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# Medicinal chemistry optimization of a diaminopurine chemotype: Towards a lead for *Trypanosoma brucei* inhibitors

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## ABSTRACT

Human African trypanosomiasis (HAT), or sleeping sickness, is caused by the protozoan parasite *Trypanosoma brucei* and transmitted through the bite of infected tsetse flies. The disease is considered fatal if left untreated. To identify new chemotypes against *T. brucei*, previously we identified 797 potent kinase-targeting inhibitors grouped into 59 clusters plus 53 singleton compounds with at least 100-fold selectivity over HepG2 cells. From this set of hits, a cluster of diaminopurine-derived compounds was identified. Herein we report our medicinal chemistry investigation involving exploration of structure-activity and structure-property relationships around one of the HTS hits, N<sup>2</sup>-(thiophen-3-yl)-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**1**, NEU-1106). This work lead to the identification of a potent lead compound (**4aa**, NEU-4854) with improved ADME properties, which was progressed into proof-of-concept translation of *in vitro* antiparasitic activity to *in vivo* efficacy.

## INTRODUCTION

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3 Neglected tropical diseases (NTDs) are a group of infectious diseases that affect more than one  
4 billion people in 149 tropical and subtropical countries.<sup>1</sup> Human African trypanosomiasis (HAT),  
5 also known as sleeping sickness, is one of the diseases classified as an NTDs by the World Health  
6 Organization. There are 65 million people at risk of HAT, and it is usually fatal if left untreated.<sup>2</sup>  
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Neglected tropical diseases (NTDs) are a group of infectious diseases that affect more than one billion people in 149 tropical and subtropical countries.<sup>1</sup> Human African trypanosomiasis (HAT), also known as sleeping sickness, is one of the diseases classified as an NTDs by the World Health Organization. There are 65 million people at risk of HAT, and it is usually fatal if left untreated.<sup>2</sup> Acc. to WHO, only 1447 new cases were reported in 2017, but HAT still threaten millions in 36 sub-Saharan African countries.<sup>4,5</sup> HAT is caused by the parasite *T. brucei* which infects the host in two stages. In the first, hemolymphatic phase, parasites stay in the bloodstream and interstitial spaces of the adipose tissue and skin, and then in the second, neurologic phase, the pathogen has crossed the blood-brain barrier and invades the brain, leading to sleep disruption, coma, and death if left untreated.<sup>6</sup> As with all NTDs, drug discovery efforts for HAT face numerous challenges, especially the large cost of developing the drug together with the poor financial incentives to big pharma companies.<sup>7</sup> Nonetheless, one compound, fexinidazole, was recently approved by the European Medicines Administration,<sup>8</sup> and a second (acoziborole) is in the midst of human clinical trials.<sup>9</sup>

In the resource-constrained field of NTDs, repurposing or repositioning existing drugs has been proven to be quite effective because of reduced costs during discovery and development, the potential to recover and repurpose previously failed compounds, and lower overall risk.<sup>7</sup> We have undertaken the repurposing of existing or investigational drugs as starting points for drug discovery. Since *T. brucei* has been known to express essential kinases that are homologous to human kinases,<sup>10-12</sup> we have found that repurposing human kinase inhibitors can be successfully utilized for uncovering antitrypanosomal lead compounds.<sup>13-17</sup>

Utilizing this so-called “lead repurposing” approach,<sup>7</sup> we have previously reported 797 potent and selective inhibitors by high-throughput screening of a kinase library with over 42,000 compounds.<sup>18</sup> One of the promising hits from this screening was NEU-1106 (**1**).<sup>18</sup> The targeted properties and profile of compound **1** are listed in **Table 1** along with the targeted properties for antitrypanosomal lead compounds. Herein, we report the structure activity relationships (SAR) and structure property relationships (SPR) for analogs of **1** that lead to the discovery of lead compound **4aa** (NEU-4854) with improved ADME and physicochemical properties.

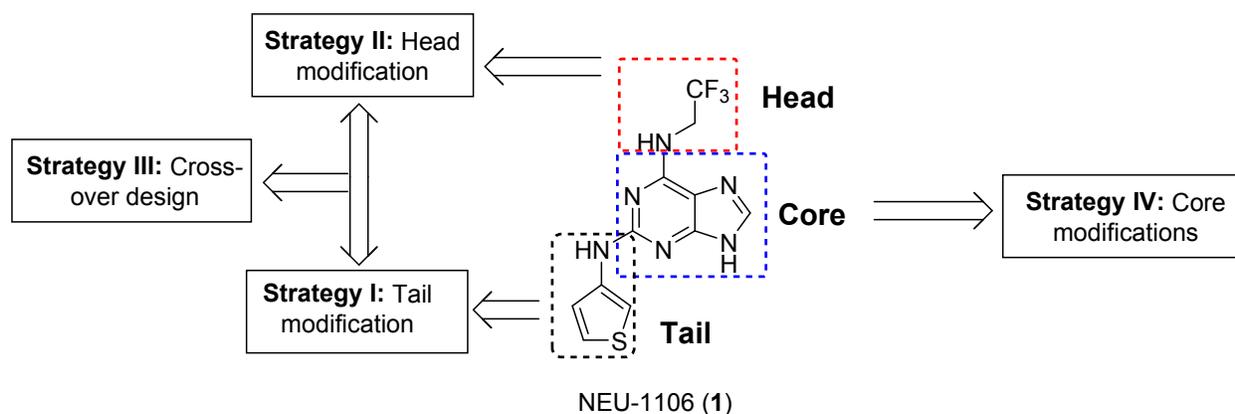
**Table 1.** Targeted parameters and corresponding values of NEU-1106 (1)

	Targeted Lead Properties	NEU-1106 (1)
<i>T. b. brucei</i> pEC <sub>50</sub>	≥7.0	7.5
MRC5 pTC <sub>50</sub>	<5.0	4.3
cLogP	≤3	2.6
PSA	40 < x < 90	79
LLE	≥4	4.9
Thermodynamic Aqueous solubility (μM)	≥10	69
MW	≤360	314
LogD <sub>7.4</sub>	≤2	3.1
CNS MPO score	≥4	4.5
Human PPB (%)	≤95	95
HLM CL <sub>int</sub> (mL/min/mg protein)	<47	64
Rat Hepatocyte CL <sub>int</sub> (mL/min/10 <sup>6</sup> )	<27	9.5

## RESULTS AND DISCUSSION

Overall, NEU-1106 (1) possesses a good profile as described in Table 1, but it suffers the fact that it has high human liver microsome clearance (64.2 μL/min/mg) and high logD<sub>7.4</sub> (3.1). Further, the presence of a thiophene ring is considered as a structural alert in drug design, as the cytochrome P450 metabolism of thiophene can lead to the formation of highly reactive metabolites *viz.* thiophene S-oxides and thiophene epoxides.<sup>19</sup> Hence, in addition to replacement of the thiophene ring, our work was focused on further improving the LLE value by reducing the lipophilicity, which was expected to lead to improved ADME properties.

We followed a four-step systematic strategy. The first approach involves C-2 modifications (tail region) of the diaminopurine core, followed by C-6 modifications (head region). In the third strategy, cross-over compounds were synthesized by choosing combination of best C-2 and C-6 substitutions (head and tail combinations); Finally, core modifications were performed in order to identify new scaffolds that could potentially be pursued against *T. brucei*.



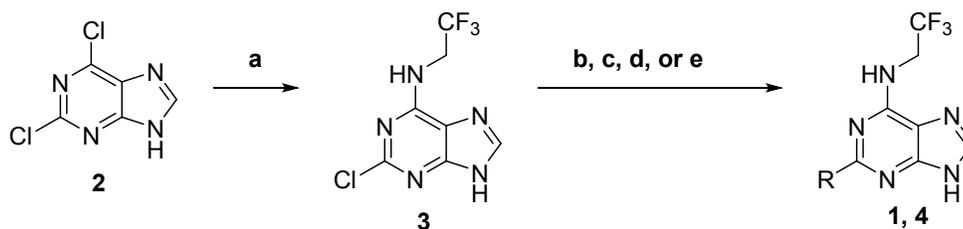
**Figure 1.** Structure of NEU-1106 (1) and our medicinal chemistry strategy

**C-2 substitutions.** The compounds **3** and **4a-4ab** were synthesized by utilizing the methods shown in **Scheme 1**. The C-6 amination of 2,6-dichloro-9*H*-purine (**2**) with 2,2,2-trifluoroethylamine under basic conditions at 90 °C yielded compound **3**. Compound **3** was then reacted with varying amines via palladium-mediated C-N coupling, nucleophilic substitution (compounds **4a-4m** and **4r-4ab**), or C-C coupling via Suzuki reaction (**4n-4q**). Initially the 3-aminothiophene moiety was replaced with aliphatic amines (**4a** and **4b**), which resulted in the improvement of aqueous solubility, but potency was lost. On the other hand, substitutions using a variety of anilines resulted in improvement in potency compared to compound **1** but led to the reduction in solubility. In an attempt to improve the solubility of the aniline-substituted compound **4c**, a variety of substitutions were made; fluoro (**4k**), trifluoromethyl (**4l**) and N-methylpiperazine (**4m**) were introduced. The N-methylpiperazine substitution (**4m**) resulted in improvement of solubility and other ADME properties but the potency was reduced by more than two log units, whereas fluoro (**4k**) and trifluoromethyl (**4l**) substitutions did not result in significant changes in potency or solubility as compared to **4c**.

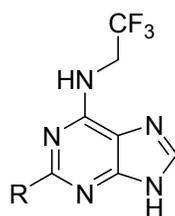
The replacement of the thiophene ring with alternative ring systems resulted in varied SAR and SPR trends. Compounds **4t**, **4w**, **4y** and **4z** showed promising activity against *T. brucei* ( $pEC_{50} > 6$ ). Interestingly, compounds **4v** and **4x** were found to be inactive, whereas methylation of the pyrimidine or pyrazine ring (compounds **4w** and **4y**) displayed improved potency. Compound **4z** showed excellent aqueous solubility (734  $\mu\text{M}$ ) but slightly lower potency compared to **1**. Subsequent replacement of the pyrazole N-ethyl with N-methyl **4aa** improved the potency and overall physicochemical and ADME properties over other compounds.

Lipophilic ligand efficiency ( $LLE = pEC_{50} - cLogP$ ) describes the quality of a lead compound, gauging how much of the potency is due to specific binding interactions versus the general lipophilicity of the compound.<sup>20</sup> LLE allows the capture of both potency and lipophilicity in a single parameter.<sup>20, 21</sup> Our targeted LLE values for a lead compound is  $\geq 4$ , as aligned with standard medicinal chemistry practices. Several tested compounds were in excess of this goal. In particular, compound **4aa** was found to be the best compound in C-2 substituted series with an LLE value of 5.7 and an appreciably improved physicochemical properties profile. The potency data and *in vitro* ADME profile of compounds **1**, **3** and **4a-4ab** are listed in Table 2.

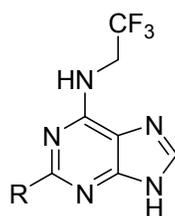
The role of the anilino N-H hydrogen bond donor was also investigated by synthesizing compounds by either deletion of the intervening -NH from the C-2 position or by N-methylation (**4n-4r**). The loss in potency suggested that a hydrogen bond donor is required at the C-2 position.



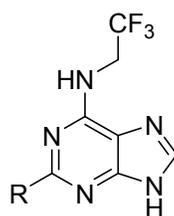
**Scheme 1.** Synthetic route for compounds **4a-4ab**. Reagents and conditions: (a) 2,2,2-trifluoroethylamine, DIPEA, *n*-BuOH, 90 °C, 16 h (17%); (b) secondary amine, IPA, 80 °C, 16h; (c) amine, HCl, dioxane, microwave, 150 °C, 40 min.; (d) amine, Pd<sub>2</sub>dba<sub>3</sub>, XPhos, KOtBu, *t*-BuOH, 100 °C, 16h; (e) boronic acid, Cs<sub>2</sub>CO<sub>3</sub>, Pd(PPh<sub>3</sub>)<sub>4</sub>, 2:1 DME/H<sub>2</sub>O, 150 °C, microwave, 60 min.

**Table 2.** Inhibition profile against *T. brucei* and *in vitro* ADME profile of C-2 substituted purines**1, 3, 4a-4ab**

Entry	R	<i>T. brucei</i> pEC <sub>50</sub> <sup>a</sup> / Log fold Selectivity <sup>b</sup>	<i>T. brucei</i> LLE <sup>c</sup>	Aq. sol. (μM)	Human Liver Microsome CL <sub>int</sub> (uL/min/mg protein)	Rat hepatocyte CL <sub>int</sub> (uL/min/10 <sup>6</sup> cells)
1		7.5 / 3.2	4.9	69	64	9.5
3	-Cl	4.4 / >0.10	2.8	705	< 3	8.7
4a		4.7 / >0.40	3.3	>1000	< 3	6.6
4b		< 4.4	--	>1000	14	14
4c		7.8 / >3.5	5.0	15	26	10
4d		7.4 / >3.1	4.1	20	32	30
4e		7.8 / >3.5	4.5	21	89	22
4f		7.6 / 3.3	5.0	10	67	48
4g		7.6 / >3.3	5.0	55	120	41
4h		7.4 / 3.1	4.7	15	26	10
4i		7.8 / 3.3	4.4	9.0	22	14
4j		7.1 / 2.6	4.0	4.0	121	47

**Table 2.** Inhibition profile against *T. brucei* and *in vitro* ADME profile of C-2 substituted purines**1, 3, 4a-4ab**

Entry	R	<i>T. brucei</i> pEC <sub>50</sub> <sup>a</sup> / Log fold Selectivity <sup>b</sup>	<i>T. brucei</i> LLE <sup>c</sup>	Aq. sol. (μM)	Human Liver Microsome CL <sub>int</sub> (uL/min/mg protein)	Rat hepatocyte CL <sub>int</sub> (uL/min/10 <sup>6</sup> cells)
4k		7.4 / >3.1	4.5	46	64	18
4l		7.7 / 3.4	4.0	5.0	40	9.0
4m		5.8 / 1.2	3.0	175	<3	9.8
4n		5.0 / >0.70	1.8	10	120	255
4o		4.9 / >0.60	1.9	55	209	88
4p		5.5 / >1.2	1.7	0.70	53	65
4q		5.2 / >0.94	1.8	3.0	79	7.0
4r		5.6 / >1.3	2.6	10	161	44
4s		4.8 / >0.50	3.2	955	<3.0	<1.0
4t		7.0 / >2.7	4.1	1.0	33	3.9
4u		5.5 / >1.2	4.0	27	58	>300
4v		4.4 / >0.10	2.8	358	30	2.2
4w		6.2 / >1.9	4.5	97	51	21

**Table 2.** Inhibition profile against *T. brucei* and *in vitro* ADME profile of C-2 substituted purines**1, 3, 4a-4ab**

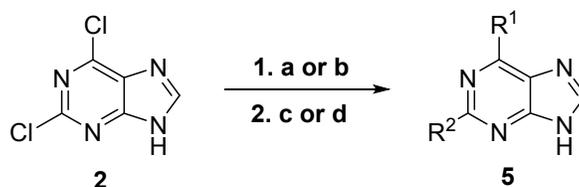
Entry	R	<i>T. brucei</i> pEC <sub>50</sub> <sup>a</sup> / Log fold Selectivity <sup>b</sup>	<i>T. brucei</i> LLE <sup>c</sup>	Aq. sol. (μM)	Human Liver Microsome CL <sub>int</sub> (uL/min/mg protein)	Rat hepatocyte CL <sub>int</sub> (uL/min/10 <sup>6</sup> cells)
4x		5.8 / >1.5	4.8	5.0	23	2.7
4y		6.4 / >2.1	5.3	3.0	162	20
4z		6.2 / >1.9	4.5	734	50	2.0
4aa		6.9 / 2.6	5.7	212	17	2.3
4ab		<5.0	--	135	12	5.0

<sup>a</sup>pEC<sub>50</sub>=-logEC<sub>50</sub>; <sup>b</sup>Log fold selectivity = *T. brucei* pEC<sub>50</sub>-MRC5 pTC<sub>50</sub>; <sup>c</sup>LLE (lipophilic ligand efficiency) = pEC<sub>50</sub>-clogP; Additional ADME data, including Log D<sub>7.4</sub> and human plasma protein binding, are included in Table S1 of the supporting information. All SD within ±0.16

**C-6 substitutions.** The compounds **5a-5aa** were synthesized as shown in **Scheme 2**. The C-6 amination of 2,6-dichloro-9*H*-purine (**2**) with the respective amines at either room temp. or heating at 80 °C followed by reaction with 3-aminothiophene or aniline via Buchwald or nucleophilic substitution yielded compound **5**. Several analogs were synthesized by replacing the C-6 amine with different primary and secondary amines. Also, some matched pair analogs (**5v-5z**) were synthesized with aniline at the C-2 position to observe the variations in SAR and SPR comparing the 3-aminothiophene substitution with its aniline bioisostere.<sup>22</sup> The replacement of the terminal CF<sub>3</sub> group of **1** with CH<sub>3</sub> (**5b**) resulted in little change in the potency, an increase in solubility, and a decrease in metabolic stability as compared to **1**.

In addition, a set of analogs with varied numbers of carbon atoms ( $n=1-4$ ) was synthesized (compounds **5a-5d**). In general, with the increase in carbon length potency increased but microsomal clearance increased as well. Compound **5d** was very potent against *T. brucei* ( $pEC_{50} = 8.0$ ) but high clearance was observed. Though we had assumed that a terminal trifluoromethyl group would improve metabolic clearance properties (c.f. **1** versus **5b**), this approach did not pay dividends in improving the properties (c.f. **5d** vs. **5e**).

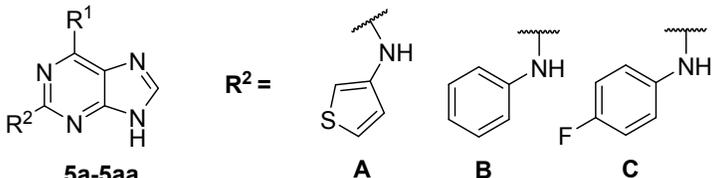
Several other primary and secondary aliphatic amines were added to the C-6 position while keeping the thiophene ring constant at the C-2 position. Similarly, a few matched pair analogs with the aniline bioisostere at the C-2 position were also synthesized. Overall, compounds **5f**, **5m**, **5q** and **5v** were found to be improved among different C-6 substituted aliphatic amines, showing low metabolic clearance, good aqueous solubility and acceptable potency range. The potency data and *in vitro* ADME profile of compounds **5a-5aa** are listed in Table 3.

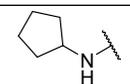
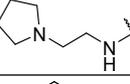
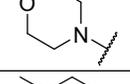
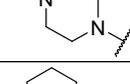
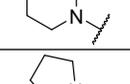
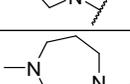
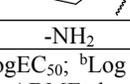


**Scheme 2.** Synthetic route for compounds **5a-5ae**. Reagents and conditions: (a) amine, THF, rt, 16 h; (b) amine THF:IPA (1:1), 80 °C, 4h; (c) amine, HCl, dioxane, microwave, 150 °C, 40 min.; (d) amine, Pd<sub>2</sub>dba<sub>3</sub>, XPhos, KOtBu, t-BuOH, 100 °C, 16h.

Table 3. Inhibition profile against <i>T. brucei</i> and <i>in vitro</i> ADME profile of C-6 substituted purines							
Entry	R <sub>1</sub>	R <sub>2</sub>	<i>T. brucei</i> pEC <sub>50</sub> <sup>a</sup> / Log fold Selectivity <sup>b</sup>	<i>T. brucei</i> LLE <sup>c</sup>	Aq. sol. (μM)	Human Liver Microsome CL <sub>int</sub> (uL/min/mg protein)	Rat hepatocyte CL <sub>int</sub> (uL/min/10 <sup>6</sup> cells)
<b>5a</b>		A	6.0 / >1.4	5.0	621	39	79
<b>5b</b>		A	7.2 / >2.3	5.0	515	135	29
<b>5c</b>		A	7.5 / >2.9	4.1	159	300	136

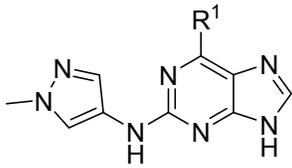


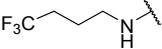
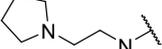
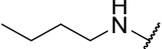
**Table 3.** Inhibition profile against *T. brucei* and *in vitro* ADME profile of C-6 substituted purines


Entry	R <sub>1</sub>	R <sub>2</sub>	<i>T. brucei</i> pEC <sub>50</sub> <sup>a</sup> / Log fold Selectivity <sup>b</sup>	<i>T. brucei</i> LLE <sup>c</sup>	Aq. sol. (μM)	Human Liver Microsome CL <sub>int</sub> (uL/min/mg protein)	Rat hepatocyte CL <sub>int</sub> (uL/min/10 <sup>6</sup> cells)
5t		A	7.6 / 3.1	4.5	33	138	38
5u		A	5.9 / >1.6	3.3	544	10	6.7
5v		B	7.1 / >2.8	4.5	35	18	13
5w		B	6.6 / >2.3	5.3	>1000	56	66
5x		B	7.5 / >3.2	2.8	41	27	86
5y		B	7.4 / >3.1	4.0	459	186	48
5z		B	6.4 / >2.1	2.6	>1000	36	17
5aa	-NH <sub>2</sub>	C	<4.4	5.7	>1000	33	45

<sup>a</sup>pEC<sub>50</sub>=-logEC<sub>50</sub>; <sup>b</sup>Log fold selectivity = *T. brucei* pEC<sub>50</sub>-MRC5 pTC<sub>50</sub>; <sup>c</sup>LLE (lipophilic ligand efficiency) = pEC<sub>50</sub>-clogP; Additional ADME data, including Log D<sub>7.4</sub> and human plasma protein binding, are included in Table S1 of the supporting information. All SD within ±0.11

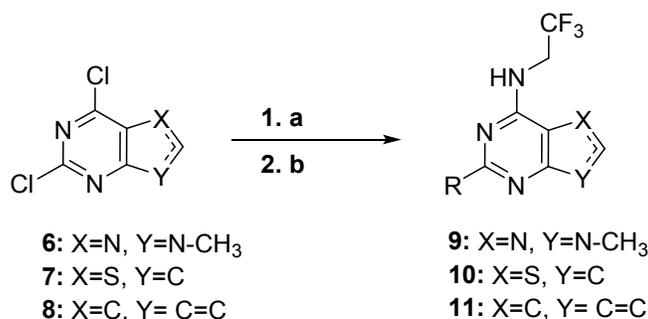
**Crossover C-2 and C-6 substitutions.** The crossover compounds (**5ab-5ad**, Table 4) were designed by selecting the best tail substitution at C-2 (4-amino-1-methylpyrazole) with other selected amines at C-6 position. The compounds were synthesized by the procedure described in Scheme 2. Compounds **5ae** and **5ac** resulted in improvement of potency compared to **4aa**, however the clearance rate was also increased. Two others, **5ad** and **5ab**, did show better solubility and ADME properties but reduced in potency by 0.6 -1.0 log unit compared to compounds **5u** and **5f** respectively.

**Table 4.** Inhibition profile against *T. brucei* and *in vitro* ADME profile of cross-over analogs


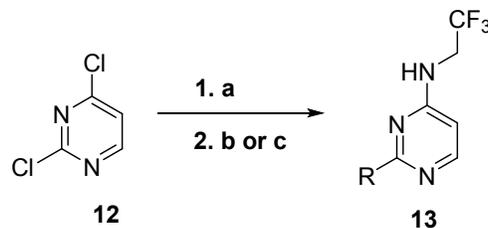
Entry	R <sub>1</sub>	<i>T. brucei</i> pEC <sub>50</sub> <sup>a</sup> / Log fold Selectivity <sup>b</sup>	<i>T. brucei</i> LLE <sup>b</sup>	Aq. sol. (μM)	Human Liver Microsome CL <sub>int</sub> (uL/min/mg protein)	Rat hepatocyte CL <sub>int</sub> (uL/min/10 <sup>6</sup> cells)
5ab		6.6 / 1.9	5.9	530	23	3.2
5ac		7.3 / 3.0	5.5	140	146	25
5ad		4.9 / >0.60	4.2	852	3	--
5ae		7.4 / >3.1	5.8	176	207	70

<sup>a</sup>pEC<sub>50</sub>=-logEC<sub>50</sub>; <sup>b</sup>Log fold selectivity = *T. brucei* pEC<sub>50</sub>-MRC5 pTC<sub>50</sub>; <sup>c</sup>LLE (lipophilic ligand efficiency) = pEC<sub>50</sub>-clogP; Additional ADME data, including Log D<sub>7.4</sub> and human plasma protein binding, are included in Table S1 of the supporting information. All SD within ±0.12

**Core modifications.** The modification of the core structure was performed as described in **Schemes 3-4** by replacing the purine scaffold with pyrimidine, quinazoline and thienopyrimidine, or by N-methylation of the imidazopyrimidine to explore the role of the hydrogen bond donor at N6. All the core modifications resulted in loss of activity except for the thienopyrimidine substitution (compound **10**) which showed moderate potency against *T. brucei* with pEC<sub>50</sub> value of 6.4. The potency data and *in vitro* ADME profile of core modified compounds **9a-11** and **13a-b** are listed in Table 5.



**Scheme 3.** Synthetic route for compounds **9-11**. Reagents and conditions: (a) 2,2,2-trifluoroethylamine, DIPEA, n-BuOH, 90 °C, 16 h; (b) amine, Pd<sub>2</sub>dba<sub>3</sub>, XPhos, KOtBu, t-BuOH, 100 °C, 16h.



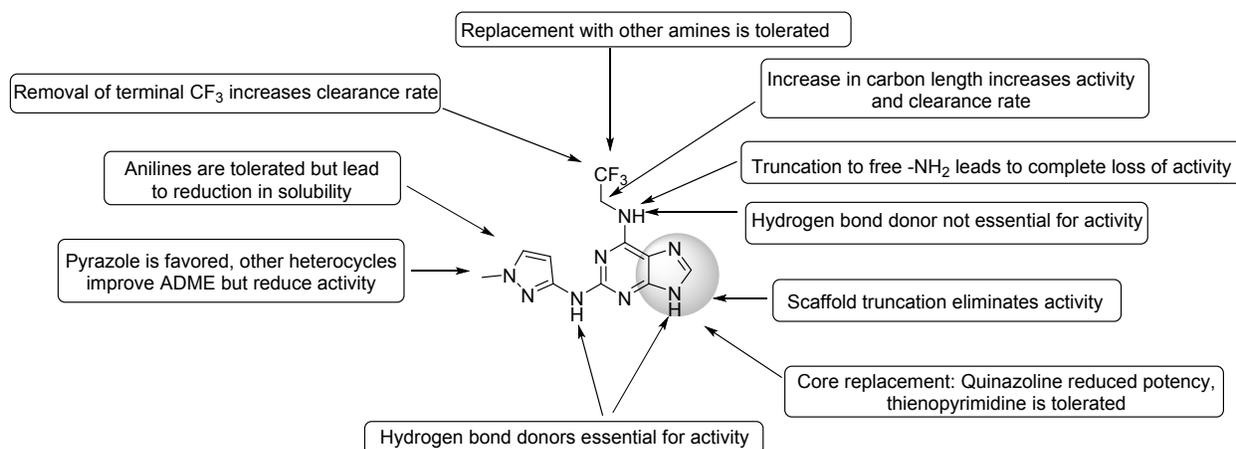
**Scheme 4.** Synthetic route for compounds **13a-13b**. Reagents and conditions: (a) 2,2,2-trifluoroethylamine, DIPEA, n-BuOH, 90 °C, 16 h; (b) aniline, HCl, dioxane, microwave, 150 °C, 40 min.; (c) 3-aminothiophene, Pd<sub>2</sub>dba<sub>3</sub>, XPhos, KOtBu, t-BuOH, 100 °C, 16h.

**Table 5.** Inhibition profile against *T. brucei* and *in vitro* ADME profile of core modified analogs

Entry	R	<i>T. brucei</i> pEC <sub>50</sub> <sup>a</sup> / Log fold Selectivity <sup>b</sup>	<i>T. brucei</i> LLE <sup>c</sup>	Aq. sol. (μM)	Human Liver Microsome CL <sub>int</sub> (uL/min/mg protein)	Rat hepatocyte CL <sub>int</sub> (uL/min/10 <sup>6</sup> cells)
9a		5.1 / >0.80	2.2	60	154	44
9b		5.3 / >1.0	3.8	47	51	8.7
10		6.4 / 2.1	2.1	12.3	144	56
11		5.4 / 0.70	1.0	6.9	58	68
13a		5.0 / >0.70	2.0	451	>300	100
13b		4.9 / >0.60	1.6	200	>300	115

<sup>a</sup>pEC<sub>50</sub> = -logEC<sub>50</sub>; <sup>b</sup>Log fold selectivity = *T. brucei* pEC<sub>50</sub>-MRC5 pTC<sub>50</sub>; <sup>c</sup>LLE (lipophilic ligand efficiency) = pEC<sub>50</sub>-clogP; Additional ADME data, including Log D<sub>7.4</sub> and human plasma protein binding, are included in Table S1 of the supporting information. All SD within ±0.090

The highlights of structure activity relationship (SAR) and structure property relationship (SPR) around lead compound **4aa** are depicted in **figure 2**.



**Figure 2.** Key SAR and SPR points around this series for anti *T. brucei* activity (**4aa**, NEU-4854)

**Cell permeability, metabolic studies and kinase profiling.** Based upon overall profile of potency and *in vitro* ADME properties, compound **4aa** was selected for further studies including Caco-2 permeability studies, mouse plasma stability, mouse liver microsome clearance and CYP450 induction. The results in Table 6 show compound **4aa** does not have CYP3A4 induction liability. Though the compound is permeable to Caco-2 cells, it is rapidly effluxed. The compound was also found to be stable in mouse liver microsome and plasma stability assays. Since this chemotype originated from a kinase-targeted library screen, we decided to profile compound **4aa** against a panel of human kinases at 5  $\mu$ M (supporting information Figure S1). Though compound **4aa** was found to inhibit several human kinases, it did not show any toxicity in animal efficacy studies and against *in vitro* cell toxicity assays. Additionally, we have tested compound **4aa** against TbMAPK6 (K5140) which is essential for the life cycle of *T. brucei* and was found to be a better inhibitor over parent compound **1** with IC<sub>50</sub> values of 1.2 and 7.6  $\mu$ M respectively (see supporting information for details). However, moderate inhibition of enzyme suggests this is not the target of action for this series.

**Table 6.** Caco-2 permeability data, mouse plasma stability, liver microsome stability and CYP450 induction of compound **4aa**

Entry	Caco-2 Mean Papp A-B (10 <sup>6</sup> cm/s)	Caco-2 Mean Papp B-A (10 <sup>6</sup> cm/s)	Efflux Ratio	Mean A-B Recovery (%)	Mean B-A Recovery (%)	A-B Permeability Ranking	Mouse Plasma Stability % remaining at 60 min	Mouse Liver Microsome (CL <sub>int</sub> ) (mL/min/g liver)	CYP3A4 fold induction (at 10 μM)	CYP3A4 % inhibition (at 10 μM)
<b>4aa</b>	5.8	44	7.6	87	102	Higher	98	4.1	0.17 (No induction)	33

**Table 7.** Summary table of compound **NEU-4854 (4aa)**

Parameters	NEU-4854 (4aa)
<i>T. b. brucei</i> pEC <sub>50</sub>	6.9
MRC5 pTC <sub>50</sub>	4.3
cLogP	1.2
PSA	96
LLE	5.7
Thermodynamic Aqueous solubility (μM)	212
MW	312
LogD <sub>7.4</sub>	1.9
CNS MPO score	5.0
Human PPB (%)	72
HLM CL <sub>int</sub> (mL/min/mg)	17
Rat Hepatocyte CL <sub>int</sub> (mL/min/10x6)	2.3
Mouse Liver Microsome (CL <sub>int</sub> ) (mL/min/g liver)	4.1
CYP3A4 Liability	No
Mouse plasma stability	Stable
Caco-2 permeability	Permeable

**Pharmacokinetics and efficacy studies of compound 4aa in acute mouse model of HAT.** All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals. The compound **4aa** was selected for *in vivo* studies based upon its overall properties (Table 7). Compound **4aa** showed the best combination of potency with selectivity over mammalian cells, favorable physicochemical and *in vitro* ADME properties. Pharmacokinetic (PK) parameters were measured in plasma of female NMRI mice following an IP dose of 10 mg/kg of **4aa**. The peripheral blood levels of **4aa** is shown in Figure 3A. The mean  $C_{\max}$  of **4aa** was found to be 3670 ng/mL (11.75  $\mu$ M) (Table S2) and this concentration is approximately 95-fold higher than the  $EC_{50}$  of **4aa** (0.123  $\mu$ M). However, the compound appears to be cleared quickly in mice with  $t_{1/2} = 0.76$  h (Figure 3A). Blood and brain ratios of **4aa** at 0.5 and 4 hours after the administration are shown in Table S3.

We aimed to achieve plasma drug levels of more than 10x  $EC_{50}$  for 4-6 h; as shown in Figure 3A, this goal is accomplished for under four hours. Noting the rapid clearance, in order to achieve the targeted drug levels and to provide a means to perform proof-of-concept efficacy experiment, we both elevated the IP dose to 30 mg/kg and utilized 1-aminobenzotriazole (ABT) pre-treatment in the mouse efficacy model of HAT disease. ABT is known to inhibit cytochrome P450 and it has been used to increase the exposure of co-administered molecules.<sup>23, 24</sup> Indeed, the modified studies indicated increased exposure levels of **4aa** with ABT pre-treatment, demonstrating 10x  $EC_{50}$  for beyond 24 hours in both infected and uninfected mice (Figure 3B). The pharmacokinetic parameters are summarized in (Table 8).

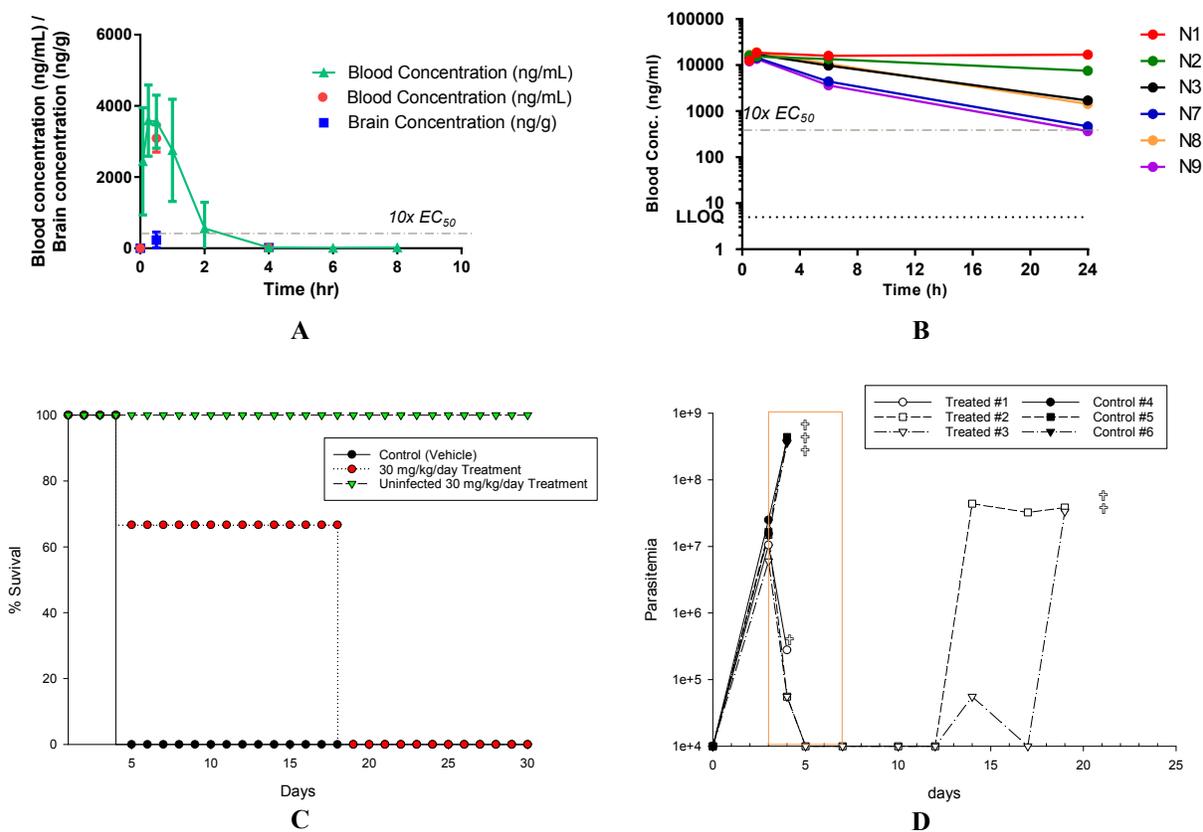
The pharmacokinetic parameters of ABT pre-treated infected and uninfected mice dosed with **4aa** were also measured on the fifth days of dosing; significant improvement in the exposure levels was observed (Table S4; and Figure S1).

**Table 8.** Blood pharmacokinetic parameters of **4aa** at intraperitoneal administration of 10 mg/kg without ABT-pretreatment and 30 mg/kg (target dose) after ABT-pretreatment to female NMRI mice (infected and non-infected) on day 1 of treatment.

Mouse type	Dose (mg/kg)	Mean $C_{\max}$ (ng/mL) $\pm$ SD	Mean $t_{\max}$ (h)	Mean $t_{1/2}$ (h)	Mean $AUC_{0-t}$ (ng·h/ml)	Mean AUC (ng·h/ml)
Non-infected	10	3670 $\pm$ 922	0.25-0.5	0.758 $\pm$ 0.290	5340 $\pm$ 2860	5350 $\pm$ 2870

<b>Non-infected</b>	30 + ABT	15767 ± 3007	0.5-2.0	0.638 ± 0.047	51799 ± 15424	52521 ± 16034
<b>Infected</b>	30 + ABT	17267 ± 1258	0.5-2.0	1.58 ± 1.11	77666 ± 15686	83443 ± 26918

The efficacy studies were performed in female NMRI mice infected with *T. brucei brucei* STIB795 parasites. All groups of animals were pre-treated with ABT (50 mg/kg po). The treatment with **4aa** (30 mg/kg, ip, once daily) was started from day 3 following infection and continued until day 7. The parasitemia levels in control (untreated) infected animals reached  $10^8$ /mL on day 4 and all control animals died on day 5, whereas treated groups of animals showed reduction in parasitemia levels compared to control animals on day 4 (Figure 3E). The survival rate of treated groups was increased by 18 days post-treatment (Figure 3D). The uninfected mouse treated with **4aa** did not show any signs of toxicity and all animals survived until the end of the studies (Figure 3D). During the experiment, one infected mouse showed some signs of lethargy after two days of treatment and was humanely euthanized. This study identifies compound **4aa** as a promising lead for the treatment of HAT, however further studies are required to identify a dosing regimen for the complete cure of infection in mice, and to increase brain penetration.



**Figure 3.** (A) *in vivo* pharmacokinetics data of **4aa** at dose 10 mg/kg ip in non-infected female NMRI mice; (B) *in vivo* pharmacokinetics data of **4aa** at dose 30 mg/kg ip after ABT-pretreatment in *Tbb* STIB795 infected mice (N1-N3) and uninfected mice (N7-N9) on day 1 of treatment; (C) percentage survival of animals in acute *in vivo* murine HAT model (NMRI mice; *T. brucei brucei* STIB795 parasites); (D) Parasitemia levels graph in murine HAT model, cross indicates the day of death, values represented on the x axis indicate undetected parasitemia (values below  $10^4$  parasites/ml which cannot be detected by counting in the Neubauer chamber). Start of infection (arrow) and treatment period (orange box) indicated.

**African animal trypanosomiasis (AAT).** *T. brucei*, *Trypanosoma congolense* and *Trypanosoma vivax* are all known to cause AAT in wild and domestic animals. AAT is a significant cause of mortality amongst livestock and has a large economic impact on the development of agriculture in Africa. We have tested our lead compound **4aa** against AAT-causing parasites, *T. congolense* and *T. vivax*, and it was found to inhibit proliferation of *T. congolense* and *T. vivax* with  $EC_{50}$  values of 1.1 and 2.7  $\mu$ M respectively. Thus, compound **4aa** can also be further utilized as a starting point for the discovery of agents for the treatment of AAT.

## CONCLUSION

Utilizing a lead repurposing approach, a collection of analogs of the HTS hit NEU-1106 (**1**) were synthesized and assessed for biological activity against *T. brucei* and their ADME properties were measured. Structure activity relationships and structure property relationships have been established, leading to the discovery of a potent anti-trypanosomal agent (**4aa**, NEU-4854) with improved ADME properties. Compound **4aa** was advanced to efficacy studies in a mouse model of HAT and it increased the survival time of infected animals by 18 days post treatment at 30 mg/kg. Work is ongoing to identify a dosing regimen of **4aa** for the complete cure of infection in mice; and to explore the potential of a new chemotype (**10**) for anti-trypanosomal activity which was identified here using scaffold replacement strategy.

## EXPERIMENTAL SECTION

**General Chemistry.** All reagents and starting materials were procured commercially from Sigma-Aldrich Inc., Fisher Scientific or Combi-blocks, and used as received. Melting points were recorded on Thermo Scientific MEL-TEMP apparatus. NMR spectra were obtained on a Varian NMR system, operating at 400 MHz and 500 MHz. Chemical data for protons is reported in parts per million (ppm) downfield from tetramethylsilane and are referenced to the residual proton in the NMR solvent [(CD<sub>3</sub>)<sub>2</sub>SO, 2.50; CD<sub>3</sub>OD, 3.31; CDCl<sub>3</sub>, 7.26; (CD<sub>3</sub>)<sub>2</sub>CO, 2.05; ppm]. LCMS analysis was performed using a Waters e2795 Alliance or Waters e2695 Alliance or Agilent 1100 reverse-phase HPLC-MS and 3.5 μm Waters SunFire C18 4.6 x 50mm column, with multiwavelength photodiode array detector (λ = 200-600 nm) and MicroMass ZQ single quadrupole mass spectrometer (electrospray ionization). Gradients for the LCMS analysis was water or acetonitrile, both with 0.1% v/v formic acid (Method A: 5% acetonitrile to 100% acetonitrile over 0-4 mins; Method B: 5% acetonitrile to 100% acetonitrile over 0-8 mins; Method C: 0% acetonitrile to 50% acetonitrile over 0-4 mins). Microwave reactions were performed in a Biotage Initiator+ or CEM Discovery SP instruments. Purification of intermediates and final compounds was performed using silica gel chromatography using the Biotage IsoleraOne flash purification system or unless otherwise noted. All newly synthesized compounds were deemed >95% pure by LCMS (PDA, λ = 200-600 nm).

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6 **Synthesis of 2-chloro-*N*-(2,2,2-trifluoroethyl)-9*H*-purin-6-amine (3).** 1 g of 2,6-  
7 dichloro-9*H*-purine (2) was dissolved in n-BuOH (7 ml) in a screw cap vial. To this  
8 diisopropylethylamine (2.0 equiv.) was added followed by addition of 2,2,2-trifluoroethylamine  
9 (1.5 equiv.). The reaction mixture was then heated at 90 °C for 16h. The reaction mixture was then  
10 allowed to cool down and concentrated under vacuum at 50 °C. Dichloromethane was added and  
11 the ppts formed were filtered and checked on LCMS. The first ppts were discarded. The filtrate  
12 was then left for 1h. The resulting ppts were filtered and washed with hexane. The solid was dried  
13 under high vacuum to yield product (3, yield 17%, light yellow solid, m.p. 244-246). <sup>1</sup>H NMR  
14 (500 MHz, Acetone-*d*<sub>6</sub>) δ 8.15 (s, 1H), 4.43 (brs, 2H). LCMS found 252.03 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.19  
15 min (Method A).  
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26 **General method for preparation of compounds 4a-b.** 2-chloro-*N*-(2,2,2-trifluoroethyl)-  
27 9*H*-purin-6-amine (3) was dissolved in isopropyl alcohol (0.03 M), To this, desired amine (10  
28 equiv.) were added and the reaction mixture was heated at 80 °C for 16h. On completion, reaction  
29 was concentrated under vacuum and desired product was purified by preparative HPLC (5–50%  
30 water–acetonitrile).  
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35 *2-(4-methyl-1,4-diazepan-1-yl)-N-(2,2,2-trifluoroethyl)-9H-purin-6-amine (4a).* (Yield:  
36 38%, light brown solid, m.p. 276-278). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 7.79 (s, 1H), 4.38-4.33  
37 (m, 2H), 4.09 (s, 2H), 3.95-3.92 (m, 2H), 3.38-3.33 (m, 2H), 3.23-3.15 (m, 2H), 2.79 (s, 3H), 2.24-  
38 2.17 (m, 2H). LCMS found 330.11 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.10 min (Method C).  
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43 *2-(4-methylpiperazin-1-yl)-N-(2,2,2-trifluoroethyl)-9H-purin-6-amine (4b).* (Yield: 34%,  
44 white solid, m.p. 266-268). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 7.78 (s, 1H), 4.35-4.31 (m, 2H), 3.89-  
45 3.77 (m, 4H), 2.54-2.52 (m, 4H), 2.36 (s, 3H). LCMS found 316.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 0.42 min (Method  
46 A).  
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53 **General method for preparation of compounds 4c-l, 4r and 4s.** 2-chloro-*N*-(2,2,2-  
54 trifluoroethyl)-9*H*-purin-6-amine (3) and desired aniline (5 equiv.) were mixed in microwave  
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sealed tube. To this, dioxane (0.02 M) and two drops of HCl (catalytic amount) were added. The reaction mixture was then heated in microwave reactor at 150 °C for 40 mins. The reaction mixture was then basified with NaHCO<sub>3</sub> solution. The solid product was filtered and dried under high vacuum to give desired product.

N<sup>2</sup>-phenyl-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4c**). (Yield: 52%, gray solid, m.p. 338-340). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.33 (s, 1H), 8.56 (brs, 1H), 8.43 (brs, 1H), 7.76 (d, *J* = 7.9 Hz, 2H), 7.27 (t, *J* = 7.8 Hz, 2H), 6.94 (t, *J* = 7.1 Hz, 1H), 4.41 (brs, 2H). LCMS found 309.02 [M+H]<sup>+</sup>, t<sub>R</sub> = 5.73 min (Method B).

N<sup>2</sup>-(*p*-tolyl)-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4d**). (Yield: 47%, light brown solid, m.p. 343-345). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.18 (s, 1H), 8.47 (brs, 1H), 8.32 (brs, 1H), 7.63 (d, *J* = 8.2 Hz, 2H), 7.26 (brs, 1H), 7.08 (d, *J* = 8.3 Hz, 2H), 4.50-4.34 (m, 2H), 2.26 (s, 3H). LCMS found 323.06 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.33 min (Method A).

N<sup>2</sup>-(*m*-tolyl)-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4e**). (Yield: 57%, off-white solid, m.p. 332-334). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.20 (s, 1H), 8.44 (brs, 1H), 8.31 (brs, 1H), 7.65 (s, 1H), 7.49 (d, *J* = 7.1 Hz, 1H), 7.14 (t, *J* = 7.8 Hz, 1H), 6.76 (d, *J* = 7.5 Hz, 1H), 4.40 (brs, 2H), 2.29 (s, 3H). LCMS found 323.06 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.40 min (Method A).

N<sup>2</sup>-(4-methoxyphenyl)-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4f**). (Yield: 50%, brown solid, m.p. 245-247). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.77 (s, 1H), 7.98 (brs, 1H), 7.87 (s, 1H), 7.67 (d, *J* = 9.0 Hz, 2H), 6.83 (d, *J* = 9.1 Hz, 2H), 4.32 (brs, 2H), 3.72 (s, 3H). LCMS found 339.12 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.05 min (Method A).

N<sup>2</sup>-(3-methoxyphenyl)-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4g**). (Yield: 56%, brown solid, m.p. 298-300). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ 8.12 (s, 1H), 7.42 (t, *J* = 2.2 Hz, 1H), 7.23-7.15 (m, 2H), 6.65-6.63 (m, 1H), 4.47-4.41 (m, 2H), 3.83 (s, 3H). LCMS found 338.99 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.27 min (Method A).

N<sup>2</sup>-(3-cyanophenyl)-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4h**). (Yield: 45%, off-white solid, m.p. 294-296). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.66 (s, 1H), 8.58 (s, 1H), 8.43 (s, 2H), 7.92 (d, *J* = 8.2 Hz, 1H), 7.47 (t, *J* = 8.0 Hz, 1H), 7.38-7.07 (m, 2H), 4.39 (brs, 2H). LCMS found 333.96 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.42 min (Method A).

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3  $N^2$ -(3-chlorophenyl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4i**). (Yield: 54%,  
4 gray solid, m.p. 323-325).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  9.52 (s, 1H), 8.55 (s, 1H), 8.48 (s,  
5 1H), 8.10 (s, 1H), 7.59 (d,  $J = 7.8$  Hz, 1H), 7.27 (t,  $J = 8.1$  Hz, 1H), 6.96 (d,  $J = 7.8$  Hz, 1H), 4.41  
6 (brs, 2H). LCMS found 343.02  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 2.71$  min (Method A).  
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11 7-((6-((2,2,2-trifluoroethyl)amino)-9H-purin-2-yl)amino)-3,4-dihydronaphthalen-1(2H)-  
12 one (**4j**). (Yield: 24%, off-white solid, m.p. 252-254).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.62  
13 (s, 1H), 9.14 (s, 1H), 8.37 (s, 1H), 8.15 (s, 1H), 7.91 (d,  $J = 13.8$  Hz, 2H), 7.22 (d,  $J = 8.4$  Hz, 1H),  
14 4.35 (brs, 2H), 3.18 (d,  $J = 5.2$  Hz, 1H), 2.88 (t,  $J = 5.9$  Hz, 1H), 2.59-2.57 (m, 2H), 2.05-2.02 (m,  
15 2H). LCMS found 377.03  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 2.27$  min (Method A).  
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20  $N^2$ -(4-fluorophenyl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4k**). (Yield: 62%,  
21 gray solid, m.p. 326-328).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  9.33 (s, 1H), 8.55 (d,  $J = 8.3$  Hz,  
22 1H), 7.77-7.74 (m, 2H), 7.33-7.26 (m, 2H), 7.13-7.09 (m, 2H), 4.39 (brs, 2H). LCMS found 327.0  
23  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 4.57$  min (Method B).  
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28  $N^6$ -(2,2,2-trifluoroethyl)- $N^2$ -(3-(trifluoromethyl)phenyl)-9H-purine-2,6-diamine (**4l**).  
29 (Yield: 49%, gray solid, m.p. 188-190).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.67 (s, 1H), 9.43 (s,  
30 1H), 8.45 (s, 1H), 8.21 (s, 1H), 7.95 (d,  $J = 8.1$  Hz, 1H), 7.87 (s, 1H), 7.45 (t,  $J = 8.0$  Hz, 1H), 7.19  
31 (d,  $J = 7.7$  Hz, 1H), 4.32 (brs, 2H). LCMS found 377.0  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 2.14$  min (Method A).  
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35  $N^2$ -methyl- $N^2$ -phenyl- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4r**). (Yield: 59%,  
36 brown solid, m.p. 232-234).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.53 (s, 1H), 7.98 (s, 1H), 7.83  
37 (s, 1H), 7.37-7.33 (m, 4H), 7.16-7.12 (m, 1H), 4.11 (bs, 2H), 3.46 (s, 3H). LCMS found 323.0  
38  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 2.23$  min (Method A).  
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43  $N^2$ -(pyridin-4-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4s**). (Yield: 36%, off-  
44 white semi-solid).  $^1\text{H}$  NMR (500 MHz,  $\text{CD}_3\text{OD}+\text{CDCl}_3$ )  $\delta$  9.24 (d,  $J = 7.8$  Hz, 2H), 6.96 (d,  $J =$   
45 7.8 Hz, 2H), 4.40-4.34 (m, 2H). LCMS found 309.96  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.31$  min (Method A).  
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51 **General method for preparation of compounds 4n-q.** A mixture of 2-chloro- $N$ -(2,2,2-  
52 trifluoroethyl)-9H-purin-6-amine (**3**), respective boronic acid (2 equiv.), cesium carbonate (4  
53 equiv.), and tetrakis(triphenylphosphine)-palladium (0) (5 mol%) in 1:2 water/DME (0.02 M) was  
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3 subjected to heating in microwave at 150 °C for 1h. The reaction mixture was then partitioned  
4 thrice with water and dichloromethane. The organic layer was dried and then purified by flash  
5 chromatography using 1% to 5% of methanol in dichloromethane gradient to get desired product.  
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9 *2-phenyl-N-(2,2,2-trifluoroethyl)-9H-purin-6-amine (4n)*. (Yield: 26%, off-white solid,  
10 m.p. 243-245). <sup>1</sup>H NMR (500 MHz, Acetone-d<sub>6</sub>) δ 8.52 (d, *J* = 7.1 Hz, 2H), 8.17 (s, 3H), 7.49 –  
11 7.46 (m, 2H), 4.67 (brs, 2H). LCMS found 294.0 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.76 min (Method A).  
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15 *2-(3-methoxyphenyl)-N-(2,2,2-trifluoroethyl)-9H-purin-6-amine (4o)*. (Yield: 42%, light  
16 yellow solid, m.p. 267-269). <sup>1</sup>H NMR (500 MHz, Acetone-d<sub>6</sub>) δ 8.18 (s, 1H), 8.10 (d, *J* = 12.3  
17 Hz, 2H), 7.39 (t, *J* = 7.9 Hz, 1H), 7.04-7.02 (m, 1H), 4.66 (brs, 2H), 2.86 (s, 3H). LCMS found  
18 324.0 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.77 min (Method A).  
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23 *2-(3-chlorophenyl)-N-(2,2,2-trifluoroethyl)-9H-purin-6-amine (4p)*. (Yield: 48%, white  
24 solid, m.p. 271-273). <sup>1</sup>H NMR (500 MHz, Acetone-d<sub>6</sub>) δ 8.50 (s, 1H), 8.46 (d, *J* = 7.2 Hz, 1H),  
25 8.21 (s, 1H), 7.52-7.50 (m, 2H), 4.67 (brs, 2H). LCMS found 328.0 [M+H]<sup>+</sup>, t<sub>R</sub> = 3.09 min (Method  
26 A).  
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31 *2-(4-fluorophenyl)-N-(2,2,2-trifluoroethyl)-9H-purin-6-amine (4q)*. (Yield: 34%, off-  
32 white solid, m.p. 240-242). <sup>1</sup>H NMR (500 MHz, Acetone-d<sub>6</sub>) δ 8.56 (t, *J* = 7.9 Hz, 1H), 8.17 (s,  
33 1H), 7.75-7.70 (m, 1H), 7.64-7.60 (m, 1H), 7.25-7.22 (m, 1H), 4.66 (brs, 2H). LCMS found 312.0  
34 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.58 min (Method A).  
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40 **General method for preparation of compounds 1, 4m and 4t-4ab.** A mixture of 2-  
41 chloro-*N*-(2,2,2-trifluoroethyl)-9*H*-purin-6-amine (**3**), respective amine (2 equiv.), Pd<sub>2</sub>(dba)<sub>3</sub> (5  
42 mol%), XPhos (15 mol%) and potassium tert-butoxide (3 equiv.) were added to a reaction vial. To  
43 this, *t*-butanol (0.02 M) was added, reaction vial was flushed with inert gas (nitrogen or argon) and  
44 stirred for 16h at 100 °C. The reaction mixture was filtered, and the residue was washed with  
45 acetone. The filtrate was dried and then purified by flash chromatography using 1% to 25% of  
46 methanol in dichloromethane gradient to get desired product.  
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53 *N*<sup>2</sup>-(*thiophen-3-yl*)-*N*<sup>6</sup>-(2,2,2-trifluoroethyl)-9*H*-purine-2,6-diamine (**1**). (Yield: 59%,  
54 light brown solid, m.p. 240-242). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.52 (s, 1H), 9.34 (s, 1H),  
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8.03 (brs, 1H), 7.89 (s, 1H), 7.59 (s, 1H), 7.38 (dd,  $J = 5.0, 3.0$  Hz, 1H), 7.20 (d,  $J = 5.0$  Hz, 1H), 4.32 (brs, 2H). LCMS found 315.06  $[M+H]^+$ ,  $t_R = 2.23$  min (Method A).

$N^2$ -(4-(4-methylpiperazin-1-yl)phenyl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4m**). (Yield: 62%, brown solid, m.p. 255-257).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.50 (s, 1H), 8.69 (s, 1H), 7.95 (s, 1H), 7.84 (s, 1H), 7.61 (d,  $J = 8.8$  Hz, 2H), 6.84 (d,  $J = 9.0$  Hz, 2H), 4.33 (brs, 2H), 3.04 (t,  $J = 4.25$  Hz, 4H), 2.45 (t,  $J = 4.55$  Hz, 4H), 2.22 (s, 3H). LCMS found 407.19  $[M+H]^+$ ,  $t_R = 1.27$  min (Method A).

$N^6$ -(2,2,2-trifluoroethyl)- $N^2$ -(6-(trifluoromethyl)pyridin-3-yl)-9H-purine-2,6-diamine (**4t**). (Yield: 62%, white solid, m.p. 283-285).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.78 (s, 1H), 9.73 (s, 1H), 9.10 (s, 1H), 8.51 (d,  $J = 8.5$  Hz, 1H), 8.00 (s, 1H), 7.76 (d,  $J = 8.7$  Hz, 1H), 4.33 (brs, 2H). LCMS found 378.0  $[M+H]^+$ ,  $t_R = 2.71$  min (Method A).

$N^2$ -(pyrimidin-4-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4u**). (Yield: 29%, off-white solid, m.p. 163-165).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.89 (s, 1H), 9.86 (s, 1H), 8.72 (s, 1H), 8.50 (d,  $J = 5.9$  Hz, 1H), 8.34 (d,  $J = 7.2$  Hz, 2H), 8.05 (s, 1H), 4.34 (brs, 2H). LCMS found 311.1  $[M+H]^+$ ,  $t_R = 1.38$  min (Method A).

$N^2$ -(pyrimidin-2-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4v**). (Yield: 40%, off-white solid, m.p. 328-330).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.89 (s, 1H), 9.53 (s, 1H), 8.53 (d,  $J = 4.8$  Hz, 2H), 8.01 (s, 1H), 6.96 (t,  $J = 4.8$  Hz, 1H), 4.35 (brs, 2H). LCMS found 311.0  $[M+H]^+$ ,  $t_R = 1.32$  min (Method A).

$N^2$ -(4-methylpyrimidin-2-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4w**). (Yield: 28%, off-white solid, m.p. 305-307).  $^1H$  NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  8.39 (d,  $J = 5.0$  Hz, 1H), 7.95 (s, 1H), 6.88 (d,  $J = 5.0$  Hz, 1H), 4.48 (brs, 2H), 2.41 (s, 3H). LCMS found 325.0  $[M+H]^+$ ,  $t_R = 1.43$  min (Method A).

$N^2$ -(pyrazin-2-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4x**). (Yield: 54%, off-white solid, m.p. 348-350).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.78 (s, 1H), 9.57 (s, 2H), 8.28 (s, 2H), 8.15 (d,  $J = 2.3$  Hz, 1H), 8.00 (s, 1H), 4.34 (s, 2H). LCMS found 311.0  $[M+H]^+$ ,  $t_R = 1.78$  min (Method A).

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$N^2$ -(6-methylpyrazin-2-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4y**). (Yield: 54%, white solid, m.p. >360 out of range).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.77 (s, 1H), 9.42 (s, 2H), 8.26 (s, 1H), 8.05 (s, 1H), 8.00 (s, 1H), 4.32 (brs, 2H), 2.40 (s, 3H). LCMS found 325.0  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.92$  min (Method A).

$N^2$ -(1-ethyl-1H-pyrazol-5-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4z**). (Yield: 62%, light orange solid, m.p. 228-230).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.57 (s, 1H), 8.62 (s, 1H), 8.09 (s, 1H), 7.87 (s, 1H), 7.34 (d,  $J = 1.8$  Hz, 1H), 6.16 (d,  $J = 1.7$  Hz, 1H), 4.26 (brs, 2H), 4.00 (q,  $J = 7.2$  Hz, 2H), 1.28 (t,  $J = 7.2$  Hz, 3H). LCMS found 327.0  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.92$  min (Method A).

$N^2$ -(1-methyl-1H-pyrazol-5-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4aa**). (Yield: 54%, off-white solid, m.p. 252-254).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.41 (s, 1H), 8.74 (s, 1H), 7.93 (s, 1H), 7.82 (s, 2H), 7.48 (s, 1H), 4.29 (brs, 2H), 3.78 (s, 3H). LCMS found 313.17  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.49$  min (Method A).

$N^2$ -(5,6-dimethyl-1,2,4-triazin-3-yl)- $N^6$ -(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (**4ab**). (Yield: 49%, off-white solid, m.p. 329-331).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.83 (s, 1H), 9.87 (s, 1H), 8.17 (s, 1H), 8.01 (s, 1H), 4.35 (brs, 2H), 2.53 (s, 3H), 2.42 (s, 3H). LCMS found 340.0  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.52$  min (Method A).

**General method for preparation of compounds 5a-e, 5k-5u and 5ac-ae.** 2,6-dichloro-9H-purine (**1**) was dissolved in THF:IPA (1:1; 0.04 M). To this, respective amine (5 equiv.) was added (if amine was in HCl salt form then 7.5 equiv. of triethylamine was added to free base the amine salt), and the reaction mixture was then stirred at 80 °C for 4h. The reaction was set aside for 1h and the ppts formed were filtered to give C-6 aminated intermediate. The C-6 aminated intermediate, 3-aminothiophene (2 equiv.),  $\text{Pd}_2(\text{dba})_3$  (5 mol%), XPhos (15 mol%) and potassium tert-butoxide (3 equiv.) were added to a reaction vial. To this, t-butanol (0.02 M) was added, reaction vial was flushed with inert gas (nitrogen or argon) and stirred for 16h at 100 °C. The reaction mixture was filtered, and the residue was washed with acetone. The filtrate was dried and then purified by flash chromatography using 1% to 25% of methanol in dichloromethane gradient to get desired product.

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3 *N*<sup>6</sup>-methyl-*N*<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (**5a**). (Yield: 55%, light brown  
4 solid, m.p. 175-177). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.34 (s, 1H), 9.23 (s, 1H), 7.77 (s, 1H),  
5 7.63 (s, 1H), 7.41 (d, *J* = 2.2 Hz, 1H), 7.34 (s, 1H), 7.18 (d, *J* = 4.3 Hz, 1H), 2.96 (s, 3H). LCMS  
6 found 247.1 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 1.51 min (Method A).  
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10 *N*<sup>6</sup>-ethyl-*N*<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (**5b**). (Yield: 65%, brown solid, m.p.  
11 196-198). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 12.31 (s, 1H), 9.16 (s, 1H), 7.75 (s, 1H), 7.59 (s, 1H),  
12 7.39 (s, 1H), 7.35 (dd, *J* = 5.0, 3.2 Hz, 1H), 7.16 (d, *J* = 4.9 Hz, 1H), 3.63-3.37 (m, 2H), 1.19 (t, *J*  
13 = 7.1 Hz, 3H). LCMS found 261.1 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 1.71 min (Method A).  
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18 *N*<sup>6</sup>-propyl-*N*<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (**5c**). (Yield: 52%, brown solid,  
19 m.p. 151-153). <sup>1</sup>H NMR (400 MHz, DMSO-*d*<sub>6</sub>) δ 12.34 (s, 1H), 9.18 (s, 1H), 7.77 (s, 1H), 7.60  
20 (s, 1H), 7.49 (d, *J* = 5.0 Hz, 1H), 7.35 (s, 1H), 7.17 (d, *J* = 3.8 Hz, 1H), 1.65-1.60 (m, 2H), 1.20  
21 (m, 2H), 0.91 (t, *J* = 7.3 Hz, 3H). LCMS found 275.1 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 1.84 min (Method A).  
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26 *N*<sup>6</sup>-butyl-*N*<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (**5d**). (Yield: 65%, light brown solid,  
27 m.p. 158-160). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 12.33 (s, 1H), 9.18 (s, 1H), 7.78 (s, 1H), 7.61  
28 (s, 1H), 7.44 (s, 1H), 7.35 (dd, *J* = 5.0, 3.3 Hz, 1H), 7.18 (d, *J* = 4.8 Hz, 1H), 3.58-3.39 (m, 2H),  
29 1.64-1.58 (m, 2H), 1.37 (dd, *J* = 14.9, 7.4 Hz, 2H), 0.93 (t, *J* = 7.4 Hz, 3H). LCMS found 289.06  
30 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 1.91 min (Method A).  
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35 *N*<sup>2</sup>-(thiophen-3-yl)-*N*<sup>6</sup>-(4,4,4-trifluorobutyl)-9H-purine-2,6-diamine (**5e**). (Yield: 47%,  
36 brown solid, m.p. 145-147). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 9.17 (s, 1H), 7.80 (s, 1H), 7.59 (s,  
37 2H), 7.34 (dd, *J* = 5.0, 3.3 Hz, 1H), 7.17 (d, *J* = 5.1 Hz, 1H), 3.58 (dd, *J* = 34.1, 18.8 Hz, 2H),  
38 2.39-2.27 (m, 2H), 1.90-1.80 (m, 2H). LCMS found 343.1 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 2.15 min (Method A).  
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43 6-(4,4-difluoropiperidin-1-yl)-*N*-(thiophen-3-yl)-9H-purin-2-amine (**5k**). (Yield: 73%,  
44 light brown solid, m.p. 209-211). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 12.57 (s, 1H), 9.31 (s, 1H),  
45 7.88 (s, 1H), 7.52 (s, 1H), 7.38 (dd, *J* = 5.1, 3.2 Hz, 1H), 7.17 (dd, *J* = 5.1, 1.2 Hz, 1H), 4.55 – 4.17  
46 (m, 4H), 2.15 – 2.03 (m, 4H). LCMS found 337.11 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 2.65 min (Method A).  
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50 *N*-(thiophen-3-yl)-6-(4-(trifluoromethyl)piperidin-1-yl)-9H-purin-2-amine (**5l**). (Yield:  
51 72%, light yellow solid, m.p. 139-141). <sup>1</sup>H NMR (500 MHz, DMSO-*d*<sub>6</sub>) δ 12.53 (s, 1H), 9.26 (s,  
52 1H), 7.85 (s, 1H), 7.52 (s, 1H), 7.38 (dd, *J* = 5.2, 3.0 Hz, 1H), 7.17 (d, *J* = 5.2 Hz, 1H), 5.55-5.44  
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(m, 1H), 3.08 (t,  $J = 12.3$  Hz, 2H), 2.74-2.70 (m, 1H), 2.10-1.94 (m, 3H), 1.51-1.42 (m, 2H). LCMS found 369.17  $[M+H]^+$ ,  $t_R = 2.70$  min (Method A).

*6-(azetidin-1-yl)-N-(thiophen-3-yl)-9H-purin-2-amine (5m)*. (Yield: 72%, brown solid, m.p. 173-175).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.37 (s, 1H), 9.28 (s, 1H), 7.78 (s, 1H), 7.61 (d,  $J = 2.9$  Hz, 1H), 7.34 (dd,  $J = 5.1, 3.3$  Hz, 1H), 7.17 (dd,  $J = 5.1, 0.7$  Hz, 1H), 4.51 – 4.11 (m, 4H), 2.47 – 2.38 (m, 2H). LCMS found 273.06  $[M+H]^+$ ,  $t_R = 1.57$  min (Method A).

*N<sup>6</sup>-cyclohexyl-N<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (5n)*. (Yield: 70%, brown solid, m.p. 177-179).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  9.16 (s, 1H), 7.78 (s, 1H), 7.59 (s, 1H), 7.35 (dd,  $J = 4.9, 3.3$  Hz, 1H), 7.18 (d,  $J = 4.5$  Hz, 2H), 4.14-4.04 (m, 1H), 1.99-1.94 (m, 2H), 1.80 – 1.75 (m, 2H), 1.67-1.64 (m, 1H), 1.39 – 1.30 (m, 3H), 1.25-1.16 (m, 2H). LCMS found 315.14  $[M+H]^+$ ,  $t_R = 2.08$  min (Method A).

*N<sup>6</sup>-isopropyl-N<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (5o)*. (Yield: 59%, brown solid, m.p. 132-134).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.34 (s, 1H), 9.17 (s, 1H), 7.78 (s, 1H), 7.61 (s, 1H), 7.35 (dd,  $J = 5.0, 3.3$  Hz, 1H), 7.18 (d,  $J = 4.9$  Hz, 1H), 7.14 (d,  $J = 6.6$  Hz, 1H), 3.18 (m, 1H), 1.25 (d,  $J = 6.6$  Hz, 6H). LCMS found 275.08  $[M+H]^+$ ,  $t_R = 1.82$  min (Method A).

*N<sup>6</sup>,N<sup>6</sup>-diethyl-N<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (5p)*. (Yield: 77%, light brown solid, m.p. 88-90).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.39 (s, 1H), 9.14 (s, 1H), 7.80 (s, 1H), 7.53 (d,  $J = 2.5$  Hz, 1H), 7.36 (dd,  $J = 5.1, 3.2$  Hz, 1H), 7.18 (d,  $J = 5.1$  Hz, 1H), 3.96 (brs, 4H), 1.22 (t,  $J = 7.0$  Hz, 6H). LCMS found 289.06  $[M+H]^+$ ,  $t_R = 1.89$  min (Method A).

*N<sup>6</sup>-cyclopropyl-N<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (5q)*. (Yield: 70%, light brown solid, m.p. 130-132).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  9.22 (s, 1H), 7.79 (s, 1H), 7.75 (d,  $J = 2.1$  Hz, 1H), 7.34 (dd,  $J = 5.0, 3.3$  Hz, 1H), 7.19 (d,  $J = 5.0$  Hz, 1H), 1.25-1.15 (m, 2H), 0.90-0.64 (m, 3H). LCMS found 273.1  $[M+H]^+$ ,  $t_R = 1.17$  min (Method A).

*N<sup>6</sup>-(tert-butyl)-N<sup>2</sup>-(thiophen-3-yl)-9H-purine-2,6-diamine (5r)*. (Yield: 59%, brown solid, m.p. 150-152).  $^1H$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  9.14 (s, 1H), 7.77 (s, 1H), 7.59 (s, 1H), 7.38 – 7.32 (m, 1H), 7.20 (d,  $J = 5.0$  Hz, 1H), 6.25 (s, 1H), 1.53 (s, 9H). LCMS found 289.2  $[M+H]^+$ ,  $t_R = 1.82$  min (Method A).

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3  $N^6$ -isobutyl- $N^2$ -(thiophen-3-yl)-9H-purine-2,6-diamine (**5s**). (Yield: 21%, off-white semi-  
4 solid).  $^1\text{H}$  NMR (500 MHz, Acetone- $d_6$ )  $\delta$  11.42 (s, 1H), 8.46 (s, 1H), 7.74 (d,  $J = 3.9$  Hz, 2H),  
5 7.33 – 7.27 (m, 1H), 7.23 (d,  $J = 4.8$  Hz, 1H), 6.67 (brs, 1H), 3.50-3.43 (m, 1H), 2.88 (d,  $J = 4.6$   
6 Hz, 2H), 0.99 (d,  $J = 6.7$  Hz, 6H). LCMS found 289.06  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.87$  min (Method A).  
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10  $N^6$ -cyclopentyl- $N^2$ -(thiophen-3-yl)-9H-purine-2,6-diamine (**5t**). (Yield: 80%, light brown  
11 solid, m.p. 126-128).  $^1\text{H}$  NMR (500 MHz, Acetone- $d_6$ )  $\delta$  11.40 (s, 1H), 8.46 (s, 1H), 7.74 (s, 2H),  
12 7.26 (d,  $J = 18.1$  Hz, 2H), 6.37 (d,  $J = 5.8$  Hz, 1H), 4.58 (s, 1H), 1.99 – 1.47 (m, 7H). LCMS found  
13 301.08  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.93$  min (Method A).  
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18  $N^6$ -(2-(pyrrolidin-1-yl)ethyl)- $N^2$ -(thiophen-3-yl)-9H-purine-2,6-diamine (**5u**). (Yield:  
19 51%, light orange solid, m.p. 162-164).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.32 (s, 1H), 9.17 (s,  
20 1H), 7.76 (s, 1H), 7.61 (s, 1H), 7.32 (dd,  $J = 5.0, 3.2$  Hz, 1H), 7.19 (s, 1H), 7.16 (t,  $J = 5.0$  Hz,  
21 1H), 3.69 – 3.49 (m, 2H), 3.15 (d,  $J = 4.6$  Hz, 1H), 2.66 (t,  $J = 6.7$  Hz, 2H), 1.72-1.62 (m, 4H).  
22 \*three aliphatic protons merged with DMSO signal. LCMS found 330.1  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.11$  min  
23 (Method A).  
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29  $N^2$ -(1-methyl-1H-pyrazol-4-yl)- $N^6$ -(4,4,4-trifluorobutyl)-9H-purine-2,6-diamine (**5ac**).  
30 (Yield: 37%, off-white solid, m.p. 275-277).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  9.28 (s, 1H), 8.58  
31 (brs, 1H), 8.24 (s, 1H), 7.84 (s, 1H), 7.52 (s, 1H), 3.80 (s, 3H), 3.65 – 3.51 (m, 2H), 2.45 – 2.37  
32 (m, 2H), 1.92 – 1.81 (m, 2H). LCMS found 341.1  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.61$  min (Method A).  
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37  $N^2$ -(1-methyl-1H-pyrazol-4-yl)- $N^6$ -(2-(pyrrolidin-1-yl)ethyl)-9H-purine-2,6-diamine  
38 (**5ad**). (Yield: 48%, brown solid, m.p. 128-130).  $^1\text{H}$  NMR (500 MHz, DMSO- $d_6$ )  $\delta$  12.26 (s, 1H),  
39 8.59 (s, 1H), 7.87 (s, 1H), 7.72 (s, 1H), 7.46 (s, 1H), 7.15 (s, 1H), 3.77 (s, 3H), 3.67 – 3.50 (m,  
40 3H), 2.69 (t,  $J = 6.8$  Hz, 2H), 2.57 - 2.53 (m, 3H), 1.75 – 1.67 (m, 4H). LCMS found 328.23  
41  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.49$  min (Method A).  
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46  $N^6$ -butyl- $N^2$ -(1-methyl-1H-pyrazol-4-yl)-9H-purine-2,6-diamine (**5ae**). (Yield: 51%, off-  
47 white solid, m.p. 205-207).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.89 (s, 1H), 7.73 (s, 1H), 7.57 (s,  
48 1H), 3.84 (s, 3H), 3.57 (d,  $J = 7.2$  Hz, 2H), 1.67 (dd,  $J = 14.6, 7.4$  Hz, 2H), 1.47 (dd,  $J = 15.0, 7.4$   
49 Hz, 2H), 0.99 (t,  $J = 7.4$  Hz, 3H). LCMS found 287.1  $[\text{M}+\text{H}]^+$ ,  $t_{\text{R}} = 1.47$  min (Method A).  
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**General method for preparation of compounds 5f-j and 5ab.** 2,6-dichloro-9H-purine (1) was dissolved in THF (0.04 M). To this, respective amine (10 equiv.) was added and the reaction mixture was then stirred at room temp. for 16h. The reaction was set aside for 1h and the ppt formed was filtered to give C-6 aminated intermediate. The C-6 aminated intermediate, 3-aminothiophene (2 equiv.), Pd<sub>2</sub>(dba)<sub>3</sub> (5 mol%), XPhos (15 mol%) and potassium tert-butoxide (3 equiv.) were added to a reaction vial. To this, t-butanol (0.02 M) was added, reaction vial was flushed with inert gas (nitrogen or argon) and stirred for 16h at 100 °C. The reaction mixture was filtered, and the residue was washed with acetone. The filtrate was dried and then purified by flash chromatography using 1% to 25% of methanol in dichloromethane gradient to get desired product.

*6-morpholino-N-(thiophen-3-yl)-9H-purin-2-amine (5f).* (Yield: 52%, light yellow solid, m.p. 250-252). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.52 (s, 1H), 9.25 (s, 1H), 7.85 (s, 1H), 7.51 (s, 1H), 7.37 (dd, *J* = 4.9, 3.3 Hz, 1H), 7.16 (d, *J* = 5.0 Hz, 1H), 4.30-4.10 (m, 4H), 3.76-3.70 (m, 4H). LCMS found 303.0 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.89 min (Method A).

*6-(4-methylpiperazin-1-yl)-N-(thiophen-3-yl)-9H-purin-2-amine (5g).* (Yield: 61%, light brown solid, m.p. 152-154). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.49 (s, 1H), 9.22 (s, 1H), 7.83 (s, 1H), 7.51 (d, *J* = 2.4 Hz, 1H), 7.37 (dd, *J* = 5.1, 3.2 Hz, 1H), 7.16 (dd, *J* = 5.0, 1.0 Hz, 1H), 4.32-4.08 (m, 4H), 2.45-2.41 (m, 4H), 2.23 (s, 3H). LCMS found 316.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.28 min (Method A).

*6-(piperidin-1-yl)-N-(thiophen-3-yl)-9H-purin-2-amine (5h).* (Yield: 59%, brown solid, m.p. 180-182). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.44 (s, 1H), 9.17 (s, 1H), 7.80 (s, 1H), 7.51 (d, *J* = 2.4 Hz, 1H), 7.36 (dd, *J* = 5.1, 3.2 Hz, 1H), 7.17 (dd, *J* = 5.1, 1.2 Hz, 1H), 4.23-4.08 (m, 4H), 3.18 (d, *J* = 4.8 Hz, 2H), 1.69-1.59 (m, 4H). LCMS found 301.0 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.19 min (Method A).

*6-(pyrrolodin-1-yl)-N-(thiophen-3-yl)-9H-purin-2-amine (5i).* (Yield: 71%, light brown solid, m.p. 208-210). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.35 (s, 1H), 9.17 (s, 1H), 7.77 (s, 1H), 7.60 (dd, *J* = 3.2, 1.2 Hz, 1H), 7.35 (dd, *J* = 5.1, 3.3 Hz, 1H), 7.18 (dd, *J* = 5.1, 1.2 Hz, 1H), 4.11-4.04 (dd, *J* = 28.2, 8.4 Hz, 3H), 3.18 (d, *J* = 5.3 Hz, 1H), 2.02 – 1.93 (m, 4H). LCMS found 287.0 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.75 min (Method A).

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*6-(4-methyl-1,4-diazepan-1-yl)-N-(thiophen-3-yl)-9H-purin-2-amine (5j)*. (Yield: 62%, brown solid, m.p. 144-146). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.43 (s, 1H), 9.18 (s, 1H), 7.80 (s, 1H), 7.52 (s, 1H), 7.36 (dd, *J* = 4.9, 3.3 Hz, 1H), 7.18 (d, *J* = 5.1 Hz, 1H), 4.61-4.28 (m, 2H), 3.88 (dd, *J* = 39.9, 22.5 Hz, 2H), 2.83 – 2.55 (m, 3H), 2.28 (s, 3H), 2.10 – 1.78 (m, 3H). LCMS found 330.1 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 1.21 min (Method A).

*N-(1-methyl-1H-pyrazol-4-yl)-6-morpholino-9H-purin-2-amine (5ab)*. (Yield: 45%, light orange solid, m.p. 261-263). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 12.38 (s, 1H), 8.61 (s, 1H), 7.75 (s, 2H), 7.43 (s, 1H), 4.21 – 4.02 (m, 4H), 3.75 (s, 3H), 3.71 – 3.67 (m, 4H). LCMS found 301.1 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 1.42 min (Method A).

**Synthesis of *N*<sup>2</sup>-(4-fluorophenyl)-9*H*-purine-2,6-diamine (5aa)**. 100 mg of 2,6-dichloro-9*H*-purine (**2**) was dissolved in 15 ml of ammonium hydroxide (1N sol in MeOH) and heated in microwave reactor at 120 °C for 90 mins. The precipitates formed were filtered and washed with 2 ml of methanol and dried to give 2-chloro-9*H*-purin-6-amine. The 2-chloro-9*H*-purin-6-amine was then added to vial containing 4-fluoroaniline (1.5 equiv.), Pd<sub>2</sub>(dba)<sub>3</sub> (2 mol%), RuPhos (5 mol%) and potassium carbonate (3 equiv.). To this, *t*-butanol (0.02 M) was added, reaction vial was flushed with inert gas (nitrogen or argon) and heated in microwave at 180 °C for 2h. The reaction mixture was then diluted with dichloromethane:methanol (1:1) and filtered. The filtrate was dried and purified by preparative HPLC (5–50% water–acetonitrile) to give desired product **5aa** (yield 17%, white solid, m.p. 214-216). <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD) δ ppm 7.83 (s, 1H), 7.70-7.68 (m, 2H), 7.02-6.99 (m, 2H). LCMS found 244.98 [M+H]<sup>+</sup>, *t*<sub>R</sub> = 2.33 min (Method A).

**General method for preparation of compounds 5v-z**. 2,6-dichloro-9*H*-purine (**1**) was dissolved in THF (0.04 M). To this, respective amine (10 equiv.) was added and the reaction mixture was then stirred at room temp. for 16h. The reaction was set aside for 1h and the ppts formed were filtered to give C-6 aminated intermediate. The C-6 aminated intermediate, and aniline (5 equiv.) were mixed in microwave sealed tube. To this, dioxane (0.02 M) and two drops of HCl (catalytic amount) were added. The reaction mixture was then heated in microwave reactor

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3 at 150 °C for 40 mins. The reaction mixture was then basified with NaHCO<sub>3</sub> solution. The solid  
4 product was filtered and dried under high vacuum to give desired product.  
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8 *6-morpholino-N-phenyl-9H-purine-2-amine (5v)*. (Yield: 59%, brown solid, m.p. 263-  
9 265). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.09 (s, 1H), 7.69 (d, *J* = 7.8 Hz, 2H), 7.51-7.29 (m, 4H),  
10 6.98 (d, *J* = 7.3 Hz, 1H), 4.26-4.16 (m, 2H), 3.79 – 3.74 (m, 4H), 3.10 – 3.06 (m, 2H). LCMS  
11 found 297.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.03 min (Method A).  
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15 *6-(4-methylpiperazin-1-yl)-N-phenyl-9H-purine-2-amine (5w)*. (Yield: 54%, gray solid,  
16 m.p. 110-112). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 7.99 (s, 1H), 7.74 (d, *J* = 8.2 Hz, 1H), 7.50-7.25  
17 (m, 5H), 6.90 (t, *J* = 7.3 Hz, 1H), 3.64-3.54 (m, 5H), 3.18-3.12 (m, 2H), 2.82 – 2.77 (m, 1H), 2.77  
18 (s, 3H). LCMS found 310.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.28 min (Method A).  
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23 *N-phenyl-6-(piperidin-1-yl)-9H-purin-2-amine (5x)*. (Yield: 28%, light brown solid, m.p.  
24 196-198). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.27 (s, 1H), 7.99 (s, 1H), 7.71 (d, *J* = 8.1 Hz, 2H),  
25 7.28 (t, *J* = 7.8 Hz, 2H), 6.95 (t, *J* = 7.2 Hz, 1H), 4.27 – 4.07 (m, 4H), 1.74 – 1.66 (m, 2H), 1.63-  
26 1.59 (m, 4H). LCMS found 295.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.22 min (Method A).  
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31 *N-phenyl-6-(pyrrolidin-1-yl)-9H-purin-2-amine (5y)*. (Yield: 68%, gray solid, m.p. 218-  
32 220). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.21 (s, 1H), 7.75 (d, *J* = 8.0 Hz, 1H), 7.43 – 7.41 (m,  
33 2H), 7.35 – 7.25 (m, 2H), 7.03 (t, *J* = 7.3 Hz, 1H), 4.12 – 3.99 (m, 2H), 3.82 – 3.72 (m, 2H), 2.09  
34 – 1.95 (m, 4H). LCMS found 281.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.78 min (Method A).  
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38 *6-(4-methyl-1,4-diazepan-1-yl)-N-phenyl-9H-purine-2-amine (5z)*. (Yield: 56%, brown  
39 solid, m.p. 222-224). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 10.75 (s, 1H), 7.98 (s, 1H), 7.76 (d, *J* =  
40 7.8 Hz, 2H), 7.45 – 7.25 (m, 3H), 6.91 (t, *J* = 7.2 Hz, 1H), 3.66 – 3.59 (m, 2H), 3.50 – 3.42 (m,  
41 2H), 3.36 – 3.18 (m, 4H), 2.79 (s, 3H), 2.43 – 2.37 (m, 1H), 2.24 – 2.20 (m, 1H). LCMS found  
42 324.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.28 min (Method A).  
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49 **General method for preparation of compounds 9-11 and 13a.** Desired dichloro-scaffold  
50 (1 equiv.) was dissolved in n-BuOH (0.03 M) in a screw cap vial. To this diisopropylethylamine  
51 (2.0 equiv.) was added followed by addition of 2,2,2-trifluoroethylamine (1.5 equiv.). The reaction  
52 mixture was then heated at 90 °C for 16h. The reaction mixture was then allowed to cool down  
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3 and concentrated under vacuum at 50 °C to an oily mass. To this, water was added and ppts formed  
4 were filtered, washed with water and dried to give 2-chloro-N-(2,2,2-trifluoroethyl)-substituted  
5 intermediate. The 2-chloro-N-(2,2,2-trifluoroethyl)-substituted intermediate was then added to the  
6 reaction vial containing desired amine (2 equiv.), Pd<sub>2</sub>(dba)<sub>3</sub> (5 mol%), XPhos (15 mol%) and  
7 potassium tert-butoxide (3 equiv.). To this, t-butanol (0.02 M) was added, reaction vial was flushed  
8 with inert gas (nitrogen or argon) and stirred for 16h at 100 °C. The reaction mixture was filtered,  
9 and the residue was washed with acetone. The filtrate was dried and then purified by flash  
10 chromatography using 1% to 25% of methanol in dichloromethane gradient to get desired product.  
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18 *9-methyl-N<sup>2</sup>-(thiophen-3-yl)-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine (9a).*  
19 (Yield: 34%, light yellow solid, m.p. 195-197). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.47 (s, 1H),  
20 8.07 (s, 1H), 7.89 (s, 1H), 7.68 (s, 1H), 7.37 (dd, *J* = 4.9, 3.3 Hz, 1H), 7.19 (d, *J* = 4.9 Hz, 1H),  
21 4.31 (brs, 2H), 3.68 (s, 3H). LCMS found 329.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 2.80 min (Method A).  
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26 *9-methyl-N<sup>2</sup>-(1-methyl-1H-pyrazol-4-yl)-N<sup>6</sup>-(2,2,2-trifluoroethyl)-9H-purine-2,6-diamine*  
27 *(9b).* (Yield: 33%, light orange solid, m.p. 235-237). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 8.92 (s,  
28 1H), 7.98 (s, 2H), 7.83 (s, 1H), 7.50 (s, 1H), 4.29 (brs, 2H), 3.79 (s, 3H), 3.66 (s, 3H). LCMS found  
29 327.1 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.99 min (Method A).  
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33 *N<sup>2</sup>-(thiophen-3-yl)-N<sup>4</sup>-(2,2,2-trifluoroethyl)thieno[3,2-d]pyrimidine-2,4-diamine (10).*  
34 (Yield: 43%, light yellow solid, m.p. 182-184). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.48 (s, 1H),  
35 8.24 (t, *J* = 6.2 Hz, 1H), 8.04 (d, *J* = 5.3 Hz, 1H), 7.63 (s, 1H), 7.38 (dd, *J* = 5.1, 3.2 Hz, 1H), 7.21  
36 (dd, *J* = 8.6, 5.2 Hz, 2H), 4.41 – 4.33 (m, 2H). LCMS found 331.0 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.99 min (Method  
37 A).  
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42 *N<sup>2</sup>-(thiophen-3-yl)-N<sup>4</sup>-(2,2,2-trifluoroethyl)quinazoline-2,4-diamine (11).* (Yield: 42%,  
43 light brown solid, m.p. 123-125). <sup>1</sup>H NMR (500 MHz, DMSO-d<sub>6</sub>) δ 9.54 (s, 1H), 8.53 (t, *J* = 5.4  
44 Hz, 1H), 8.12 (d, *J* = 8.2 Hz, 1H), 7.83 (brs, 1H), 7.64 (t, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 8.2 Hz, 1H),  
45 7.40 (dd, *J* = 5.0, 3.3 Hz, 1H), 7.24 – 7.21 (m, 2H), 4.49 – 4.37 (m, 2H). LCMS found 325.01  
46 [M+H]<sup>+</sup>, t<sub>R</sub> = 1.78 min (Method A).  
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51 *N<sup>2</sup>-(thiophen-3-yl)-N<sup>4</sup>-(2,2,2-trifluoroethyl)pyrimidine-2,4-diamine (13a).* (Yield: 30%,  
52 brown solid, m.p. 85-87). <sup>1</sup>H NMR (500 MHz, Acetone-d<sub>6</sub>) δ 8.67 (s, 1H), 7.99 (d, *J* = 5.7 Hz,  
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3 1H), 7.68 (s, 1H), 7.34 (dd,  $J = 5.2, 3.2$  Hz, 1H), 7.29 – 7.26 (m, 1H), 6.95 (s, 1H), 6.16 (d,  $J = 5.7$   
4 Hz, 1H), 4.33 – 4.28 (m, 2H). LCMS found 275.08  $[M+H]^+$ ,  $t_R = 1.59$  min (Method A).  
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10 **Synthesis of  $N^2$ -phenyl- $N^4$ -(2,2,2-trifluoroethyl)pyrimidine-2,4-diamine (13b).** 2,4-  
11 dichloropyrimidine (1 equiv.) was dissolved in n-BuOH (0.03 M) in a screw cap vial. To this  
12 diisopropylethylamine (2.0 equiv.) was added followed by addition of 2,2,2-trifluoroethylamine  
13 (1.5 equiv.). The reaction mixture was then heated at 90 °C for 16h. The reaction mixture was then  
14 allowed to cool down and concentrated under vacuum at 50 °C to an oily mass. To this, water was  
15 added and ppts formed were filtered, washed with water and dried to give intermediate 2-chloro-  
16 N-(2,2,2-trifluoroethyl)pyrimidin-4-amine. The intermediate and aniline (5 equiv.) were mixed in  
17 microwave sealed tube. To this, dioxane (0.02 M) and two drops of HCl (catalytic amount) were  
18 added. The reaction mixture was then heated in microwave reactor at 150 °C for 40 mins. The  
19 reaction mixture was then basified with  $\text{NaHCO}_3$  solution. The solid product was filtered and dried  
20 under high vacuum to give desired product (13b, yield: 30%, off-white solid, m.p. 253-255).  $^1\text{H}$   
21 NMR (500 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.76 (d,  $J = 7.1$  Hz, 1H), 7.50 - 7.46 (m, 4H), 7.36 (d,  $J = 8.0$  Hz,  
22 1H), 6.35 (d,  $J = 7.2$  Hz, 1H), 4.31 - 4.25 (m, 2H). LCMS found 269.1  $[M+H]^+$ ,  $t_R = 1.69$  min  
23 (Method A).  
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35 **Strains and media.** Bloodstream *Trypanosoma brucei brucei* Lister 427 (STIB 795) was  
36 cultured in Hirumi's modified Iscove's medium (HMI-9), supplemented with 10% heat-  
37 inactivated FBS, at 37 °C and 5%  $\text{CO}_2$  in T-25 vented flask (Corning®). MRC5-SV2 cell line  
38 (SV40-transformed human lung fibroblast cell line) was cultured in DMEM medium supplemented  
39 with 10% FBS at 37 °C and 5%  $\text{CO}_2$  in T-75 vented flask (Corning®). The assays were run in  
40 sterile 96-well transparent Nunclon plates.  
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50 **Preparation of compound plates.** For dose-response experiments, compound plates were  
51 prepared for each analogue by serial 3-fold dilutions in 100% DMSO. Five concentration points  
52 (mammalian cytotoxicity) or ten concentration points (parasite growth inhibition), were made in  
53 96-well transparent Nunclon plates, and plates were stored sealed at -20 °C for no more than four  
54 weeks.  
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5 **Growth inhibition assay for *T. brucei brucei*.** 4  $\mu\text{L}$  per well from compound master plates  
6 were dispensed into a new plate and 96  $\mu\text{L}$  of HMI-9 per well were added to generate a 4% DMSO  
7 intermediate plate. Cultures of *T. b. brucei* in logarithmic phase were diluted to a working cell  
8 density of 2,750 cells/mL and 90  $\mu\text{L}$ /well dispensed into 96-well flat-bottom transparent assay  
9 plates (Nunc®). 10  $\mu\text{L}$ /well from intermediate plates were added so final cell concentration was  
10 2,500 cell/mL, and final top concentration of compounds was 40  $\mu\text{M}$  in 0.4% DMSO per well.  
11 Assay plates were incubated for 72 h at 37 °C and 5% CO<sub>2</sub>. After 68 hours of incubation, 20  $\mu\text{L}$   
12 of a 440  $\mu\text{M}$  resazurin solution in pre-warmed HMI-9 was added to each well and incubated for  
13 another 4 h. Fluorescence was then measured in an Infinite F200 plate reader (Tecan®) at 550 nm  
14 (excitation filter) and 590 nm (emission filter). A four-parameter equation was employed to fit the  
15 dose-response curves and determine EC<sub>50</sub> using the SigmaPlot® 13.0 software. Assays were  
16 performed in duplicate at least twice, to achieve a minimal n=4 per dose response, and pentamidine  
17 was routinely included in compound plates as internal quality control.  
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29 **Cytotoxicity assay in MRC5 cells.** Fresh intermediate plates were made as described,  
30 adding 95  $\mu\text{L}$  of DMEM complete media to 5  $\mu\text{L}$  of compound per well setting a 5% DMSO  
31 amount. Log-phase MRC5 cells were removed from a T-75 TC flask using TrypLE® Express  
32 (Thermo®) and dispersed by gentle pipetting. Cell density was adjusted to working concentration  
33 in prewarmed DMEM medium: 25,000 cells in 90  $\mu\text{L}$  of culture were plated in 96-well transparent  
34 Nunclon plates and let to settle for 24 h at 37 °C and 5% CO<sub>2</sub>. After settling incubation, 10  $\mu\text{L}$  of  
35 freshly made intermediate plate were added per well: final maximal concentration for compounds  
36 was 50  $\mu\text{M}$  in 0.5% DMSO per well. Plates were incubated for 48 h at 37 °C and 5% CO<sub>2</sub>. At 4 h  
37 prior to fluorescence measurement, 20  $\mu\text{L}$  of 500  $\mu\text{M}$  resazurin solution was added. Fluorescence  
38 was read in an Infinite F200 plate reader (Tecan®) at 550 nm (excitation filter) and 590 nm  
39 (emission filter). A four-parameter equation was used to fit the dose-response curves and  
40 determination of EC<sub>50</sub> by SigmaPlot ® 13.0 software. Assays were performed in duplicate at least  
41 twice for positive compounds, to achieve a minimal n=3 per dose response.  
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53 **Pharmacokinetics Protocols.** Compound was administered intraperitoneally (ip) to two  
54 groups of female NMRI mice (group 1, n = 3; group 2, n = 6) supplied by Charles River (Germany)  
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3 Ltd. The compound was prepared in 1% (v/v) DMSO/99% (v/v) 20% (w/v) sulfobutyl ether- $\beta$ -  
4 cyclodextrin (SBE $\beta$ -CD)/Captisol, used as a solubilizing agent) in water, and the dosing volume  
5 was 10 mL/kg for a total dose of 10 mg/kg. Food and tap water were available *ad libitum*.  
6 Following ip dosing, blood samples were collected from the tail vein into capillary tubes  
7 containing K2EDTA at the following time points: 0.0833, 0.25, 0.5, 1, 2, 4, 6, 8, and 24 h. In order  
8 to obtain simultaneous blood and brain samples, group 2 mice were placed under terminal  
9 anesthetic (isoflurane), and blood samples (0.3 mL) were collected from the retro-orbital sinus into  
10 K2EDTA tubes at 0.5 h (n = 3) and 4 h (n = 3) after compound administration. Immediately  
11 following blood sample collection, death was confirmed by cervical dislocation, and the brain  
12 removed. Aliquots of each blood sample were diluted in an equal volume of water. Mouse brain  
13 samples were weighed, water was added at a 1/2 (w/v) ratio (brain/water), and then samples were  
14 homogenized. Both blood and brain samples were stored -80 °C until analysis. For PK analysis of  
15 ABT-pretreated animals, blood samples from treated mice (infected and non-infected) were taken  
16 at 0.5, 2, 4 and 6 h of treatment on day 1 of treatment; and at 0.5 h of day 5 of treatment. Diluted  
17 blood and brain homogenates were processed under standard liquid-liquid extraction procedures  
18 using CAN containing an internal standard (nifedipine) and analyzed by LC-MS/MS.  
19 Noncompartmental analysis was performed using the Phoenix pharmacokinetic software, version  
20 1.4 (Certara), and  $C_{\max}$ ,  $t_{\max}$ ,  $AUC_{\text{last}}$ ,  $AUC$ , and  $t_{1/2}$  were estimated.  
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36 **Blood-stage efficacy model.** All procedures were performed in the Institute of  
37 Parasitology and Biomedicine Lopez-Neyra (Spanish National Research Council, CSIC) and  
38 approved according with the European and Spanish ethical regulations. Infection was carried out  
39 in female NMRI mice (Charles River Laboratories) by i.p. injection of  $10^4$  bloodstream forms of  
40 *T. brucei brucei* (STIB795) in 0.2 mL TDB glucose from a cryopreserved stock. Three days later,  
41 infected animals with confirmed parasitemia were distributed into two groups: control (infected  
42 mice treated with vehicle (4 % DMSO, 20 % Captisol®), n=3) and treated (infected mice treated  
43 with 30 mg/kg/day of 4aa, n=3). An additional group was used for PK analysis (non-infected mice  
44 treated with 30 mg/kg/day of 4aa, n=3). All mice received a 0.2 mL i.p. injection at day 3 from  
45 infection during 5 consecutive days. Pre-treatment with 50 mg/kg/day ABT (1-  
46 Aminobenzotriazole, Sigma-Aldrich), a known inhibitor of CYP enzymes, 1 h prior compound  
47 injection was administered. Blood samples from tail tip were mixed with sterile water (ratio 1:2).  
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To eliminate the risk of infections, the samples undergo 3 freeze-thaw cycles. Parasitemia was periodically examined, diluting 2  $\mu\text{L}$  of blood from infected mice tail in 100  $\mu\text{L}$  of TDB glucose and counting in a Neubauer chamber in a light microscope, and the day of death was recorded.

## ASSOCIATED CONTENT

**Supporting Information.** Supplemental biological (TbMAPK6 and human kinase), PK and ADME data (annotated with NEU registry numbers), experiment protocols and HPLC traces of all compounds tested. Compound ID numbers and their associated SMILES strings.

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**Abbreviations.** HAT, Human African trypanosomiasis; ADME, absorption, distribution, metabolism, and excretion; NTDs, Neglected tropical diseases; SAR, structure activity relationship; SPR, structure property relationship; PPB, plasma protein binding; CNS, central nervous system; MPO, multiparameter optimization; PSA, polar surface area; LLE, lipophilic ligand efficiency; HLM, human liver microsome;  $\text{CL}_{\text{int}}$ , intrinsic clearance; Aq. sol.,

thermodynamic aqueous solubility; DIPEA, N,N-diisopropylethylamine; TbMAPK6, *T. brucei* mitogen-activated protein kinase 6; PK, pharmacokinetics, ABT, 1-aminobenzotriazole; IP, intraperitoneal; AAT, African animal trypanosomiasis; AUC, area under the curve.

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