 1 H) 8.76 (d, *J*=5.4 Hz, 1 H) 8.63 - 8.67 (m, 1 H) 8.18 (d, *J*=5.4 Hz, 1 H) 7.52 (dd, *J*=9.3, 4.4 Hz, 1 H) 5.76 (d, *J*=6.4 Hz, 1 H) 5.25 (quin, *J*=6.4 Hz, 1 H) 1.62 (d, *J*=6.4 Hz, 3 H). LC-MS (*m/z*): 276.0 (³⁵Cl), 278.0 (³⁷Cl) [M+H]⁺.

3-(2-Chloropyrimidin-4-yl)-2-isopropylpyrazolo[1,5-*b*]pyridazine (7k). (Aminooxy) sulfonic acid (1.83 g, 16.2 mmol) was dissolved in 5 mL water, saturated sodium bicarbonate was added to the clear solution until effervescence ceased (pH ~ 6). The clear colorless solution was heated to 70 °C and pyridazine (778 μL, 10.8 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then solution of **6j** (487 mg, 2.7 mmol) in DCM (7 mL) was added followed by KOH (151 mg, 2.70 mmol) and the reaction was stirred for 16 h at RT, at which point more KOH (302 mg, 5.4 mmol) was added and stirring was continued for another 3 h. The reaction mixture was diluted with DCM was washed with water and brine, then dried over magnesium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 20-50% to afford the title compound as a yellow solid (471 mg, 64%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.87 (d, *J*=9.3 Hz, 1 H) 8.60 (d, *J*=5.4 Hz, 1 H) 8.39 (d, *J*=4.4 Hz, 1 H) 7.47 (d, *J*=5.4 Hz, 1 H) 7.24 (dd, *J*=9.3, 4.4 Hz, 1 H) 3.54 - 3.64 (m, 1 H) 1.51 (d, *J*=6.4 Hz, 6 H). LC-MS (*m/z*): 274.1 (³⁵Cl), 276.0 (³⁷Cl) [M+H]⁺.

(3-(2-Chloropyrimidin-4-yl)-6-methoxypyrazolo[1,5-*b*]pyridazin-2-yl)methanol (7l). (Aminooxy)

sulfonic acid (909 mg, 8.0 mmol) was dissolved in 4 mL water, saturated sodium bicarbonate was added to the clear solution until effervescence ceased (pH ~ 6). The clear colorless solution was heated at 70 °C and **4b** (590 mg, 5.36 mmol) was added then the reaction was stirred at 70 °C for 2 h. The reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then a solution of **6g** (452 mg, 2.68 mmol) in DCM (15 mL) was added followed by KOH (150 mg, 2.7 mmol) and the reaction mixture was stirred for 16 h at RT. The reaction mixture was diluted with DCM and washed with water and brine then dried over sodium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 25-50% to afford the title compound as an orange solid (265 mg, 34%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.76 (d, *J*=5.4 Hz, 1 H) 8.72 (d, *J*=9.8 Hz, 1 H) 8.01 (d, *J*=5.4 Hz, 1 H) 7.25 (d, *J*=9.8 Hz, 1 H) 5.69 (t, *J*=5.4 Hz, 1 H) 4.81 (d, *J*=5.4 Hz, 2 H) 4.00 (s, 3 H). LC-MS (m/z): 292.0 (³⁵Cl), 294.0 (³⁷Cl) [M+H]⁺.

(3-(2-Chloropyrimidin-4-yl)-6-morpholinopyrazolo[1,5-*b*]pyridazin-2-yl)methanol (7m). (Aminooxy)

sulfonic acid (590 mg, 5.2 mmol) was dissolved in 5 mL water, saturated sodium bicarbonate was added to the clear solution until effervescence ceased (pH ~ 6). The clear colorless solution was heated to 70 °C and **4a** (570 mg, 3.5 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. Reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then solution of **6g** (290 mg, 5.2 mmol) in DCM (15 mL) was added followed by KOH (98 mg, 1.7 mmol) and the reaction mixture was stirred for 16 h at RT. The reaction mixture was diluted with DCM and washed with water and brine then dried over sodium sulfate, filtered and evaporated to get crude product which was purified by column chromatography run isocratically 100% EtOAc, to afford the title compound as a yellow solid (88.1 mg, 15%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.71 (d, *J*=5.4 Hz, 1 H) 8.58 (d, *J*=9.8 Hz, 1 H) 7.97 (d, *J*=5.4 Hz, 1 H) 7.52 (d, *J*=9.8 Hz, 1 H) 5.62 (t, *J*=5.4 Hz, 1 H) 4.78 (d, *J*=5.4 Hz, 2 H) 3.76 (t, *J*=4.9 Hz, 4 H) 3.52 (t, *J*=4.9 Hz, 4 H). LC-MS (m/z): 347.1 (³⁵Cl), 349.0 (³⁷Cl) [M+H]⁺.

3-(2-Chloropyrimidin-4-yl)-2-cyclopropyl-6-methoxypyrazolo[1,5-*b*]pyridazine (7n). (Aminooxy) sulfonic acid (380 mg, 3.36 mmol) was dissolved in 4 mL water, to the clear solution saturated sodium bicarbonate was added until effervescence ceased (pH ~ 6). The clear colorless solution was heated to 70 °C and **4b** (247 mg, 2.24 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then a solution of 2-chloro-4-(cyclopropylethynyl)pyrimidine (200 mg, 1.1 mmol) in DCM (10 mL) was added followed by KOH (63 mg, 1.1 mmol) and the reaction was stirred for 16 h at RT, partial conversion to product was observed so additional KOH (63 mg, 1.1 mmol) was added and the reaction was stirred for another 24 h. The reaction mixture was diluted with DCM and washed with water and brine, then dried over sodium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 10-30% to yield the desired compound as a white solid (99.1 mg, 29%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.73 (d, *J*=5.4 Hz, 1 H) 8.63 (d, *J*=9.8 Hz, 1 H) 7.99 (d, *J*=5.4 Hz, 1 H) 7.20 (d, *J*=9.8 Hz, 1 H) 3.98 (s, 3 H) 2.40 - 2.46 (m, 1 H) 1.09 - 1.14 (m, 2 H) 1.00 - 1.05 (m, 2 H). LC-MS (m/z): 302.1 (³⁵Cl), 304.1 (³⁷Cl) [M+H]⁺.

4-(3-(2-Chloropyrimidin-4-yl)-2-cyclopropylpyrazolo[1,5-*b*]pyridazin-6-yl)morpholine (7o). (Aminooxy) sulfonic acid (439 mg, 3.89 mmol) was dissolved in 4 mL water, saturated sodium bicarbonate was added to the clear solution until effervescence ceased (pH ~ 5). The clear colorless solution was heated to 70 °C and **4a** (428 mg, 2.59 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then a solution of **6d** (231 mg, 1.3 mmol) in DCM (20 mL) was added followed by KOH (73 mg, 1.3 mmol) and the reaction was stirred for 16 h at RT. The reaction mixture was diluted with DCM and washed with water and brine then dried over magnesium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 10-50% to provide the desired compound as a yellow solid (183.7 mg, 40%). ¹H NMR (400 MHz, chloroform-*d*) δ ppm 8.71 (d, *J*=9.5 Hz,

1 H) 8.55 (d, $J=5.1$ Hz, 1 H) 7.84 (d, $J=5.1$ Hz, 1 H) 6.98 (d, $J=9.5$ Hz, 1 H) 3.86 (t, $J=5.1$ Hz, 4 H) 3.58 (t, $J=5.1$ Hz, 4 H) 2.13 - 2.23 (m, 1 H) 1.20 - 1.28 (m, 2 H) 1.08 - 1.18 (m, 2 H). LC-MS (m/z): 357.1 (^{35}Cl), 359.0 (^{37}Cl) $[\text{M}+\text{H}]^+$.

***N*-(4-Chlorobenzyl)-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (10a).** In a reaction vial **7a** (20 mg, 86 μmol) was stirred in *n*-BuOH (1 mL), and 4-chlorobenzylamine (25 mg, 170 μmol) was added. The reaction mixture was stirred at 115 $^{\circ}\text{C}$ for 16 h. The solution changed from a red/brown color to clear yellow on heating. Upon completion of the reaction, the mixture was concentrated and taken up in DCM and washed with water and brine, then dried over sodium sulfate, decanted and concentrated to get crude product, which was purified by column chromatography using EtOAc: hexane 20-100%, 12 CV to yield the product as a colorless solid (7 mg, 24%). ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.46 (s, 1 H) 8.35 (dd, $J=4.2$, 1.7 Hz, 1 H) 8.31 (d, $J=5.4$ Hz, 1 H) 7.30 - 7.41 (m, 4 H) 6.99 - 7.07 (m, 1 H) 6.94 (d, $J=5.4$ Hz, 1 H) 5.54 - 5.68 (m, 1 H) 4.70 (d, $J=5.9$ Hz, 2 H). LC-MS (m/z): 337.0 (^{35}Cl), 339.1 (^{37}Cl) $[\text{M}+\text{H}]^+$.

***N*-(3-Morpholinopropyl)-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (10b).** In a microwave vial containing **7a** (30 mg, 129 μmol), EtOH (1 mL) was added followed by 3-morpholinopropan-1-amine (37 mg, 260 μmol) and the reaction mixture was subjected to microwave irradiation at 150 $^{\circ}\text{C}$ for 15 min. Upon completion of the reaction, the reaction mixture was concentrated and taken up in DCM which was washed with water and brine then dried over sodium sulfate, decanted and concentrated to get crude product, which was purified by column chromatography using EtOAc: MeOH 0-20%, 12 CV to yield the product as a dark yellow solid (15 mg, 34%). ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.97 (dd, $J=9.3$, 2.0 Hz, 1 H) 8.45 (s, 1 H) 8.37 (dd, $J=4.4$, 2.0 Hz, 1 H) 8.27 (d, $J=4.9$ Hz, 1 H) 7.16 (dd, $J=9.3$, 4.4 Hz, 1 H) 6.86 (d, $J=4.9$ Hz, 1 H) 6.01 (br. s, 1 H) 3.79 (t, $J=4.2$ Hz, 4 H) 3.59 (q, $J=6.3$ Hz, 2 H) 2.57 - 2.65 (m, 6 H) 1.91 (quin, $J=6.4$ Hz, 2 H). LC-MS (m/z): 340.1 $[\text{M}+\text{H}]^+$.

***N*-Cyclopropyl-4-(6-morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (10d).** In a vial **7b** (15 mg, 47 μ mol), and cyclopropylamine (1.0 mL, 14 mmol) were stirred in 1 mL of *n*-BuOH. Reaction mixture was subjected to microwave irradiation at 150 $^{\circ}$ C for 1 h. The reaction mixture was then evaporated to dryness and column purification performed using EtOAc: hex 50-100% 12 CV followed by EtOAc: MeOH 1-20% 5 CV to afford the title compound as a brown solid (8.9 mg, 55%). ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.83 - 8.91 (m, 1 H) 8.28 (d, J =4.9 Hz, 1 H) 8.23 (s, 1 H) 6.92 (d, J =9.3 Hz, 1 H) 6.90 (d, J =4.9 Hz, 1 H) 5.38 (br. s., 1 H) 3.87 (t, J =4.9 Hz, 4 H) 3.59 (t, J =4.9 Hz, 4 H) 2.82 - 2.87 (m, 1 H) 0.85 - 0.90 (m, 2 H) 0.62 - 0.66 (m, 2 H). LC-MS (m/z): 338.1 [$\text{M}+\text{H}$] $^{+}$.

***N*-Phenyl-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (10e).** An oven-dried flask containing **7a** (10 mg, 43 μ mol) in DMSO (0.1 mL), was heated until the solution became clear, then *n*-BuOH (0.3 mL) and aniline (8 μ L, 86 μ mol) were added to the flask. The reaction mixture was heated to 120 $^{\circ}$ C for 16 h. After completion of reaction mixture was evaporated to dryness and column purification was performed using EtOAc: hex 20-100% 15 CV to obtain the product as an orange solid (10 mg, 80%). m.p. = 207-209 $^{\circ}$ C. ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.91 (dd, J =9.3, 2.0 Hz, 1 H) 8.52 (s, 1 H) 8.43 (d, J =5.4 Hz, 1 H) 8.40 (dd, J =4.4, 2.0 Hz, 1 H) 7.61 - 7.65 (m, 2 H) 7.37 - 7.43 (m, 2 H) 7.18 (br. s, 1 H) 7.11 - 7.16 (m, 2 H) 7.08 (d, J =5.37 Hz, 1 H). LC-MS (m/z): 289.3 [$\text{M}+\text{H}$] $^{+}$.

***N*-Phenyl-4-(2-(4-(trifluoromethyl)phenyl)pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (10f).** To an oven-dried flask containing a solution of **7d** (20 mg, 53 μ mol) in *n*-BuOH (1 mL), aniline (10 μ L, 106 μ mol) was added and the reaction was heated to 110 $^{\circ}$ C for 16 h. After completion of reaction, reaction mixture was evaporated to dryness and column purification was performed using EtOAc: hex, 20-100% 12 CV to afford the title compound as a colorless solid (20 mg, 87%). ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.69 (dd, J =9.3, 2.0 Hz, 1 H) 8.43 (dd, J =4.4, 2.0 Hz, 1 H) 8.26 (d, J =5.4 Hz, 1 H) 7.87 (d, J =8.3 Hz, 2 H) 7.74 (d, J =8.3 Hz, 2 H) 7.59 - 7.62 (m, 2 H) 7.33 - 7.37 (m, 2 H) 7.09 - 7.16 (m, 2 H) 6.60 (d, J =5.4 Hz, 1 H). LC-MS (m/z): 433.1 [$\text{M}+\text{H}$] $^{+}$.

4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(*o*-tolyl)pyrimidin-2-amine (10g). To an oven-dried flask containing *o*-toluidine (19 μ L, 173 μ mol) and **7a** (20 mg, 86 μ mol), *n*-BuOH was added. The reaction mixture was heated at 100 °C for 16 h. After completion of reaction mixture was evaporated to dryness and column purification was performed using EtOAc: hex 20-100% 15 CV to yield the title compound as an off-white solid (7 mg, 27%). m.p. = 177-178 °C. ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.64 (dd, *J*=9.3, 2.0 Hz, 1 H) 8.49 (s, 1 H) 8.40 (d, *J*=5.4 Hz, 1 H) 8.35 (dd, *J*=4.4, 2.0 Hz, 1 H) 7.82 (d, *J*=7.8 Hz, 1 H) 7.28 - 7.32 (m, 2 H) 7.16 (td, *J*=7.3, 1.5 Hz, 1 H) 7.01 - 7.05 (m, 2 H) 6.86 (br. s, 1 H) 2.36 (s, 3 H). LC-MS (m/z): 303.3 [M+H]⁺.

***N*-(3-Nitrophenyl)-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (10h).** In a vial **7a** (50 mg, 216 μ mol), 3-nitroaniline (52 mg, 540 μ mol) were stirred in *n*-BuOH (1.5 mL) and the reaction mixture was subjected to microwave irradiation at 150 °C for 2 h. After completion of reaction mixture was filtered and washed with MeOH. The precipitate was purified using column chromatography using EtOAc: hex, 50-100% to yield the title compound as a yellow solid (52 mg, 72%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 10.14 (s, 1 H) 9.15 - 9.21 (m, 1 H) 8.98 (s, 1 H) 8.92 (t, *J*=2.4 Hz, 1 H) 8.66 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.59 (d, *J*=5.4 Hz, 1 H) 8.11 (dd, *J*=8.3, 2.0 Hz, 1 H) 7.84 (dd, *J*=8.3, 2.0 Hz, 1 H) 7.64 (t, *J*=8.3 Hz, 1 H) 7.53 (d, *J*=5.4 Hz, 1 H) 7.49 (dd, *J*=8.8, 4.4 Hz, 1 H). LC-MS (m/z): 334.0 [M+H]⁺.

***N*-(3-Methoxyphenyl)-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (10i).** To an oven-dried flask containing 3-methoxyaniline (22 μ L, 194 μ mol), a solution of **7a** (30 mg, 130 μ mol) and acetic acid (1 mL) in dioxane (2 mL) was added. The reaction mixture was stirred at 110 °C for 23 h and monitored by TLC. The reaction mixture appeared dark red/brown and some solid particles were observed. Upon completion, the mixture was extracted with DCM (3 \times 100 mL). The combined organic phase was washed with brine and dried over sodium sulfate. Column purification was performed by preparative reverse phase

HPLC using 5-100 % ACN: water system, to afford the pure product as white solid (15 mg, 36%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.57 (s, 1 H) 9.17 - 9.24 (m, 1 H) 8.90 (s, 1 H) 8.62 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.49 (d, *J*=5.4 Hz, 1 H) 7.43 - 7.49 (m, 2 H) 7.39 (d, *J*=5.4 Hz, 1 H) 7.33 (d, *J*=8.3 Hz, 1 H) 7.23 (t, *J*=8.3 Hz, 1 H) 6.58 (dd, *J*=7.8, 2.4 Hz, 1 H) 3.76 (s, 3 H). LC-MS (*m/z*): 319.0 [M+H]⁺.

4-(2-cyclopropylpyrazolo[1,5-*b*]pyridazin-3-yl)-N-(3,4-difluorophenyl)pyrimidin-2-amine (10n). In a vial, **7e** (30 mg, 110 μmol) and 3,4-difluoroaniline (21 mg, 170 μmol) were dissolved in *n*-BuOH (1.5 mL). The vial was sealed, and the reaction was subjected to microwave irradiation at 150 °C for 2 h. The reaction was cooled to room temperature, then filtered, and washed with diethyl ether. The precipitate was taken up in DCM, and washed with saturated sodium bicarbonate. The organic layer was dried over sodium sulfate, filtered and evaporated *in vacuo*, to yield the product (22 mg, 55%). ¹H NMR (400 MHz, chloroform-*d*) δ ppm 8.66 (dd, *J*=8.8, 1.5 Hz, 1 H) 8.48 (d, *J*=5.1 Hz, 1 H) 8.32 (dd, *J*=4.4, 1.5 Hz, 1 H) 7.86 (dd, *J*=8.1, 2.2 Hz, 1 H) 7.36 (d, *J*=5.1 Hz, 1 H) 7.09 - 7.15 (m, 3 H) 2.32 - 2.42 (m, 1 H) 1.29 - 1.35 (m, 2 H) 1.13 - 1.21 (m, 2 H). LC-MS (*m/z*): 365.0 [M+H]⁺.

N-(4-(*tert*-Butyl)phenyl)-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (10s). To an oven-dried flask added 4-*tert*butylaniline (28 μL, 173 μmol) to a solution of **7a** (20 mg, 86 μmol) in *n*-BuOH. The reaction mixture was heated to 100 °C for 16 h. After completion of reaction mixture was evaporated to dryness and column purification was performed using EtOAc: hex 20-100% 15 CV to afford the title compound as a yellow solid (16 mg, 53%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.83 (dd, *J*=8.8, 2.0 Hz, 1 H) 8.51 (s, 1 H) 8.39 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.36 (d, *J*=5.4 Hz, 1 H) 7.70 (br. s, 1 H) 7.49 - 7.54 (m, 2 H) 7.40 - 7.45 (m, 2 H) 7.09 (dd, *J*=8.8, 4.4 Hz, 1 H) 7.05 (d, *J*=5.4 Hz, 1 H) 1.37 (s, 9 H). LC-MS (*m/z*): 345.4 [M+H]⁺.

3-(2-Chloropyrimidin-4-yl)pyrazolo[1,5-*a*]pyridine (11). (Aminoxy) sulfonic acid (490 mg, 4.33 mmol) was dissolved in 2 mL water, to the clear solution added 2.5 M sodium bicarbonate until effervescence ceased (pH ~ 5). The clear colorless solution was heated to 70 °C and pyridine (0.233 mL, 2.89 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction was cooled to RT, neutralized with 2.5 M sodium bicarbonate (until effervescence ceased) and then solution of **6b** (185 mg, 1.34 mmol) in DCM (10 mL) was added followed by KOH (100 mg, 1.78 mmol). The reaction was stirred for 16 h at RT. The reaction mixture was then diluted with DCM, and washed with water and brine then dried over sodium sulfate, decanted and concentrated to get crude product, which was purified by column chromatography using EtOAc: hex, 50-100% 12 CV, followed by EtOAc: MeOH 0-20% 5 CV to obtain the desired compound as a beige solid (85 mg, 51%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.95 (s, 1 H) 8.90 (d, *J*=6.8 Hz, 1 H) 8.62 (d, *J*=5.4 Hz, 1 H) 8.49 (d, *J*=8.8 Hz, 1 H) 7.97 (d, *J*=5.4 Hz, 1 H) 7.62 - 7.68 (m, 1 H) 7.19 (td, *J*=6.8, 1.5 Hz, 1 H). LC-MS (*m/z*): 230.9 (³⁵Cl), 232.9 (³⁷Cl) [M+H]⁺.

***N*-Cyclopropyl-4-(pyrazolo[1,5-*a*]pyridin-3-yl)pyrimidin-2-amine (12a).** In a vial **11** (10 mg, 43 μmol), cyclopropylamine (6 μL, 87 μmol), and DIPEA (16 μL, 87 μmol) were stirred in *n*-BuOH and subjected to microwave irradiation at 150 °C for 1 h. The reaction mixture was evaporated to dryness and purified by column chromatography using EtOAc: hex, 40-100%, 7 CV, followed by MeOH: EtOAc, 0-20% 5 CV to yield the title compound as a colorless solid (8 mg, 66%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.69 - 8.79 (m, 1 H) 8.52 (d, *J*=6.8 Hz, 1 H) 8.42 (s, 1 H) 8.28 (d, *J*=4.9 Hz, 1 H) 7.33 (ddd, *J*=8.8, 6.8, 1.0 Hz, 1 H) 6.87 - 6.95 (m, 2 H) 2.82 - 2.92 (m, 1 H) 0.84 - 0.93 (m, 2 H) 0.60 - 0.68 (m, 2 H). LC-MS (*m/z*): 252.0 [M+H]⁺.

***N*-Phenyl-4-(pyrazolo[1,5-*a*]pyridin-3-yl)pyrimidin-2-amine (12b).** In a vial added **11** (10 mg, 43 μmol), aniline (8 μL, 87 mmol) were stirred in *n*-BuOH at 110 °C for 16 h. The reaction mixture was

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3 evaporated to dryness and purified by column chromatography using EtOAc: hex, 40-100% 12 CV
4 followed by EtOAc: MeOH 1-20% 5 CV to afford the title compound as a beige solid (11 mg, 88%). ¹H
5 NMR (500 MHz, chloroform-*d*) δ ppm 8.51 - 8.57 (m, 2 H) 8.46 (s, 1 H) 8.35 - 8.40 (m, 1 H) 7.67 (d, *J*=7.3
6 Hz, 2 H) 7.36 - 7.41 (m, 2 H) 7.28 - 7.35 (m, 2 H) 7.10 (t, *J*=7.3 Hz, 1 H) 7.04 (d, *J*=5.4 Hz, 1 H) 6.89 -
7 6.95 (m, 1 H). LC-MS (m/z): 288.0 [M+H]⁺.
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16 ***N*-(3-Methoxyphenyl)-4-(pyrazolo[1,5-*a*]pyridin-3-yl)pyrimidin-2-amine (12c).** In a vial **11** (20 mg, 87
17 μmol) and 3-anisidine (20 μL, 0.173 mmol) were stirred in *n*-BuOH at 110 °C for 16 h. The reaction mixture
18 was then evaporated to dryness and column purification was performed using EtOAc: hex 40-100% 12 CV
19 followed by EtOAc: MeOH 1-20% 5 CV to afford the title compound as a beige solid (11 mg, 40%). ¹H
20 NMR (500 MHz, DMSO-*d*₆) δ ppm 9.49 (s, 1 H) 8.81 - 8.86 (m, 2 H) 8.77 (d, *J*=8.8 Hz, 1 H) 8.41 (d, *J*=5.4
21 Hz, 1 H) 7.46 - 7.52 (m, 2 H) 7.30 - 7.37 (m, 2 H) 7.22 (t, *J*=8.1 Hz, 1 H) 7.11 (t, *J*=6.8 Hz, 1 H) 6.57 (dd,
22 *J*=8.3, 2.2 Hz, 1 H) 3.76 (s, 3 H). LC-MS (m/z): 318.0 [M+H]⁺.
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33 **3-((4-(Pyrazolo[1,5-*a*]pyridin-3-yl)pyrimidin-2-yl)amino)benzonitrile (12d).** In a vial **11** (20 mg, 87
34 μmol), K₃PO₄ (37 mg, 173 μmol), XPhos (6 mg, 13 μmol), tris(dibenzylideneacetone)palladium(0) (4.0
35 mg, 4.3 μmol) and 3-aminobenzonitrile (20.5 mg, 173 μmol) were added. The vial was evacuated then
36 backfilled with N₂ three times. Degassed, anhydrous toluene (1 mL) was added to a sealed vial. The reaction
37 mixture was degassed for another 5 minutes and left stirring at 110 °C for 16 h. The heterogeneous red
38 reaction mixture, slowly turned yellow and clear with some floating particles. The reaction mixture was
39 evaporated to dryness and purified by using preparative reverse phase HPLC to afford the title compound
40 as a pale-yellow solid (6 mg, 22%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 9.19 (s, 1 H), 8.86 (d, *J*=7.0
41 Hz, 1 H), 8.85 (s, 1 H), 8.71 (d, *J*=9.0 Hz, 1 H), 8.53 (s, 1 H), 8.48 (d, *J*=5.0 Hz, 1 H), 8.43 (t, *J*=2.0 Hz, 1
42 H), 7.96 (dt, *J*=8.0, 1.5 Hz, 1 H), 7.56-7.52 (m, 2 H), 7.42-7.41 (m, 2 H), 7.13 (td, *J*=7.0, 1.5 Hz, 1 H). LC-
43 MS (m/z): 313.0 [M+H]⁺.
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Methyl pyrazolo[1,5-*b*]pyridazine-3-carboxylate (14a). (Aminooxy) sulfonic acid (8.1 g, 71 mmol) was dissolved in 13 mL water, to the clear colorless solution added saturated sodium bicarbonate until effervescence ceased (pH ~ 6). The clear colorless solution was heated to 70 °C and pyridazine (3.4 mL, 48 mmol) was added dropwise and the reaction was stirred at 70 °C for 2 h. The reaction was cooled to RT, neutralized with sat. sodium bicarbonate (until effervescence ceased) and then solution of **13** (1.1 mL, 12 mmol) in DCM (50 mL) was added followed by KOH (1.3 g, 24 mmol). The reaction was stirred for 16 h at RT. After completion of reaction the reaction mixture was diluted with DCM, washed with water, brine and dried over magnesium sulfate, filtered and evaporated to obtain the desired compound as a tan solid (1.5 g, 71%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.56 (dd, *J*=9.0, 1.7 Hz, 1 H) 8.49 (s, 1 H) 8.44 (dd, *J*=4.2, 1.7 Hz, 1 H) 7.25 - 7.29 (m, 1 H) 3.94 (s, 3 H). LC-MS (*m/z*): 177.9 [M+H]⁺.

Pyrazolo[1,5-*b*]pyridazine-3-carboxylic acid hydrochloride (14b). In a round bottom flask **14a** (1.0 g, 5.6 mmol), was dissolved in water (15 mL), sodium hydroxide (677 mg, 16.9 mmol) was added and the reaction was heated to 70 °C for 1 h. The reaction was cooled to room temperature and 1 M HCl was added, which caused a precipitate to form. The precipitate was filtered to yield the desired product as a white solid (414 mg, 37%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.62 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.52 (dd, *J*=9.3, 2.0 Hz, 1 H) 8.47 (s, 1 H) 7.48 (dd, *J*=9.3, 4.4 Hz, 1 H). LC-MS (*m/z*): 163.8 [M+H]⁺.

3-Bromopyrazolo[1,5-*b*]pyridazine (15). In a round bottom flask **14b** (410 mg, 2.1 mmol) was dissolved in anhydrous DMF (10 mL), *N*-bromosuccinimide (520 mg, 2.9 mmol) was added and the reaction was stirred at RT for 3 h. The reaction was poured into water and extracted with EtOAc, then the organic layer was washed three times with 1 M LiCl, followed by brine, then dried over magnesium sulfate, filtered and evaporated *in vacuo*, to yield the compound as a white solid (327 mg, 80 %). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.51 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.27 (s, 1 H) 8.19 (dd, *J*=8.8, 2.0 Hz, 1 H) 7.30 (dd, *J*=8.8, 4.4 Hz, 1 H). LC-MS (*m/z*): 197.8 (⁷⁹Br), 199.8 (⁸¹Br) [M+H]⁺.

3-Phenylpyrazolo[1,5-*b*]pyridazine (16a). In a vial **15** (42.1 mg, 213 μ mol) was added to 1.5 mL of 3:1 mixture of degassed dioxane/water, 0.25 mL of DCM was added followed by phenyl boronic acid (31.1 mg, 255 μ mol), Pd(dppf)₂Cl₂ (9 mg, 11 μ mol) and potassium carbonate (88 mg, 628 μ mol). The reaction mixture was subjected to microwave irradiation and heated to 130 °C for 1 h. The reaction was diluted with DCM and water and extracted 3 times into DCM, washed with brine, dried over magnesium sulfate, filtered and evaporated *in vacuo*. Column purification was performed using EtOAc: hex 0-50% to afford the product as a white solid (6.2 mg, 15%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.31 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.24 (s, 1 H) 8.21 (dd, *J*=8.8, 2.0 Hz, 1 H) 7.56 - 7.60 (m, 2 H) 7.47 - 7.51 (m, 2 H) 7.34 - 7.38 (m, 1 H) 7.05 (dd, *J*=8.8, 4.4 Hz, 1 H). LC-MS (*m/z*): 196.0 [M+H]⁺.

3-(3-Chlorophenyl)pyrazolo[1,5-*b*]pyridazine (16b). In a sealed vial **15** (100 mg, 505 μ mol), **28** (103 mg, 656 μ mol), potassium acetate (209 mg, 1.51 mmol) and PdCl₂(dppf)·CH₂Cl₂ (21 mg, 25 μ mol) were stirred in 4 mL of 3:1 mixture of degassed dioxane/water. The reaction was evacuated and backfilled with N₂ three times, then subjected to microwave irradiation at 130 °C for 1 h. The reaction was cooled and filtered through celite, and washed with EtOAc, then concentrated *in vacuo*. Column purification was performed using EtOAc: hex 25-50% to yield the desired compound as a yellow solid (83 mg, 72%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.34 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.23 (s, 1 H) 8.19 (dd, *J*=9.3, 2.0 Hz, 1 H) 7.55 - 7.57 (m, 1 H) 7.44 - 7.48 (m, 1 H) 7.41 (t, *J*=7.8 Hz, 1 H) 7.31 - 7.35 (m, 1 H) 7.09 (dd, *J*=9.3, 4.4 Hz, 1 H). LC-MS (*m/z*): 230.2 (³⁵Cl), 232.0 (³⁷Cl) [M+H]⁺.

3-((3-(Pyrazolo[1,5-*b*]pyridazin-3-yl)phenyl)amino)benzonitrile (17). In a sealed vial **16b**, 3-aminobenzonitrile (51 mg, 435 μ mol), cesium carbonate (212 mg, 653 μ mol), palladium(II) acetate (2.4 mg, 10.9 μ mol) and Xantphos (12.6 mg, 21.7 μ mol) were stirred in anhydrous dioxane (2 mL), the reaction mixture was evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to

microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was performed using EtOAc: hex 20-50%, to afford the title compound as a yellow solid (31.9 mg, 47%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.32 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.21 (s, 1 H) 8.18 (dd, *J*=8.8, 2.0 Hz, 1 H) 7.43 (t, *J*=7.8 Hz, 1 H) 7.33 - 7.38 (m, 2 H) 7.27 - 7.29 (m, 2 H) 7.24 - 7.25 (m, 1 H) 7.17 - 7.21 (m, 1 H) 7.10 (dd, *J*=7.8, 2.0 Hz, 1 H) 7.07 (dd, *J*=8.8, 4.4 Hz, 1 H) 5.94 (br. s, 1 H). LC-MS (*m/z*): 312.1 [M+H]⁺.

3-(Pyridin-2-yl)pyrazolo[1,5-*b*]pyridazine (18a). In a vial, (Aminoxy) sulfonic acid (658 mg, 5.82 mmol) was dissolved in 1 mL water, to the clear colorless solution saturated sodium bicarbonate was added until effervescence ceased (pH ~ 6). The clear colorless solution was heated to 70 °C and pyridazine (0.280 mL, 3.88 mmol) was added dropwise and the reaction was stirred at 70 °C for 2 h. Reaction mixture then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then solution of 2-ethynylpyridine (100 mg, 0.716 μmol) in DCM (8 mL) was added followed by 1,8-diazabicyclo[5.4.0]undec-7-ene (0.145 mL, 970 μmol). The reaction mixture was subjected to microwave irradiation at 100 °C for 30 min, then 120 °C for 30 min. The reaction was then left stirring at 40 °C for 72 h. After completion, the reaction mixture was extracted in DCM, washed with water, brine and dried over sodium sulfate, filtered and evaporated to get crude product. Column purification was performed using EtOAc: hex 20-50%, followed by a second column purification using MeOH: DCM 0-5% to afford the title compound as an orange solid (7.1 mg, 4%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 9.03 (dd, *J*=9.3, 2.0 Hz, 1 H) 8.65 - 8.69 (m, 1 H) 8.47 (s, 1 H) 8.37 (dd, *J*=4.4, 2.0 Hz, 1 H) 7.76 (td, *J*=7.3, 2.0 Hz, 1 H) 7.68 - 7.72 (m, 1 H) 7.18 (ddd, *J*=7.3, 4.9, 1.0 Hz, 1 H) 7.15 (dd, *J*=9.3, 4.4 Hz, 1 H). LC-MS (*m/z*): 196.9 [M+H]⁺.

3-((6-(Pyrazolo[1,5-*b*]pyridazin-3-yl)pyridin-2-yl)amino)benzonitrile (18b). In a sealed vial **29** (27.5 mg, 119 μmol), cesium carbonate (117 mg, 358 μmol), 3-aminobenzonitrile (28 mg, 238 μmol),

palladium(II) acetate (1.3 mg, 6.0 μmol), and Xantphos (6.9 mg, 11.9 μmol) were stirred in anhydrous dioxane (1.5 mL), the reaction mixture was evacuated and backfilled with N_2 three times. The reaction mixture was then subjected to MW irradiation at 160 $^\circ\text{C}$ for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was performed using EtOAc: hex 20-50%, to afford the product as a yellow solid (16.0 mg, 43%). ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.84 (dd, $J=9.3$, 2.0 Hz, 1 H) 8.45 (s, 1 H) 8.36 (dd, $J=4.4$, 2.0 Hz, 1 H) 8.09 (s, 1 H) 7.65 (t, $J=7.8$ Hz, 1 H) 7.52 - 7.57 (m, 1 H) 7.44 (t, $J=7.8$ Hz, 1 H) 7.32 - 7.36 (m, 1 H) 7.15 (dd, $J=9.3$, 4.4 Hz, 1 H) 6.66 (d, $J=7.8$ Hz, 1 H) 6.54 - 6.60 (m, 1 H). LC-MS (m/z): 313.1 $[\text{M}+\text{H}]^+$.

3-(Pyridin-4-yl)pyrazolo[1,5-*b*]pyridazine (18c). (Aminoxy) sulfonic acid (486 mg, 4.30 mmol) was dissolved in 2 mL water, to the clear colorless solution saturated sodium bicarbonate was added until effervescence ceased (pH \sim 6). The clear colorless solution was heated to 70 $^\circ\text{C}$ and pyridazine (0.207 mL, 2.87 mmol) was added dropwise, then the reaction was stirred at 70 $^\circ\text{C}$ for 2 h. The reaction mixture was then cooled to RT, and neutralized with saturated sodium bicarbonate (until effervescence ceased), then a solution of 4-ethynylpyridine HCl (100 mg, 0.716 μmol) in DCM (5 mL) was added followed by KOH (40 mg, 716 μmol). The reaction mixture was stirred at RT for 16 h. After completion of reaction the reaction mixture was diluted with DCM, washed with water, brine and dried over sodium sulfate, filtered and evaporated to get crude product. Column purification was performed using EtOAc: hex 50-100% to yield the product as an off-white solid (16 mg, 11%). ^1H NMR (500 MHz, chloroform-*d*) δ ppm 8.68 (dd, $J=4.4$, 2.0 Hz, 2 H) 8.39 (dd, $J=4.4$, 2.0 Hz, 1 H) 8.35 (s, 1 H) 8.27 (dd, $J=9.3$, 2.0 Hz, 1 H) 7.50 (dd, $J=4.4$, 2.0 Hz, 2 H) 7.17 (dd, $J=9.3$, 4.4 Hz, 1 H). LC-MS (m/z): 196.9 $[\text{M}+\text{H}]^+$.

3-((4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)pyridin-2-yl)amino)benzonitrile (18d). In a vial **30** (50 mg, 217 μmol), cesium carbonate (212 mg, 650 μmol), palladium(II) acetate (2.43 mg, 11 μmol), Xantphos (12.5 mg, 22 μmol) and 3-aminobenzonitrile (25.6 mg, 217 μmol) were added followed by anhydrous dioxane

(1.5 mL) and the vial was sealed. The reaction mixture was evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h, then filtered through celite and washed with EtOAc, and evaporated to dryness. Column purification was performed using MeOH: DCM 2-10% followed by reverse phase column purification using ACN: water 5-95% with 0.1% formic acid, to afford the title compound as a yellow solid (6.6 mg, 10%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.39 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.30 - 8.36 (m, 2 H) 8.24 (dd, *J*=8.8, 2.0 Hz, 1 H) 7.99 - 8.04 (s, 1 H) 7.64 (d, *J*=7.8 Hz, 1 H) 7.43 (t, *J*=7.8 Hz, 1 H) 7.31 (d, *J*=7.8 Hz, 1 H) 7.17 (dd, *J*=8.8, 4.4 Hz, 1 H) 7.07 (d, *J*=5.4 Hz, 1 H) 6.98 (s, 1 H) 6.84 (br. s., 1 H). LC-MS (*m/z*): 313.1 [M+H]⁺.

3-((4-Methyl-6-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (19a). In a vial **26** (30 mg, 122 μmol), and 3-aminobenzonitrile (29 mg, 244 μmol) were stirred in *n*-BuOH (1.5 mL) and subjected to microwave irradiation at 150 °C for 2 h. Reaction was filtered and washed with diethyl ether, the precipitate was taken up in DCM and washed with a saturated sodium bicarbonate solution and brine, then dried over magnesium sulfate, filtered and evaporated *in vacuo*, to yield the product as a white solid (19.8 mg, 50%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.89 (s, 1 H) 9.05 - 9.11 (m, 1 H) 8.87 (s, 1 H) 8.61 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.34 - 8.37 (m, 1 H) 7.97 (d, *J*=7.8 Hz, 1 H) 7.53 (t, *J*=7.8 Hz, 1 H) 7.45 (dd, *J*=9.3, 4.4 Hz, 1 H) 7.38 - 7.42 (m, 2 H) 2.43 (s, 3 H). LC-MS (*m/z*): 328.2 [M+H]⁺.

3-((5-Methyl-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (19b). In a vial **29** (35 mg, 140 μmol), 3-aminobenzonitrile (34 mg, 430 μmol), cesium carbonate (139 mg, 427 μmol), palladium(II) acetate (1.6 mg, 7.1 μmol) and Xantphos (8.2 mg, 14 μmol) were stirred in 1.5 mL of anhydrous dioxane. The reaction mixture was sealed and then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 130 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was performed using MeOH: DCM 0-5%, followed by another column purification using MeOH: DCM 1-3%, to afford the title compound as a white solid (7.4 mg, 16%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.87 (s,

1 H) 9.11 (dd, $J=9.3, 2.0$ Hz, 1 H) 8.71 (s, 1 H) 8.66 (dd, $J=4.4, 2.0$ Hz, 1 H) 8.46 - 8.49 (m, 1 H) 8.36 (t, $J=2.0$ Hz, 1 H) 7.93 - 7.98 (m, 1 H) 7.51 (t, $J=7.8$ Hz, 1 H) 7.46 (dd, $J=9.3, 4.4$ Hz, 1 H) 7.36 - 7.40 (m, 1 H) 2.46 (s, 3 H). LC-MS (m/z): 328.1 $[M+H]^+$.

3-((4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)oxy)benzonitrile (20a). 3-Hydroxybenzonitrile (8 mg, 65 μ mol) in anhydrous DMF was stirred with potassium carbonate (13 mg, 97 μ mol) at RT under N_2 for 30 min then **7a** (15 mg, 65 μ mol) was added and the reaction mixture was stirred at 80 °C for 16 h. After completion of reaction, the reaction mixture was dumped in water and extracted with EtOAc. Organic layer was then washed with water, brine and dried over sodium sulfate, decanted and concentrated to get crude product which was purified by column chromatography using acetone: hex 20-50% 15 CV then 50-100% 5 CV to afford the title compound as a white solid (11 mg, 51%). 1H NMR (500 MHz, chloroform-*d*) δ ppm 8.56 (d, $J=5.4$ Hz, 1 H) 8.54 (s, 1 H) 8.39 - 8.45 (m, 2 H) 7.59 - 7.66 (m, 3 H) 7.53 - 7.57 (m, 1 H) 7.40 (d, $J=5.4$ Hz, 1 H) 7.11 (dd, $J=8.8, 4.4$ Hz, 1 H). LC-MS (m/z): 315.0 $[M+H]^+$.

3-(Methyl(4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (20b).

In a vial **7a** (100 mg, 430 μ mol), cesium carbonate (420 mg, 1.3 mmol), 3-(methylanino)benzonitrile (110 mg, 860 μ mol), palladium (II) acetate (4.8 mg, 22 μ mol), and Xantphos (27 mg, 43 μ mol) were added in 5 mL of anhydrous dioxane. The reaction mixture was sealed then evacuated and backfilled with N_2 three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was done using MeOH: DCM 0-4%, followed by isocratic column purification 80% EtOAc: hexanes affording the title compound as an off-white solid (18.8 mg, 13%). 1H NMR (500 MHz, DMSO-*d*₆) δ ppm 8.88 (s, 1 H) 8.58 (dd, $J=4.4, 2.0$ Hz, 1 H) 8.45 (d, $J=5.4$ Hz, 1 H) 8.38 (dd, $J=9.3, 2.0$ Hz, 1 H) 8.00 (s, 1 H) 7.83 (d, $J=7.8$ Hz, 1 H) 7.75 (d, $J=7.8$ Hz, 1 H) 7.66 (t, $J=7.8$ Hz, 1 H) 7.38 (d, $J=5.4$ Hz, 1 H) 7.30 (dd, $J=9.3, 4.4$ Hz, 1 H) 3.59 (s, 3 H). LC-MS (m/z): 328.2 $[M+H]^+$.

4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(3-(trifluoromethyl)phenyl)pyrimidin-2-amine (20c). In a vial **7a** (50 mg, 216 μ mol), 3-(trifluoromethyl)aniline (67 μ L, 540 μ mol) were stirred in *n*-BuOH (1.5 mL) and the reaction mixture was subjected to microwave irradiation at 150 °C for 2 h. After completion the reaction was evaporated to dryness and column purification was performed using EtOAc: hex, 33-66% to afford the title compound as a white solid (52 mg, 68%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.95 (s, 1 H) 9.10 - 9.18 (m, 1 H) 8.93 (s, 1 H) 8.64 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.55 (d, *J*=5.4 Hz, 1 H) 8.30 (s, 1 H) 7.97 (d, *J*=8.3 Hz, 1 H) 7.57 (t, *J*=8.3 Hz, 1 H) 7.43 - 7.50 (m, 2 H) 7.33 (d, *J*=8.3 Hz, 1 H). LC-MS (*m/z*): 357.0 [M+H]⁺.

2-Fluoro-3-((4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (20d). In a vial 3-(2-chloropyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazine **7a** (40 mg, 173 μ mol), cesium carbonate (169 mg, 518 μ mol), 3-amino-2-fluorobenzonitrile (47 mg, 345 μ mol), palladium(II) acetate (2 mg, 8.6 μ mol), and Xantphos (10 mg, 17 μ mol) were added in anhydrous dioxane (1.5 mL), the reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. The reaction mixture was taken up in DCM and water, and extracted 2X into DCM, then washed with brine, dried with Na₂SO₄, filtered and evaporated in vacuo. Column purification was performed using EtOAc: hex 25-100% followed by reverse phase column purification using ACN: water 5-95% with 0.1% ammonium hydroxide, to afford the title compound as a white solid (5.0 mg, 9%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.62 (s, 1 H) 8.94 - 9.01 (m, 1 H) 8.90 (s, 1 H) 8.56 - 8.63 (m, 1 H) 8.45 (d, *J*=5.1 Hz, 1 H) 8.19 (t, *J*=7.3 Hz, 1 H) 7.65 (t, *J*=7.3 Hz, 1 H) 7.43 (s, 3 H). LC-MS 332.0 (*m/z*): [M+H]⁺.

4-Fluoro-3-((4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (20e). In a vial **7a** (40 mg, 173 μ mol), cesium carbonate (169 mg, 518 μ mol), 3-amino-4-fluorobenzonitrile (47 mg, 345

μmol), palladium(II) acetate (2 mg, 8.6 μmol), and Xantphos (10 mg, 17 μmol) were added with anhydrous dioxane (1.5 mL), the reaction mixture was sealed, then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was done using EtOAc: hex 25-100%, followed by MeOH: DCM 4% with 5% ammonium hydroxide, followed by reverse phase column purification using ACN: water 5-95% with 0.1% ammonium hydroxide, to afford the title compound as a white solid (5.0 mg, 8.7%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.60 (s, 1 H) 9.11 (d, *J*=8.8 Hz, 1 H) 8.93 (s, 1 H) 8.63 (dd, *J*=4.4, 2.2 Hz, 1 H) 8.49 - 8.58 (m, 2 H) 7.62 - 7.67 (m, 1 H) 7.49 - 7.59 (m, 2 H) 7.43 (dd, *J*=8.8, 4.4 Hz, 1 H). LC-MS (*m/z*): 332.0 [M+H]⁺.

4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyridin-2-yl)pyrimidin-2-amine (20f). In a vial **7a** (40 mg, 173 μmol), cesium carbonate (169 mg, 518 μmol), 2-aminopyridine (33 mg, 350 μmol), palladium(II) acetate (2 mg, 8.6 μmol), and Xantphos (10 mg, 17 μmol) were added to anhydrous dioxane (1.5 mL), the reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was performed using EtOAc: hex 50-100%, to afford the title compound as a pale-yellow solid (20.2 mg, 41%). ¹H NMR (400 MHz, chloroform-*d*) δ ppm 9.00 (dd, *J*=8.8, 2.2 Hz, 1 H) 8.54 (s, 1 H) 8.51 (d, *J*=5.1 Hz, 1 H) 8.43 (dd, *J*=4.4, 2.2 Hz, 1 H) 8.40 (d, *J*=8.1 Hz, 1 H) 8.34 (d, *J*=5.1 Hz, 1 H) 7.96 (br. s., 1 H) 7.69 - 7.76 (m, 1 H) 7.22 (dd, *J*=8.8, 4.4 Hz, 1 H) 7.16 (d, *J*=5.1 Hz, 1 H) 6.98 (dd, *J*=8.1, 5.1 Hz, 1 H). LC-MS (*m/z*): 290.1 [M+H]⁺.

4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyridin-3-yl)pyrimidin-2-amine (20g). In a vial **7a** (50 mg, 216 μmol), cesium carbonate (140 mg, 431 μmol), palladium (II) acetate (0.9 mg, 4 μmol), and Xantphos (4 mg, 7 μmol) were dissolved in 2 mL of anhydrous dioxane, the reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at

160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then concentrated *in vacuo*. Reverse phase preparative HPLC purification was performed using ACN: water 5-95% with 0.1% formic acid, to afford the title compound as a white solid (9.1 mg, 15%), isolated as a 0.5 eq formic acid solvate. ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.79 (s, 1 H) 9.12 - 9.18 (m, 1 H) 8.92 (s, 1 H) 8.89 (d, *J*=2.0 Hz, 1 H) 8.63 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.51 (d, *J*=5.4 Hz, 1 H) 8.41 (br. s., 1 H) 8.24 (d, *J*=8.3 Hz, 1 H) 8.20 (d, *J*=4.9 Hz, 1 H) 7.48 (dd, *J*=8.8, 4.4 Hz, 1 H) 7.44 (d, *J*=5.4 Hz, 1 H) 7.37 (dd, *J*=8.3, 4.9 Hz, 1 H). LC-MS (m/z): 290.2 [M+H]⁺.

4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyridin-4-yl)pyrimidin-2-amine (20h). In a vial **7a** (30 mg, 130 μmol), and 4-aminopyridine (18 mg, 190 μmol) were stirred in *n*-BuOH (1.5 mL) and heated to 150 °C for 2 h. The reaction was concentrated *in vacuo*. Column purification was performed using EtOAc: hexanes 50-75% then changed to MeOH: DCM 0-20%, a second column was run using an isocratic method MeOH: DCM 30%, to afford the title compound as a yellow solid (5.2 mg, 14%). ¹H NMR (400 MHz, methanol-*d*₄) δ ppm 9.39 (d, *J*=7.3 Hz, 2 H) 9.05 (d, *J*=9.5 Hz, 1 H) 8.93 (s, 1 H) 8.85 (d, *J*=5.1 Hz, 1 H) 8.61 (d, *J*=4.4 Hz, 1 H) 8.03 (d, *J*=5.1 Hz, 1 H) 7.55 (dd, *J*=9.5, 4.4 Hz, 1 H) 7.09 (d, *J*=7.3 Hz, 2 H). LC-MS (m/z): 290.1 [M+H]⁺.

4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyrimidin-5-yl)pyrimidin-2-amine (20i). In a vial added **7a** (50 mg, 220 μmol), 5-aminopyrimidine (205 mg, 2.2 mmol), palladium(II) acetate (2.4 mg, 11 μmol), Xantphos (12.5 mg, 21.6 μmol), and cesium carbonate (211 mg, 648 μmol) were stirred in anhydrous dioxane (5 mL). The reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction was then subjected to microwave irradiation at 160 °C for 1.5 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was performed using MeOH: DCM 0-5%, followed by reverse phase preparative HPLC column purification using ACN: water 5-70% with 0.1% formic acid, to afford the title compound as a white solid (3.6 mg, 6%). ¹H NMR (500 MHz, chloroform-

d) δ ppm 9.14 (s, 2 H) 8.97 (s, 1 H) 8.85 (dd, $J=8.8$, 2.0 Hz, 1 H) 8.56 (s, 1 H) 8.50 (d, $J=5.4$ Hz, 1 H) 8.44 (dd, $J=4.9$, 2.0 Hz, 1 H) 7.25 (dd, $J=8.8$, 4.4 Hz, 1 H) 7.20 (d, $J=5.4$ Hz, 1 H) 7.05 (br. s, 1 H). LC-MS (m/z): 291.2 [M+H]⁺.

***N*-(4-(Pyrrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)pyridazin-4-amine (20j).** In a vial **7a** (50 mg, 216 μ mol), and 4-aminopyridazine (41 mg, 432 μ mol) were stirred in *n*-BuOH (1.5 mL) and subject to microwave irradiation to 120 °C for 30 min. The reaction was concentrated *in vacuo* and column purification was performed MeOH: DCM 2-20% followed by reverse phase preparative HPLC purification using ACN: water 5-50% with 0.1% formic acid, to afford the title compound as a yellow solid (5.5 mg, 11%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.21 - 9.26 (m, 1 H) 9.13 (dd, $J=8.78$, 2.0 Hz, 1 H) 9.10 (s, 1 H) 8.87 (d, $J=5.4$ Hz, 1 H) 8.69 (dd, $J=4.4$, 2.0 Hz, 1 H) 8.35 - 8.41 (m, 2 H) 8.05 (d, $J=5.4$ Hz, 1 H) 7.58 (dd, $J=8.8$, 4.4 Hz, 1 H) 6.84 - 6.89 (m, 1 H). LC-MS (m/z): 291.1 [M+H]⁺.

***N*-(6-Methylpyridin-3-yl)-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (20k).** In a vial **7a** (50 mg, 220 μ mol), 3-amino-6-methylpyridine (47 mg, 430 μ mol), cesium carbonate (211 mg, 648 μ mol), palladium(II) acetate (2.4 mg, 11 μ mol) and Xantphos (12.5 mg, 21.6 μ mol) were stirred in anhydrous dioxane (1.5 mL). The reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Reverse phase preparative HPLC column purification was performed using ACN: water 5-70% with 0.1% formic acid. The compound was then taken up in DCM and stirred with resin-bound silicon carbonate for 12 h then filtered and evaporated *in vacuo*, to afford the title compound as an off-white solid (25.9 mg, 40%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.64 (s, 1 H) 9.09 - 9.16 (m, 1 H) 8.90 (s, 1 H) 8.73 (d, $J=2.4$ Hz, 1 H) 8.62 (dd, $J=4.4$, 2.0 Hz, 1 H) 8.48 (d, $J=5.4$ Hz, 1 H) 8.11 (dd, $J=8.8$, 2.4 Hz, 1 H) 7.47 (dd, $J=8.8$, 4.4 Hz, 1 H) 7.40 (d, $J=5.4$ Hz, 1 H) 7.22 (d, $J=8.8$ Hz, 1 H) 2.44 (s, 3 H). LC-MS (m/z): 304.2 [M+H]⁺.

3-((4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)propan-1-ol (20l). To an oven-dried flask containing 3-aminopropan-1-ol (7 mg, 90 μ mol, and **7a** (10 mg, 43 μ mol) added in *n*-BuOH. The reaction mixture was stirred at 110 °C for 16 h. After completion of reaction mixture was evaporated to dryness and the solid obtained was titrated with cold methanol, filtered and dried under high vacuum affording the title compound as a solid (6 mg, 68%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.01 - 9.25 (m, 1 H) 8.82 (s, 1 H) 8.58 (d, *J*=5.1 Hz, 1 H) 8.27 (d, *J*=4.4 Hz, 1 H) 7.41 (dd, *J*=8.8, 4.4 Hz, 1 H) 7.19 (br. s., 1 H) 7.10 (d, *J*=5.1 Hz, 1 H) 4.53 (br. s., 1 H) 3.37 - 3.60 (m, 4 H) 1.64 - 1.82 (m, 2 H). LC-MS (*m/z*): 271.1 [M+H]⁺.

***N*-(4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)acrylamide (20m).** To the solution of (10 mg, 47 μ mol, prepared by refluxing 3-(2-chloropyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazine in liq. NH₃, added triethylamine (10 μ l, 71 μ mol) in 1 mL THF followed by acryloyl chloride (6 μ l, 71 μ mol) and reaction mixture was stirred at 50 °C overnight. The reaction mixture then evaporated to dryness and taken up in DCM, washed with water and brine then dried over sodium sulfate, decanted and concentrated to get crude product which was purified by column chromatography using EtOAc: hex 30-100% 12 CV to afford the title compound as a solid (6 mg, 48%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 10.93 (s, 1 H) 9.71 (d, *J*=8.8 Hz, 1 H) 8.97 (s, 1 H) 8.57 - 8.70 (m, 2 H) 7.71 (d, *J*=5.1 Hz, 1 H) 7.53 (dd, *J*=8.8, 4.4 Hz, 1 H) 6.71 (dd, *J*=16.9, 10.3 Hz, 1 H) 6.40 (d, *J*=16.9 Hz, 1 H) 5.85 (d, *J*=10.3 Hz, 1 H). LC-MS (*m/z*): 267.1 [M+H]⁺.

4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (20n). In a vial **10a** (34.3 mg, 103 μ mol) was dissolved in TFA (2 mL) and stirred at 80 °C for 20 min. The reaction was quenched with saturated sodium bicarbonate and filtered. The precipitate was purified using column purification using MeOH: DCM 5% to afford the title compound as a white solid (8.1 mg, 37%). ¹H NMR (400 MHz, chloroform-*d*) δ ppm 8.99 (d, *J*=8.8 Hz, 1 H) 8.49 (s, 1 H) 8.41 (d, *J*=4.4 Hz, 1 H) 8.32 (d, *J*=5.1 Hz, 1 H) 7.20 (dd, *J*=8.8, 4.4 Hz, 1 H) 6.99 (d, *J*=5.1 Hz, 1 H) 5.02 (br. s., 2 H). LC-MS (*m/z*): 213.0 [M+H]⁺.

***N*-Methyl-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (20o).** In a vial **7a** (30 mg, 130 μ mol) and methylamine (2.0 M solution in THF) (129.5 μ L, 259 μ mol), were stirred in *n*-BuOH and subjected to microwave irradiation at 150 $^{\circ}$ C for 2 h. The reaction was concentrated *in vacuo*, then subjected to column purification using EtOAc: hexanes 50-100% to afford the title compound as an off-white solid (22.8 mg, 78%). ^1H NMR (400 MHz, methanol- d_4) δ ppm 9.12 (dd, $J=8.8$, 2.2 Hz, 1 H) 8.65 (s, 1 H) 8.49 (dd, $J=4.4$, 2.2 Hz, 1 H) 8.22 (d, $J=5.1$ Hz, 1 H) 7.36 (dd, $J=8.8$, 4.4 Hz, 1 H) 7.07 (d, $J=5.1$ Hz, 1 H) 3.02 (s, 3 H). LC-MS (m/z): 227.0 $[\text{M}+\text{H}]^+$.

***N*-Isopropyl-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (20p).** In a vial **7a** (50 mg, 220 μ mol), and isopropylamine (35 μ L, 430 μ mol) were stirred in *n*-BuOH (1.5 mL) and the reaction mixture was subjected to microwave irradiation at 150 $^{\circ}$ C for 2 h. After completion of reaction mixture was evaporated to dryness. Column purification performed using reverse phase preparative HPLC using 5-35 % ACN: water system, affording the title compound as a yellow solid (7.0 mg, 15%). ^1H NMR (500 MHz, methanol- d_4) δ ppm 9.00 - 9.13 (m, 1 H) 8.88 (s, 1 H) 8.64 (dd, $J=4.4$, 2.0 Hz, 1 H) 8.16 (d, $J=6.4$ Hz, 1 H) 7.57 (dd, $J=8.8$, 4.4 Hz, 1 H) 7.38 (d, $J=6.4$ Hz, 1 H) 4.89 - 4.90 (m, 1 H) 1.40 (d, $J=6.35$ Hz, 6 H). LC-MS (m/z): 255.1 $[\text{M}+\text{H}]^+$.

***N*-Cyclohexyl-4-(pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (20q).** In a vial **7a** (50 mg, 216 μ mol) and cyclohexylamine (49 μ L, 432 μ mol) were stirred in 1.5 mL *n*-BuOH and the reaction was subjected to microwave irradiation at 150 $^{\circ}$ C for 2 h, the reaction was then conventionally heated to 50 $^{\circ}$ C for 12 h, and then subjected to microwave irradiation at 150 $^{\circ}$ C for 30 min. The reaction was filtered and washed with diethyl ether to give the crude product in the precipitate. Reverse phase preparative HPLC purification was performed using ACN: water 5-95% with 0.1% formic acid to afford the title compound as a white solid (26.2 mg, 41%). ^1H NMR (500 MHz, chloroform- d) δ ppm 8.96 (dd, $J=8.8$, 2.0 Hz, 1 H) 8.46 (s, 1 H) 8.38 (dd, $J=4.4$, 2.0 Hz, 1 H) 8.23 - 8.32 (m, 1 H) 7.16 (dd, $J=8.8$, 4.5 Hz, 1 H) 6.86 (d, $J=5.4$

Hz, 1 H) 5.12 (br. s., 1 H) 3.84 - 3.95 (m, 1 H) 2.09 - 2.19 (m, 2 H) 1.76 - 1.87 (m, 2 H) 1.64 - 1.73 (m, 1 H) 1.41 - 1.53 (m, 2 H) 1.22 - 1.38 (m, 3 H). LC-MS (m/z): 295.1 [M+H]⁺.

4-((4-(Pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)cyclohexan-1-ol (20r). In a vial **7a** (0.040 g, 173 μmol), 4-aminocyclohexanol (0.040 g, 347 μmol) were stirred in *n*-BuOH (1.5 mL) and the reaction mixture was subjected to microwave irradiation at 150 °C for 2 h. After completion of reaction mixture was filtered and washed with diethyl ether, the filtrate was evaporated to dryness and column purification was performed using MeOH: DCM 2-4% to yield the title compound as a white solid (6.0 mg, 11%). ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 9.04 - 9.13 (m, 1 H) 8.64 (s, 1 H) 8.49 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.21 (d, *J*=5.4 Hz, 1 H) 7.36 (dd, *J*=8.8, 4.4 Hz, 1 H) 7.04 (d, *J*=5.4 Hz, 1 H) 3.76 - 3.87 (m, 1 H) 3.58 - 3.67 (m, 1 H) 2.09 - 2.20 (m, 2 H) 1.98 - 2.08 (m, 2 H) 1.35 - 1.53 (m, 4 H). LC-MS (m/z): 311.1 [M+H]⁺.

3-((4-(2-(4-Methoxyphenyl)pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino) benzonitrile (21a).

In a vial added **7f** (20 mg, 59 μmol), anhydrous K₃PO₄ (25 mg, 118 μmol), XPhos (4 mg, 9 μmol), tris(dibenzylideneacetone)palladium(0) (3 mg, 3.0 μmol) and 3-aminobenzonitrile (14 mg, 118 μmol). The vial was sealed with a stir bar and evacuated and backfilled with N₂ three times. Degassed anhydrous toluene (1 mL, dried over molecular sieves and stored under N₂) was added to a sealed vial and the reaction mixture was degassed with nitrogen for an additional 5 minutes. The reaction was stirred at 101 °C for 16 h. The reaction mixture was evaporated to dryness and then extracted with DCM and washed with water, and brine then dried over sodium sulfate, decanted and concentrated to get crude product. Purification was done by column chromatography using EtOAc: hex 40-100% 12 CV to yield the title compound as a yellow solid (3 mg, 12%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.71 (dd, *J*=9.0, 1.5 Hz, 1 H), 8.40 (dd, *J*=4.5, 2.0 Hz, 1 H), 8.31 (s, 1 H), 8.27 (d, *J*=5.0 Hz, 1 H), 7.66 (d, *J*=8.0 Hz, 1 H), 7.63 (d, *J*=9.0 Hz, 2 H), 7.414 (t, *J*=8.0 Hz, 1 H), 7.36-7.32 (m, 2 H), 7.20 (dd, *J*=9.0, 4.5 Hz, 1 H), 7.00 (d, *J*=9.0 Hz, 2 H), 6.76 (d, *J*=5.5 Hz, 1 H), 3.89 (s, 3 H). LC-MS (m/z): 420.0 [M+H]⁺.

3-((4-(2-(4-(Trifluoromethyl)phenyl)pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-

yl)amino)benzonitrile (21b). In a vial **7d** (15 mg, 40 μ mol), cesium carbonate (39 mg, 120 μ mol), 3-aminobenzonitrile (9 mg, 80 μ mol), palladium (II) acetate (0.5 mg, 4.0 μ mol), and Xantphos (2.3 mg, 4 μ mol) were added in 1.5 mL of anhydrous dioxane. The reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was performed using EtOAc: hexanes 25-100%, to yield the title compound as a pale-yellow solid (11.7 mg, 64%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 10.06 (s, 1 H) 8.80 - 8.85 (m, 1 H) 8.69 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.50 (d, *J*=5.4 Hz, 1 H) 8.19 (s, 1 H) 7.89 (d, *J*=8.3 Hz, 2 H) 7.82 - 7.87 (m, 3 H) 7.49 (dd, *J*=8.8, 4.4 Hz, 1 H) 7.41 (t, *J*=7.8 Hz, 1 H) 7.36 (d, *J*=7.8 Hz, 1 H) 6.79 (d, *J*=5.37 Hz, 1 H). LC-MS (*m/z*): 458.1 [M+H]⁺.

3-((4-(2-(2-Hydroxypropan-2-yl)pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile

(21c). In a vial **7g** (71 mg, 170 μ mol), and 3-aminobenzonitrile (87 mg, 740 μ mol) were stirred in *n*-BuOH (2 mL) and the reaction mixture was subjected to microwave irradiation at 150 °C for 2 h. After completion of reaction mixture was evaporated to dryness and column purification was done using MeOH: DCM 0-5%, followed by column purification 25-100% EtOAc: hexanes, followed by preparative reverse phase HPLC purification 30-100% ACN: water, affording the title compound as a white solid (2.9 mg, 4%). ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 8.54 - 8.60 (m, 2 H) 8.47 (dd, *J*=4.4, 1.5 Hz, 1 H) 8.26 (s, 1 H) 7.89 (d, *J*=8.3 Hz, 1 H) 7.51 (d, *J*=5.4 Hz, 1 H) 7.47 (t, *J*=8.3 Hz, 1 H) 7.31 - 7.38 (m, 2 H) 1.69 (s, 6 H). LC-MS (*m/z*): 372.1 [M+H]⁺.

3-((4-(2-(Hydroxymethyl)pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (21d).

In a vial added **7h** (33.5 mg, 128 μ mol), and 3-aminobenzonitrile (37.8 mg, 320 μ mol) were stirred in *n*-BuOH (2 mL) and subjected to microwave irradiation at 150 °C for 1 h. The reaction mixture was filtered, and the

precipitate was washed with MeOH. The precipitate was found to contain the desired product as a brown solid (8 mg, 18%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.96 (s, 1 H) 9.02 - 9.09 (m, 1 H) 8.58 - 8.64 (m, 2 H) 8.38 (s, 1 H) 7.96 (dd, *J*=8.3, 2.0 Hz, 1 H) 7.49 - 7.56 (m, 2 H) 7.40 - 7.47 (m, 2 H) 5.64 (br. s, 1 H) 4.87 (s, 2 H). LC-MS (m/z): 344.2 [M+H]⁺.

3-((4-(2-(Methoxymethyl)pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (21e). In a vial added **7i** (100 mg, 362 μmol) and 3-aminobenzonitrile (85.7 mg, 725 μmol) with 1.5 mL of *n*-BuOH. The reaction mixture was subjected to microwave irradiation at 150 °C for 2 h. The reaction was cooled to room temperature, filtered and washed with diethyl ether to yield the desired product in the precipitate as an off-white solid (116.8 mg, 90%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.98 (s, 1 H) 9.03 - 9.09 (m, 1 H) 8.65 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.62 (d, *J*=5.4 Hz, 1 H) 8.38 (s, 1 H) 7.94 - 7.99 (m, 1 H) 7.53 (t, *J*=8.1 Hz, 1 H) 7.46 (dd, *J*=8.8, 4.4 Hz, 1 H) 7.42 (d, *J*=7.3 Hz, 1 H) 7.33 (d, *J*=5.4 Hz, 1 H) 4.84 (s, 2 H) 3.37 (s, 3 H). LC-MS (m/z): 358.1 [M+H]⁺.

3-((4-(2-(1-Hydroxyethyl)pyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (21f). In a vial added **7j** (100 mg, 363 μmol) and 3-aminobenzonitrile (86 mg, 725 μmol) with 1.5 mL of *n*-BuOH. The reaction mixture was subjected to microwave irradiation at 150 °C for 2 h. The reaction was then concentrated *in vacuo*. Column purification was performed using EtOAc: hex 45-100% to afford the title compound as a white solid (95.5 mg, 74%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.96 (s, 1 H) 8.93 (d, *J*=8.8 Hz, 1 H) 8.58 - 8.63 (m, 2 H) 8.38 (s, 1 H) 7.97 (d, *J*=8.3 Hz, 1 H) 7.59 (d, *J*=5.4 Hz, 1 H) 7.51 (t, *J*=8.3 Hz, 1 H) 7.39 - 7.44 (m, 2 H) 5.63 (d, *J*=6.4 Hz, 1 H) 5.28 (quin, *J*=6.4 Hz, 1 H) 1.62 (d, *J*=6.4 Hz, 3 H). LC-MS (m/z): 358.1 [M+H]⁺.

3-((4-(2-Isopropylpyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (21g). In a vial **7k** (100 mg, 365 μmol), and 3-aminobenzonitrile (86 mg, 731 μmol) were stirred in 1.5 mL of *n*-BuOH. The reaction mixture was subjected to microwave irradiation and heated to 150 °C for 2 h. The reaction mixture

was concentrated *in vacuo*. Column purification was performed using 1-5% MeOH in DCM, followed by reverse phase preparative HPLC purification using ACN: water 5-95% with 0.1% formic acid, providing the title compound as a tan solid (36.8 mg, 28%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.98 (s, 1 H) 8.75 - 8.82 (m, 1 H) 8.59 (d, *J*=5.4 Hz, 1 H) 8.55 (d, *J*=4.4 Hz, 1 H) 8.37 (s, 1 H) 7.97 (d, *J*=8.3 Hz, 1 H) 7.51 (t, *J*=8.3 Hz, 1 H) 7.37 - 7.43 (m, 2 H) 7.20 (d, *J*=5.4 Hz, 1 H) 3.79 (spt, *J*=6.8 Hz, 1 H) 1.38 (d, *J*=6.8 Hz, 6 H). LC-MS (*m/z*): 356.1 [M+H]⁺.

3-((4-(2-Cyclopropylpyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (21h). In a vial **7e** (100 mg, 368 μmol) and 3-aminobenzonitrile (87 mg, 736 μmol) were stirred in 1.5 mL of *n*-BuOH. The reaction mixture was subjected to microwave irradiation at 150 °C for 2 h. The reaction was filtered and washed with diethyl ether to give the crude product in the precipitate. Column purification was performed using MeOH: DCM 0-3% followed by reverse phase purification using ACN: water 5-95% with 0.1% formic acid to afford the title compound as a white solid (46.1 mg, 35%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.68 (dd, *J*=8.8, 2.0 Hz, 1 H) 8.52 (d, *J*=5.4 Hz, 1 H) 8.34 - 8.35 (m, 1 H) 8.33 (dd, *J*=4.4, 2.0 Hz, 1 H) 7.65 - 7.70 (m, 1 H) 7.41 - 7.45 (m, 3 H) 7.34 (d, *J*=7.8 Hz, 1 H) 7.16 (dd, *J*=8.8, 4.4 Hz, 1 H) 2.31 - 2.39 (m, 1 H) 1.30 - 1.35 (m, 2 H) 1.15 - 1.20 (m, 2 H). LC-MS (*m/z*): 354.1 [M+H]⁺.

4-(6-Morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-phenylpyrimidin-2-amine (22a). In a reaction vial added **7b** (20 mg, 63 μmol) and aniline (12 μL, 126 μmol) in *n*-BuOH (1 mL), the reaction mixture was subjected to microwave irradiation at 150 °C for 1 h. Then the reaction mixture was evaporated to dryness and column purification done using EtOAc: hex 50-100% 12 CV followed by EtOAc: MeOH 1-20% 5 CV to afford the title compound as a yellow/brown solid (10 mg, 42%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.65 (d, *J*=9.8 Hz, 1 H) 8.37 (d, *J*=5.4 Hz, 1 H) 8.26 (s, 1 H) 7.62 (d, *J*=7.8 Hz, 2 H) 7.38 (t, *J*=7.8 Hz, 2 H) 7.25 (br. s, 1 H) 7.11 (t, *J*=7.8 Hz, 1 H) 7.02 (d, *J*=5.4 Hz, 1 H) 6.87 (d, *J*=9.8 Hz, 1 H) 3.86 (t, *J*=4.4 Hz, 4 H) 3.59 (t, *J*=4.4 Hz, 4 H). LC-MS (*m/z*): 374.0 [M+H]⁺.

***N*-(3-Methoxyphenyl)-4-(6-morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-amine (22b).** In a vial **7b** (0.020 g, 63.14 μ mol), and *m*-anisidine (15 μ L, 126 μ mol) were stirred in *n*-BuOH. The reaction mixture was subjected to microwave irradiation at 150 °C for 1 h. On cooling solid precipitation seen, which was filtered and washed with diethyl ether and air dried to afford the title compound as a dark brown solid (14 mg, 55%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.70 (d, *J*=9.8 Hz, 1 H) 8.38 (d, *J*=5.4 Hz, 1 H) 8.27 (s, 1 H) 7.36 (t, *J*=2.4 Hz, 1 H) 7.27 - 7.31 (m, 1 H) 7.10 - 7.15 (m, 2 H) 7.03 (d, *J*=5.4 Hz, 1 H) 6.89 (d, *J*=9.8 Hz, 1 H) 6.66 (dd, *J*=8.3, 2.4 Hz, 1 H) 3.87 (t, *J*=4.9 Hz, 4 H) 3.83 (s, 3 H) 3.60 (t, *J*=4.9 Hz, 4 H). LC-MS (*m/z*): 404.0 [*M*+*H*]⁺.

3-((4-(6-Morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (22c). In a vial **7b** (20 mg, 63 μ mol), K₃PO₄ (27 mg, 126 μ mol), XPhos (5 mg, 9.5 μ mol), tris(dibenzylideneacetone)palladium(0) (3 mg, 3.2 μ mol) and *m*-aminobenzonitrile (15 mg, 126 μ mol) were added. The vial was sealed with a stir bar and then evacuated and backfilled with N₂ three times, 1 mL of *n*-BuOH was added to a sealed vial. The reaction was left stirring at 101 °C for 16 h. Solid precipitation was seen in the reaction vial, which was filtered and washed with diethyl ether. Column purification was undertaken on the precipitate using EtOAc: hex 70-100% 5 CV followed by MeOH: EtOAc 1-20% 10 CV to afford the title compound as a white solid (6.3 mg, 25%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.68 (d, *J*=9.8 Hz, 1 H) 8.39 - 8.45 (m, 2 H) 8.28 (s, 1 H) 7.56 (ddd, *J*=7.8, 2.4, 1.0 Hz, 1 H) 7.45 (t, *J*=7.8 Hz, 1 H) 7.36 (dt, *J*=7.8, 1.0 Hz, 1 H) 7.32 (br. s, 1 H) 7.12 (d, *J*=5.4 Hz, 1 H) 7.06 (d, *J*=9.8 Hz, 1 H) 3.87 (t, *J*=4.9 Hz, 4 H) 3.61 (t, *J*=4.9 Hz, 4 H). LC-MS (*m/z*): 399.0 [*M*+*H*]⁺.

3-((4-(6-Methoxypyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (22d). In a vial **3-7c** (15 mg, 57 μ mol) and 3-aminobenzonitrile (34 mg, 287 μ mol) were stirred in *n*-BuOH (1.5 mL) and subjected to microwave irradiation at 150 °C for 2 h. The reaction mixture was then heated to 110 °C using

conventional heating for an additional 96 h. The mixture was then concentrated, and column purification was undertaken using EtOAc: hex, 25-33% to yield the title compound as a white solid (7.0 mg, 37%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.74 (d, *J*=9.8 Hz, 1 H) 8.45 (d, *J*=5.4 Hz, 1 H) 8.32 - 8.36 (m, 2 H) 7.62 - 7.67 (m, 1 H) 7.45 (t, *J*=7.8 Hz, 1 H) 7.36 (d, *J*=7.8 Hz, 1 H) 7.20 (br. s, 1 H) 7.14 (d, *J*=5.4 Hz, 1 H) 6.95 (d, *J*=9.8 Hz, 1 H) 4.11 (s, 3 H). LC-MS (*m/z*): 344.1 [M+H]⁺.

3-((4-(2-(Hydroxymethyl)-6-methoxypyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-

yl)amino)benzonitrile (23a). In a vial **7l** (20 mg, 69 μmol) and 3-aminobenzonitrile (24 mg, 206 μmol) were added to *n*-BuOH (1 mL) and the reaction was heated to 150 °C for 2 h, then 180 °C for 3 hrs. The reaction was filtered and washed with diethyl ether to yield the desired product in the precipitate as a white solid (7.2 mg, 28%). ¹H NMR (400 MHz, DMSO-*d*₆) δ ppm 9.96 (s, 1 H) 8.93 (d, *J*=9.5 Hz, 1 H) 8.59 (d, *J*=5.1 Hz, 1 H) 8.38 (t, *J*=1.5 Hz, 1 H) 7.93 - 7.98 (m, 1 H) 7.47 - 7.56 (m, 2 H) 7.41 (dt, *J*=7.3, 1.5 Hz, 1 H) 7.14 (d, *J*=9.5 Hz, 1 H) 5.59 (t, *J*=5.1 Hz, 1 H) 4.80 (d, *J*=5.1 Hz, 2 H) 4.00 (s, 3 H). LC-MS (*m/z*): 374.1 [M+H]⁺.

3-((4-(2-(Hydroxymethyl)-6-morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-

yl)amino)benzonitrile (23b). In a vial **7m** (30 mg, 87 μmol), and 3-aminobenzonitrile (20 mg, 173 μmol) were stirred in 1.5 mL *n*-BuOH and the reaction was subjected to microwave irradiation at 150 °C for 3 h. The reaction was filtered and washed with diethyl ether to give the crude product in the precipitate. Column purification was undertaken using MeOH: DCM 2-30% to afford the title compound as a white solid (20.8 mg, 56%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.94 (s, 1 H) 8.76 (d, *J*=9.8 Hz, 1 H) 8.56 (d, *J*=5.4 Hz, 1 H) 8.36 (s, 1 H) 7.95 - 7.99 (m, 1 H) 7.53 (t, *J*=8.3 Hz, 1 H) 7.46 (d, *J*=5.4 Hz, 1 H) 7.36 - 7.42 (m, 2 H) 5.52 (t, *J*=4.9 Hz, 1 H) 4.76 (d, *J*=4.9 Hz, 2 H) 3.76 (t, *J*=4.9 Hz, 4 H) 3.52 (t, *J*=4.9 Hz, 4 H). LC-MS (*m/z*): 429.1 [M+H]⁺.

3-((4-(2-Cyclopropyl-6-morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)benzonitrile (23c). In a vial **7o** (25 mg, 70 μ mol), cesium carbonate (69 mg, 220 μ mol), 3-aminopyridine (17 mg, 140 μ mol), palladium(II) acetate (0.8 mg, 3.5 μ mol) and Xantphos (4.1 mg, 7.0 μ mol) were stirred in anhydrous dioxane (2 mL), the reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Column purification was performed using EtOAc: hex 25-75%, to afford the title compound as an orange solid (21.5 mg, 70%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.51 (d, *J*=9.8 Hz, 1 H) 8.46 (d, *J*=5.4 Hz, 1 H) 8.39 (s, 1 H) 7.62 (dd, *J*=8.3, 2.0 Hz, 1 H) 7.40 - 7.49 (m, 2 H) 7.34 (d, *J*=7.8 Hz, 1 H) 7.22 - 7.25 (s, 1 H) 6.95 (d, *J*=9.8 Hz, 1 H) 3.85 (t, *J*=4.4 Hz, 4 H) 3.57 (t, *J*=5.4 Hz, 4 H) 2.22 - 2.29 (m, 1 H) 1.23 - 1.28 (m, 2 H) 1.08 - 1.14 (m, 2 H) LC-MS (*m/z*): 439.1 [M+H]⁺.

4-(2-Cyclopropylpyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyridin-3-yl)pyrimidin-2-amine (23d). In a vial 3-(2-chloropyrimidin-4-yl)-2-cyclopropylpyrazolo[1,5-*b*]pyridazine **7e** (30 mg, 110 μ mol), cesium carbonate (108 mg, 331 μ mol), 3-aminopyridine (21 mg, 220 μ mol), filtered and evaporated (1.2 mg, 5.5 μ mol) and Xantphos (6.4 mg, 11 μ mol) were stirred in anhydrous dioxane (1.5 mL), the reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Reverse phase column purification was done using ACN: water 5-95% with 0.1% formic acid, to afford the title compound as a white solid (17.0 mg, 47%). ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 8.92 (d, *J*=2.4 Hz, 1 H) 8.89 (dd, *J*=8.8, 2.0 Hz, 1 H) 8.52 (d, *J*=5.4 Hz, 1 H) 8.40 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.32 (ddd, *J*=8.3, 2.4, 1.5 Hz, 1 H) 8.17 (dd, *J*=4.6, 1.5 Hz, 1 H) 7.47 (d, *J*=5.4 Hz, 1 H) 7.39 (dd, *J*=8.3, 4.6 Hz, 1 H) 7.28 (dd, *J*=8.8, 4.4 Hz, 1 H) 2.45 - 2.53 (m, 1 H) 1.11 - 1.23 (m, 4 H). LC-MS (*m/z*): 330.1 [M+H]⁺.

4-(6-Methoxypyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyridin-3-yl)pyrimidin-2-amine (23e). In a vial **7c** (30 mg, 115 μ mol), cesium carbonate (112 mg, 344 μ mol), 3-aminopyridine (22 mg, 230 μ mol), filtered and evaporated (1.3 mg, 5.7 μ mol), and Xantphos (6.6 mg, 12 μ mol) were stirred in anhydrous dioxane (1.5 mL). The reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with MeOH, then evaporated to dryness. Reverse phase column purification was performed ACN: water 5-95% with 0.1% formic acid, affording the title compound as a yellow solid (10.3 mg, 28%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.75 (s, 1 H) 9.00 (d, *J*=9.8 Hz, 1 H) 8.88 (d, *J*=2.9 Hz, 1 H) 8.71 (s, 1 H) 8.49 (d, *J*=5.4 Hz, 1 H) 8.23 (ddd, *J*=8.3, 2.9, 1.5 Hz, 1 H) 8.19 (dd, *J*=4.9, 1.5 Hz, 1 H) 7.39 (d, *J*=5.4 Hz, 1 H) 7.36 (dd, *J*=8.3, 4.9 Hz, 1 H) 7.18 (d, *J*=9.8 Hz, 1 H) 4.02 (s, 3 H). LC-MS (*m/z*): 320.1 [M+H]⁺.

(6-Methoxy-3-(2-(pyridin-3-ylamino)pyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazin-2-yl)methanol (23f).

In a vial **7l** (30 mg, 103 μ mol), cesium carbonate (101 mg, 309 μ mol), 3-aminopyridine (19.4 mg, 206 μ mol), palladium(II) acetate (1.2 mg, 8.2 μ mol), and Xantphos (6.0 mg, 10.3 μ mol) were stirred in anhydrous dioxane (1.5 mL). The reaction mixture was sealed then evacuated and backfilled with nitrogen 3X. The reaction mixture was then subjected to MW irradiation at 160 °C for 1.5 h. The reaction was filtered through celite and washed with MeOH, then evaporated to dryness. Reverse phase column purification was performed ACN: water 5-95% with 0.1% formic acid, to afford the title compound as a yellow solid (15.5 mg, 43%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm 9.75 (s, 1 H) 8.92 (d, *J*=9.8 Hz, 1 H) 8.88 (d, *J*=2.4 Hz, 1 H) 8.54 (d, *J*=5.4 Hz, 1 H) 8.24 (ddd, *J*=8.3, 2.4, 1.5 Hz, 1 H) 8.18 (dd, *J*=4.4, 1.5 Hz, 1 H) 7.45 (d, *J*=5.4 Hz, 1 H) 7.34 (dd, *J*=8.3, 4.4 Hz, 1 H) 7.13 (d, *J*=9.8 Hz, 1 H) 5.53 - 5.58 (m, 1 H) 4.80 (d, *J*=2.4 Hz, 2 H) 4.00 (s, 3 H). LC-MS (*m/z*): 350.2 [M+H]⁺.

4-(2-Cyclopropyl-6-methoxypyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyridin-3-yl)pyrimidin-2-amine

(23g). In a vial **7n** (30 mg, 99 μmol), cesium carbonate (97 mg, 298 μmol), 3-aminopyridine (19 mg, 199 μmol), filtered and evaporated (1.1 mg, 5.0 μmol), and Xantphos (5.8 mg, 10.0 μmol) were stirred in anhydrous dioxane (1.5 mL). The reaction mixture was sealed then evacuated and backfilled with N_2 three times. The reaction mixture was then subjected to microwave irradiation at 160 $^\circ\text{C}$ for 1 h. The reaction was filtered through celite and washed with MeOH, then evaporated to dryness. Reverse phase preparative HPLC purification was performed using ACN: water 5-95% with 0.1% formic acid, to provide the title compound as a yellow solid (7.4 mg, 21%). ^1H NMR (500 MHz, methanol- d_4) δ ppm 8.90 (d, $J=2.9$ Hz, 1 H) 8.77 (d, $J=9.3$ Hz, 1 H) 8.49 (d, $J=5.4$ Hz, 1 H) 8.31 (ddd, $J=8.3, 2.9, 1.5$ Hz, 1 H) 8.17 (dd, $J=4.9, 1.5$ Hz, 1 H) 7.45 (d, $J=5.4$ Hz, 1 H) 7.39 (dd, $J=8.3, 4.9$ Hz, 1 H) 6.94 (d, $J=9.3$ Hz, 1 H) 4.04 (s, 3 H) 2.36 - 2.43 (m, 1 H) 1.11 - 1.17 (m, 2 H) 1.05 - 1.10 (m, 2 H). LC-MS (m/z): 360.2 $[\text{M}+\text{H}]^+$.

4-(2-Cyclopropyl-6-morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyridin-3-yl)pyrimidin-2-amine

(23h). In a vial **7o** (30 mg, 84 μmol), cesium carbonate (82 mg, 252 μmol), 3-aminopyridine (16 mg, 168 μmol), palladium(II) acetate (0.9 mg, 4.2 μmol) and Xantphos (5 mg, 8.4 μmol) were stirred in anhydrous dioxane (1.5 mL), the reaction mixture was sealed then evacuated and backfilled with N_2 three times. The reaction mixture was then subjected to microwave irradiation at 160 $^\circ\text{C}$ for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Reverse phase preparative HPLC purification was performed using ACN: water 5-95% with 0.1% formic acid, to afford the title compound as a pale-yellow solid (11.0 mg, 32%). ^1H NMR (500 MHz, methanol- d_4) δ ppm 8.91 (d, $J=2.9$ Hz, 1 H) 8.62 (d, $J=9.8$ Hz, 1 H) 8.46 (d, $J=5.4$ Hz, 1 H) 8.29 (ddd, $J=8.3, 2.9, 1.5$ Hz, 1 H) 8.16 (dd, $J=4.9, 1.5$ Hz, 1 H) 7.43 (d, $J=5.4$ Hz, 2 H) 7.40 (dd, $J=8.3, 4.9$ Hz, 1 H) 7.17 (d, $J=9.8$ Hz, 1 H) 3.83 (t, $J=4.9$ Hz, 4 H) 3.55 (t, $J=4.9$ Hz, 4 H) 2.32 - 2.42 (m, 1 H) 1.10 - 1.15 (m, 2 H) 1.04 - 1.08 (m, 2 H). LC-MS (m/z): 415.1 $[\text{M}+\text{H}]^+$.

4-(6-Morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)-*N*-(pyridin-3-yl)pyrimidin-2-amine (23i). In a vial **7b** (30 mg, 95 μ mol), cesium carbonate (93 mg, 284 μ mol), 3-aminopyridine (18 mg, 189 μ mol), palladium(II) acetate (1.1 mg, 4.7 μ mol) and Xantphos (5.5 mg, 9.5 μ mol) were stirred in anhydrous dioxane (1.5 mL), the reaction mixture was sealed then evacuated and backfilled with N₂ three times. The reaction mixture was then subjected to microwave irradiation at 160 °C for 1 h. The reaction was filtered through celite and washed with EtOAc, then evaporated to dryness. Reverse phase preparative HPLC purification was performed using ACN: water 5-95% with 0.1% formic acid, affording the title compound as a white solid (7.2 mg, 20%). ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 8.89 - 8.93 (m, 1 H) 8.79 (d, *J*=9.8 Hz, 1 H) 8.38 - 8.42 (m, 2 H) 8.26 (ddd, *J*=8.3, 2.9, 1.5 Hz, 1 H) 8.15 - 8.21 (m, 1 H) 7.40 (dd, *J*=8.3, 4.9 Hz, 1 H) 7.24 - 7.29 (m, 2 H) 3.85 (t, *J*=4.9 Hz, 4 H) 3.60 (t, *J*=4.9 Hz, 4 H). LC-MS (*m/z*): 375.1 [M+H]⁺.

4-((4-(6-Morpholinopyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)cyclohexan-1-ol (23j). In a vial **2-4b** (20 mg, 63 μ mol), and *trans*-4-amino-cyclohexanol (36 mg, 316 μ mol) were stirred in *n*-BuOH (2 mL) and heated to 110 °C for 72 h. Reaction mixture was then concentrated and column purification was undertaken using MeOH: DCM, 2-5% to afford the title compound as a white solid (16.3 mg, 65.3%). ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 8.82 (d, *J*=9.8 Hz, 1 H) 8.37 (s, 1 H) 8.18 (d, *J*=5.4 Hz, 1 H) 7.30 (d, *J*=9.8 Hz, 1 H) 6.99 (d, *J*=5.4 Hz, 1 H) 3.80 - 3.90 (m, 5 H) 3.56 - 3.68 (m, 5 H) 2.12 - 2.20 (m, 2 H) 2.01 - 2.09 (m, 2 H) 1.38 - 1.55 (m, 4 H). LC-MS (*m/z*): 396.2 [M+H]⁺.

4-((4-(6-Methoxypyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)cyclohexan-1-ol (23k). In a vial **7c** (50 mg, 191 μ mol), and *trans*-4-aminocyclohexanol (44 mg, 392 μ mol) were stirred in *n*-BuOH (1.5 mL) subjected to microwave irradiation at 150 °C for 2.5 h. The reaction was concentrated *in vacuo* then subjected to column purification MeOH: DCM 0-5%, to afford the title compound as a yellow solid (38.5 mg, 59%). ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 8.88 - 8.95 (m, 1 H) 8.42 (s, 1 H) 8.18 (d, *J*=5.4 Hz, 1 H) 6.98 - 7.03 (m, 2 H) 4.07 (s, 3 H) 3.75 - 3.84 (m, 1 H) 3.58 - 3.66 (m, 1 H) 2.08 - 2.17 (m, 2 H) 1.99 - 2.06 (m, 2 H) 1.35 - 1.52 (m, 4 H). LC-MS (*m/z*): 341.2 [M+H]⁺.

4-((4-(2-Cyclopropyl-6-methoxypyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)cyclohexan-1-ol (23l). In a vial **7n** (30 mg, 99 μ mol), and *trans*-4-aminocyclohexanol (23 mg, 199 μ mol) were stirred in *n*-BuOH (1.5 mL) subjected to microwave irradiation at 150 °C for 2 h. The reaction was concentrated *in vacuo* then subjected to column purification MeOH: DCM 0-10%, to afford the title compound as a white solid (17.3 mg, 66% based on recovered starting material). ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 8.69 - 8.76 (m, 1 H) 8.23 (d, *J*=5.4 Hz, 1 H) 7.16 (d, *J*=5.4 Hz, 1 H) 6.92 (d, *J*=9.3 Hz, 1 H) 4.03 (s, 3 H) 3.75 - 3.85 (m, 1 H) 3.57 - 3.65 (m, 1 H) 2.33 - 2.41 (m, 1 H) 2.07 - 2.16 (m, 2 H) 1.99 - 2.05 (m, 2 H) 1.36 - 1.48 (m, 4 H) 1.09 - 1.13 (m, 2 H) 1.04 - 1.07 (m, 2 H). LC-MS (*m/z*): 381.2 [M+H]⁺.

4-((4-(2-(Hydroxymethyl)-6-methoxypyrazolo[1,5-*b*]pyridazin-3-yl)pyrimidin-2-yl)amino)cyclohexan-1-ol (23m). In a vial **7l** (50 mg, 171 μ mol), and *trans*-4-aminocyclohexanol (39.5 mg, 343 μ mol) were stirred in *n*-BuOH (1.5 mL) subjected to microwave irradiation at 150 °C for 2 h. The reaction was concentrated *in vacuo* then subjected to column purification EtOAc: hex 25-100%, to afford the title compound as a yellow solid (33.2 mg, 52%). ¹H NMR (500 MHz, methanol-*d*₄) δ ppm 8.70 (d, *J*=9.8 Hz, 1 H) 8.26 (d, *J*=5.4 Hz, 1 H) 7.09 (d, *J*=5.4 Hz, 1 H) 7.00 (d, *J*=9.8 Hz, 1 H) 4.91 (s, 2 H) 4.06 (s, 3 H) 3.75 - 3.83 (m, 1 H) 3.57 - 3.65 (m, 1 H) 2.07 - 2.15 (m, 2 H) 2.00 - 2.03 (m, 2 H) 1.35 - 1.50 (m, 4 H). LC-MS (*m/z*): 371.2 [M+H]⁺.

2-Chloro-4-methyl-6-((trimethylsilyl)ethynyl)pyrimidine (24a). In a reaction vial 2,4-dichloro-6-methylpyrimidine (1 g, 6.1 mmol), Pd(PPh₃)₂Cl₂ (86 mg, 123 μ mol), and CuI (35 mg, 184 μ mol) were added. A degassed solution of triethylamine (2.6 mL, 18.4 mmol) and THF (12 mL) were added under N₂ to the reaction vial, followed by ethynyltrimethylsilane (961 μ L, 6.75 mmol). The reaction mixture was heated to 50 °C for 16 h. Reaction mixture was then filtered through celite and washed with EtOAc. The reaction mixture was concentrated *in vacuo* then subjected to column purification EtOAc: hexanes 0-10%,

to yield the desired product as an off-white solid (370 mg, 27%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 7.16 (s, 1 H) 2.48 (s, 3 H) 0.24 (s, 9 H). LC-MS (m/z): 225.0 (³⁵Cl), 227.0 (³⁷Cl) [M+H]⁺.

2-Chloro-4-ethynyl-6-methylpyrimidine (24b). In a vial **24a** (370 mg, 1.65 mmol) was stirred in MeOH (10 mL) and KOH (0.46 mg, 8.2 μmol) was added. After 30 min the reaction was stopped and concentrated *in vacuo*, to yield the product as a pale-yellow solid (240 mg, 96%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 7.23 (s, 1 H) 3.40 (s, 1 H) 2.55 (s, 3 H). LC-MS (m/z): 152.7 (³⁵Cl), 154.7 (³⁷Cl) [M+H]⁺.

3-(2-Chloro-6-methylpyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazine (25). (Aminooxy) sulfonic acid (889 mg, 7.86 mmol) was dissolved in 7 mL water, to the clear solution added saturated sodium bicarbonate till effervescence ceased (pH ~ 6). The clear colorless solution was heated to 70 °C and pyridazine (378 μL, 5.24 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction was cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then solution of **24b** (200 mg, 1.31 mmol) in DCM (20 mL) was added followed by KOH (74 mg, 1.3 mmol) and the reaction was stirred at RT for 16 h. The reaction mixture was diluted with DCM and washed with water and brine then dried over sodium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 30-50% to provide the desired compound as a pink solid (121 mg, 38%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 9.06 (dd, *J*=9.3, 2.0 Hz, 1 H) 8.53 (s, 1 H) 8.46 (dd, *J*=4.4, 2.0 Hz, 1 H) 7.40 (s, 1 H) 7.29 (dd, *J*=9.3, 4.4 Hz, 1 H) 2.59 (s, 3 H). LC-MS (m/z): 246.1 (³⁵Cl), 248.0 (³⁷Cl) [M+H]⁺.

2-Chloro-5-methyl-4-((trimethylsilyl)ethynyl)pyrimidine (26a). In a reaction vial 2,4-dichloro-5-methylpyrimidine (1 g, 6.1 mmol), Pd(PPh₃)₂Cl₂ (86 mg, 123 μmol), and CuI (35 mg, 184 μmol) were added. A degassed solution of triethylamine (2.6 mL, 18.4 mmol) in THF (12 mL) was added under N₂ to the reaction vial, followed by ethynyltrimethylsilane (961 μL, 6.75 mmol). The reaction mixture was heated to 50 °C for 16 h. The reaction was then filtered through celite and washed with EtOAc. The reaction

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3 mixture was concentrated *in vacuo* and subjected to column purification EtOAc: hexanes 0-10%, to provide
4 the compound as a white solid (713 mg, 52%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 8.46 (s, 1 H) 2.36
5 (s, 3 H) 0.28 (s, 9 H). LC-MS (m/z): 225.0 (³⁵Cl), 227.0 (³⁷Cl) [M+H]⁺.
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11 **2-Chloro-4-ethynyl-5-methylpyrimidine (26b)**. In a vial **26a** (713 mg, 3.2 mmol) was stirred in MeOH
12 (20 mL) and KOH (0.9 mg, 16 μmol) was added and the reaction was stirred at RT. After 30 min the
13 reaction was stopped and concentrated *in vacuo*, then subjected to column purification EtOAc: hex 10-25%
14 to obtain the desired compound as a white solid (361 mg, 75%). ¹H NMR (500 MHz, DMSO-*d*₆) δ ppm
15 8.74 (s, 1 H) 5.13 (s, 1 H) 2.31 (s, 3 H). LC-MS (m/z): 152.8 (³⁵Cl), 154.7 (³⁷Cl) [M+H]⁺.
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24 **3-(2-Chloro-5-methylpyrimidin-4-yl)pyrazolo[1,5-*b*]pyridazine (27)**. (Aminooxy) sulfonic acid (889
25 mg, 7.86 mmol) was dissolved in 7 mL water, saturated sodium bicarbonate was added to the clear solution
26 until effervescence ceased (pH ~ 6). The clear colorless solution was heated at 70 °C and pyridazine (378
27 μL, 5.24 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction was then
28 cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then a solution
29 of **26b** (200 mg, 1.31 mmol) in DCM (20 mL) was added followed by KOH (148 mg, 2.6 mmol) and the
30 reaction was stirred at RT for 16 h. The reaction mixture was diluted with DCM and washed with water
31 and brine then dried over sodium sulfate, filtered and evaporated to get crude product which was purified
32 by column chromatography using MeOH: DCM 0-2% to yield the desired compound as a brown solid (206
33 mg, 64%). ¹H NMR (500 MHz, chloroform-*d*) δ ppm 9.13 (dd, *J*=9.3, 2.0 Hz, 1 H) 8.55 (s, 1 H) 8.50 (dd,
34 *J*=4.4, 2.0 Hz, 1 H) 8.45 (s, 1 H) 7.32 (dd, *J*=9.3, 4.4 Hz, 1 H) 2.60 (s, 3 H). LC-MS (m/z): 246.1 (³⁵Cl),
35 248.0 (³⁷Cl) [M+1].
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51 **2-(3-Chlorophenyl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (28)**. In a vial 3-chloriodobenzene (52
52 μL, 420 μmol), bis(pinacolato)diboron (160 mg, 629 μmol), potassium acetate (144 mg, 1.47 mmol) and
53 PdCl₂(dppf)·CH₂Cl₂ (17 mg, 21 mmol) were combined and the vial was sealed. Anhydrous dioxane (3 mL)
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was added and the reaction was evacuated and backfilled with N₂ three times, then subjected to microwave irradiation at 160 °C for 30 min. The reaction was cooled and filtered through celite, concentrated *in vacuo* and taken forward without purification. LC-MS (m/z): 156.7 (³⁵Cl), 158.8 (³⁷Cl) [M+H]⁺ (boronic acid).

3-(6-Chloropyridin-2-yl)pyrazolo[1,5-*b*]pyridazine (29). (Aminooxy) sulfonic acid (296 mg, 2.62 mmol) was dissolved in 2 mL water, to the clear solution added saturated sodium bicarbonate until effervescence ceased (pH ~ 6). The clear colorless solution was heated to 70 °C, then pyridazine (126 µL, 1.74 mmol) was added and the reaction mixture was stirred at 70 °C for 2 h. The reaction mixture was then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then solution of 2-chloro-6-ethynylpyridine (120 mg, 872 µmol) in DCM (10 mL) was added followed by KOH (49 mg, 872 µmol) and the reaction mixture was stirred at 40 °C for 72 h, partial conversion to product was observed. Additional KOH (49 mg, 872 µmol) was added and the reaction was stirred for another 24 h at 40 °C. The reaction mixture was diluted with DCM and water and extracted. The organic layer was washed with brine then dried over sodium sulfate, filtered and evaporated to get crude product which was purified by column chromatography using EtOAc: hex 25% to yield the product as a tan solid (18.5 mg, 15% based on recovered starting material). ¹H NMR (400 MHz, chloroform-*d*) δ ppm 9.00 (dd, *J*=8.8, 2.2 Hz, 1 H) 8.44 (s, 1 H) 8.38 (dd, *J*=4.4, 2.2 Hz, 1 H) 7.69 (t, *J*=8.1 Hz, 1 H) 7.58 (d, *J*=8.1 Hz, 1 H) 7.13 - 7.22 (m, 2 H). LC-MS (m/z): 231.0 (³⁵Cl), 233.0 (³⁷Cl) [M+H]⁺.

3-(2-Chloropyridin-4-yl)pyrazolo[1,5-*b*]pyridazine (30). (Aminooxy) sulfonic acid (2.06 g, 18.2 mmol) was dissolved in 4 mL water, saturated sodium bicarbonate was added to the clear solution until effervescence ceased (pH ~ 6). The clear colorless solution was heated at 70 °C and pyridazine (875 µL, 12.1 mmol) was added. The reaction mixture heated at 70 °C for 2 h, then cooled to RT, neutralized with saturated sodium bicarbonate (until effervescence ceased) and then a solution of 2-chloro-4-ethynylpyridine (417 mg, 3.03 mmol) in DCM (15 mL) and KOH (170 mg, 3.0 mmol) was added then reaction mixture was

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3 stirred for 16 h at RT. The reaction mixture was diluted with DCM and washed with water and brine then
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5 dried over magnesium sulfate, filtered and evaporated to get crude product which was purified by column
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7 chromatography using MeOH: DCM 0-2% followed by an additional column purification using EtOAc:
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9 hexanes 25-100% to afford the desired compound as a brown solid (173 mg, 25%). ¹H NMR (500 MHz,
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11 chloroform-*d*) δ ppm 8.46 (d, *J*=5.4 Hz, 1 H) 8.43 (dd, *J*=4.4, 2.0 Hz, 1 H) 8.36 (s, 1 H) 8.28 (dd, *J*=9.3,
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13 2.0 Hz, 1 H) 7.53 - 7.56 (m, 1 H) 7.44 (dd, *J*=5.4, 1.5 Hz, 1 H) 7.22 (dd, *J*=9.3, 4.4 Hz, 1 H). LC-MS (*m/z*):
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15 230.9 (³⁵Cl), 233.0 (³⁷Cl) [M+H]⁺.
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20 Supporting Information

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22 Cidal assay results, additional compounds and associated biological data not presented in the manuscript,
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24 comparison of *T. b. brucei* pEC₅₀ inhibition data to literature values for GSK-3β pIC₅₀, CDK-2 pIC₅₀, and
25
26 CDK-4 pIC₅₀, additional ADME data (including mouse plasma stability, CYP450 inhibition and induction,
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28 Caco-2), kinase panel profiling of **20g**, blood and brain concentration of **20r** as measured in the PK study,
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30 parasitemia levels measured during efficacy study, biological data of compound set against *T. cruzi*, *L.*
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32 *donovani*, *S. mansoni*, L6 and THP-1 cells, HPLC traces of all compounds tested, and molecular formula
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34 strings file.
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39 Acknowledgements

40
41 This work was supported by National Institutes of Health grants (R01AI114685 (M.P.P and M.N),
42
43 R01AI082577 (M.P.P), R56AI099476 (M.P.P), R01AI124046 (M.P.P), R21AI127594 (M.P.P), the
44
45 Spanish Ministerio de Economía, Industria y Competitividad, (MN: SAF2015-71444-P; DG-P: SAF2016-
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47 79957-R. <http://www.mineco.gob.es>) and Subdirección General de Redes y Centros de Investigación
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49 Cooperativa (RICET) (MN: RD16/0027/0019; DG-P: RD16/0027/0014. <https://bit.ly/2OegOnX>) RTI2018-
50
51 097210-B-100 (MINCIU-FEDER) to FG. CRC acknowledges grant support from the NIH-NIAID
52
53 (R21AI126296) and the Bill and Melinda Gates Foundation (OPP1171488), as well as the technical
54
55 assistance of Brian M. Suzuki for screening adult *S. mansoni*. We are grateful to AstraZeneca for
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performing the *in vitro* ADME experiments presented throughout and to Charles River Labs for the *in vitro* ADME data presented in Tables S3-S5 in the Supplementary Information. We would like to thank GSK Tres Cantos open lab foundation for running the PK studies discussed in this publication. An academic license for ChemAxon (<https://www.chemaxon.com>) is gratefully acknowledged. We would like to thank Dr. Melissa Buskes for help in the preparation of this manuscript.

Abbreviations used

BBB: blood-brain barrier; NECT: nifurtimox-eflornithine combination therapy; IV: intravenous; HTS: high-throughput screen; CNS: central nervous system; MPO: multiparameter optimization; ADME: absorption, distribution, metabolism, and excretion; RH: rat hepatocytes; Cl_{int} : intrinsic clearance; HAT: human African trypanosomiasis; HLM: human liver microsomes; GSK-3 β : Glycogen synthase kinase 3 beta; CDK: cyclin dependent kinases; PPB: plasma protein binding; aq. sol.: thermodynamic aqueous solubility; HBD: hydrogen bond donor; SAR: structure-activity relationship; PK: pharmacokinetic; IP: intraperitoneal.

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Table of contents graphic

