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Design and synthesis of novel, potent and selective hypoxanthine analogs as adenosine A_1 receptor antagonists and their biological evaluation

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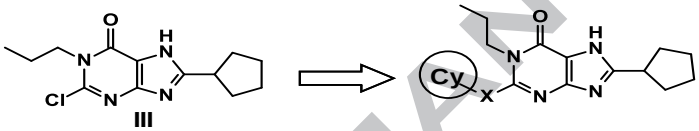
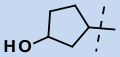
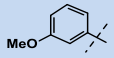


Compound	14	23	33	38
Cy				
X	O	O	O	Absent
A_1 Binding K_i (nM)	23	6.6	57	16

Design and synthesis of novel, potent and selective hypoxanthine analogs as adenosine A_1 receptor antagonists and their biological evaluation

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Abstract: Multipronged approach was used to synthesize a library of diverse C-8 cyclopentyl hypoxanthine analogs from a common intermediate **III.** Several potent and selective compounds were identified and evaluated for pharmacokinetic (PK) properties in Wistar rats. One of the compounds **14** with acceptable PK parameters was selected for testing in *in-vivo* primary acute diuresis model. The compound demonstrated significant diuretic activity in this model.

Keywords: Adenosine, Rolofylline, BG-9928, diuresis, hypoxanthine, glomerular

1. Introduction

Adenosine is an endogenous signaling nucleoside distributed in a wide variety of tissues and plays a key role in a variety of physiological processes.[1-4] The effects elicited by adenosine are mediated by its interactions with four receptor subtypes named A_1, A_{2A}, A_{2B} and A_3. These receptors belong to the superfamily of G protein coupled receptors and modulate a number of effector systems, including adenylate cyclase, potassium and calcium channels, phospholipase C or D, and guanylate cyclase.[5] Activation of A_1 and A_3AR leads to an inhibition of adenylyl cyclase activity, while activation of A_{2A} andA_{2B}AR causes a stimulation of adenylyl cyclase. Different therapeutic applications have been identified in preclinical and clinical studies for A_1AR antagonists, which are effective as potassium-sparing diuretic agents with kidney-protecting properties.[6] The ability of A_1R antagonists to induce diuresis and natriuresis while not compromising glomerular filtration rate is an attractive therapeutic option for the treatment of fluid retention disorders, e.g. in kidney disease and heart failure, especially in conditions

associated with diuretic resistance. Adenosine, via A_1 receptors (A_1R), also serves as a mediator for triggering the hepatorenal reflex leading to renal water and sodium retention.[7, 8] . Blockade of hepatic and renal A_1R could therefore provide a new therapeutic option for sodium and water retention in conditions such as liver cirrhosis [9] as well. Berthold Hocher and his coworkers have demonstrated long term safety and efficacy of A_1R-specific antagonist SLV329 in an animal model of liver cirrhosis.[10] In clinics, SLV329 is known to increase renal sodium and water excretion in patients with liver cirrhosis without affecting the glomerular filtration rate. [11, 12] Despite progression of A_1 receptor antagonists to clinics (including a large Phase 3 trial), [13] development of clinically successful A_1 receptor antagonists has been hampered by safety concerns. Seizures have been reported in patients treated with Rolofylline [14]. Adenosine via A_1 receptors located in central nervous system modulates seizure threshold [15]. Selective renal A_1 receptor antagonism with compounds that do not cross blood brain barrier may offer an opportunity to develop safe A_1 receptor antagonists. This provides impetus to discover and develop novel selective A_1 receptor antagonists for therapeutic intervention.

A_1R antagonists can be mainly divided into two structural subgroups, xanthines and non-xanthines. The compounds, Rolofylline (**1**) [16] and BG-9928 (**2**) [17] that entered Phase III clinical trials (**Fig. 1**) are xanthine based A_1R antagonists. SLV320 (**3**), another A_1R antagonist in clinical trials is an adenine analog.[18] Other non xanthine based antagonists are pyrazolo pyridine derivatives [19, 20] and quinoxaline analogs.[21]

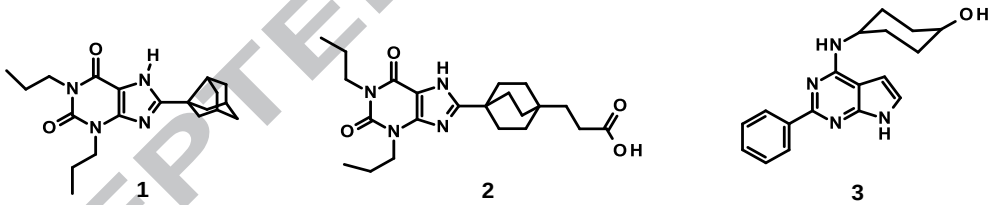


Figure 1. A_1R antagonists that entered clinical trials.

As described above, xanthine and adenine analogs are well known in literature as potent A_1R antagonists that follow a particular metabolic pathway as shown in **Fig. 2**. However, hypoxanthine analogs that fall between adenine and xanthine on the pathway have not been much explored in general as adenosine receptor antagonists. Hence, we rationalized that hypoxanthines can provide us with a novel chemotype forA_1R antagonism. Herein we report discovery of novel N-1 propyl-C-8 cyclopentyl hypoxanthine analogs that are potent and selective A_1R antagonists.

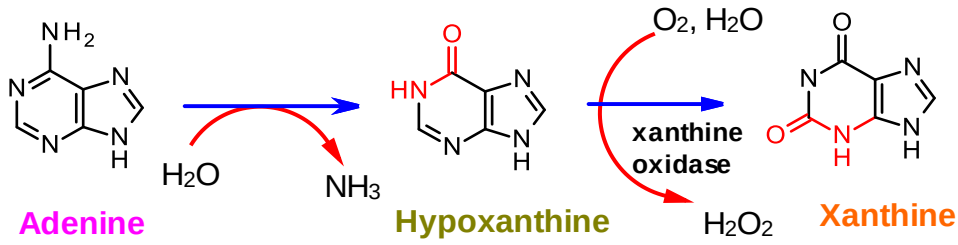


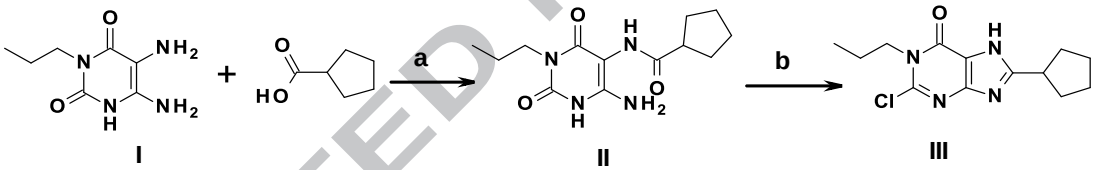
Figure 2. Metabolic Pathway of Adenine to Xanthine.

The structure activity relationship studies, pharmacokinetic properties of selected compounds and their *in-vivo* efficacy results are also described

2. Results and Discussion

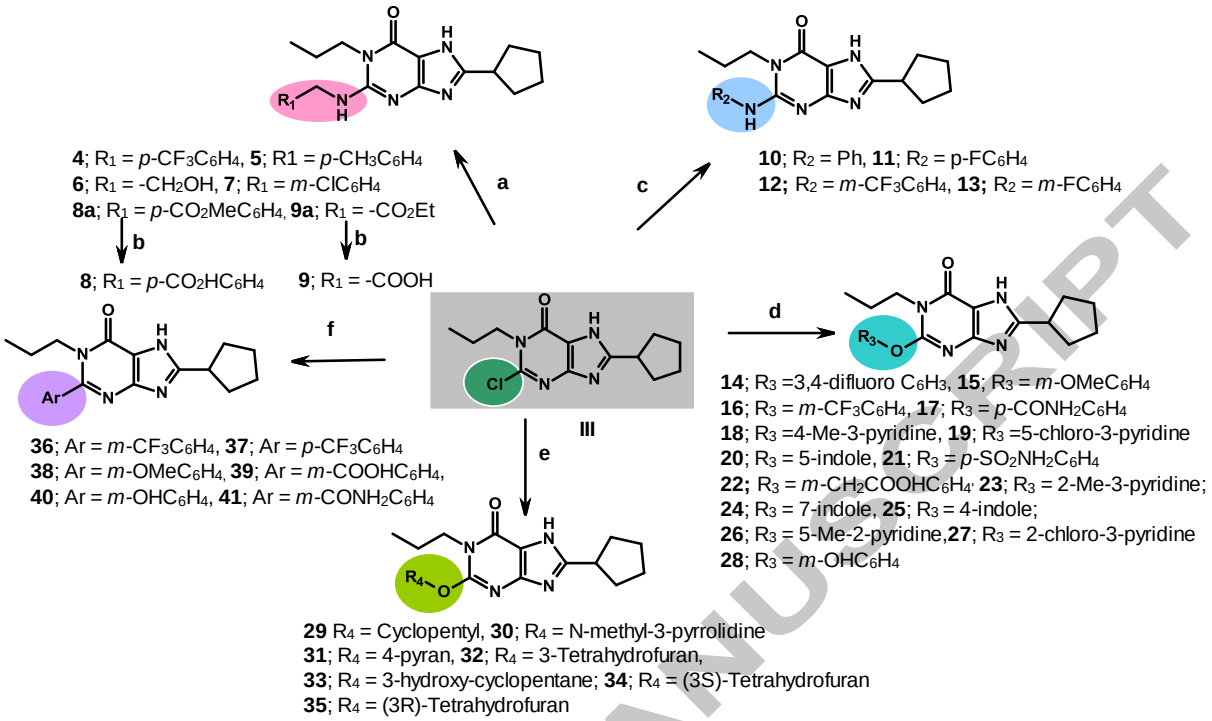
2.1. Chemistry

Multipronged approach was used to synthesize a library of about 40 compounds from Intermediate **III** (**Scheme 2**). Intermediate **III** was prepared from 5, 6-diamino-3-propyl-1H-pyrimidine-2, 4-Dione [22, 23] (**I**) in two steps by EDCI coupling of **I** with cyclopentane carboxylic acid followed by concerted cyclization and chlorination with $POCl_3$ (**Scheme 1**)



Scheme 1. General synthesis of common intermediate, **III**. Reagents and conditions: (a) EDCI.HCl, MeOH, 0 ^{0}C → rt; (b) $POCl_3$, PCl_5, reflux overnight, 19% over 2 steps.

Compounds **4-7, 8a and 9a** were synthesized in moderate yields in one step by heating Intermediate **III** with the corresponding aliphatic or aromatic amine in N-methyl pyrrolidine in presence of diisopropylethylamine. **8a** and **9a** on base mediated hydrolysis afforded acids **8** and **9** in good yields. Intermediate **III** on treatment with the appropriately substituted anilines and phenols in N-methyl pyrrolidine at elevated temperatures afforded compounds **10-13 and 14-28** respectively in overall good yields. Compounds **29-35** were prepared in moderate yields from **III** by heating it with corresponding alcohol in presence of sodium hydride. Finally compounds **36-41** were synthesized by palladium catalyzed Suzuki coupling of **III** with corresponding boronic acids using sodium bicarbonate as a base.

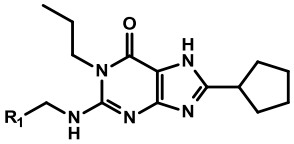


Scheme 2. General synthesis of A_1R antagonists **4-41**. Conditions: (a) Corresponding amines, N-methyl pyrrolidine, DIPEA, 130 ^oC, 2 h, 15-30%; (b) NaOH, EtOH/THF/H_2O (1:1:1), 50-60 ^oC, 12 h; (c) Corresponding anilines, N-methyl-pyrrolidine, 150 ^oC, 12 h, 47%; (d) Corresponding phenols, N-methyl-pyrrolidine, 130 ^oC, 12 h, 52%; (e) Corresponding alcohols, NaH (60% dispersion in mineral oil), 120 ^oC – 130 ^oC, 3 h, 22-34%; (f) Corresponding boronic acids, Pd $(PPh_3)_4$, $NaHCO_3$, THF, 100 ^oC, 12 h, 20-40%.

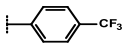
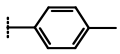
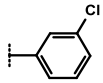
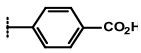
2.2. Structure Activity Relationship

Compounds **4** and **5** with hydrophobic substituents at *para* positions of N-benzyl group, were identified as the most potent (K_i = 23 and 27 nM) and selective A_1R antagonists (**Table 1**). *Meta* substitution on the aryl ring (compound **7**) retained potency against A_1R (Ki = 120 nM) though found to be less potent as compared to compounds **4** and **5.** Substituting the *para* position with hydrophilic carboxylic group as in compound **8** resulted in drastic loss in potency**.** Compounds **6** and **9** with aliphatic ethanol amine and N-acetic acid moieties lost potency against A_1R though **9** was found to be slightly more potent in comparison to its aromatic counterpart **8.**

Table 1
In-vitro data of C-2 substituted N-benzyl and N-alkyl analogs



Compound	R_1	hA_1 K_i (nM) / %	% Inhibition at 1 µM conc[a]

		inhibition at 1μM conc[a]	hA_{2B}	hA_{2A}	hA_3
4		23	0%	0%	23%
5		27	6%	0%	24%
6	$-CH_2OH$	36%	31%	5%	0%
7		120	nt	nt	nt
8		54%	16%	31%	nt
9	-COOH	72%	9%	0%	0%

All data points were evaluated in triplicates. Each compound was evaluated at 8 concentrations with each data point in triplicates for Ki determination

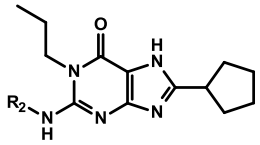
[a]Binding affinities were determined by using membrane preparations from HEK-293 cells overexpressing the relevant human AdoR subtype.

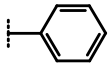
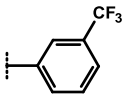
nt - not tested.

Compounds **10**-**13** with substituted anilines at C-2 position were found to be less potent as compared to N-benzyl analogs (**Table 2**). In this series, compound **12** with *meta*-CF_3 substitution was identified as the most potent compound (K_i = 370 nM) against A_1R.

Table 2

In- vitro data of C-2 substituted N-aryl analogs



Compound	R_2	hA_1 K_i (nM)	% Inhibition at 1 μM conc.[a]		
			hA_{2B}	hA_{2A}	hA_3
10		> 1000	nt	nt	nt
11		> 1000	6%	0%	24%
12		370	5%	27%	nt

13		4500	16%	31%	nt

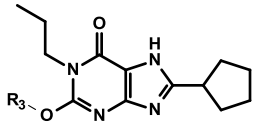
All data points were evaluated in triplicates. Each compound was evaluated at 8 concentrations with each data point in triplicates for Ki determination

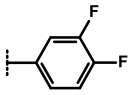
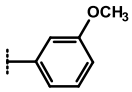
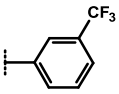
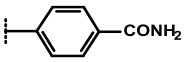
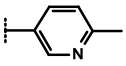
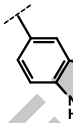
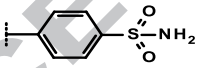
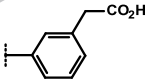
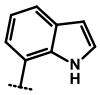
[a]Binding affinities were determined by using membrane preparations from HEK-293 cells overexpressing the relevant human AdoR subtype.

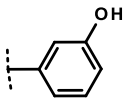
nt - not tested.

A set of about 15 O-aryl and heteroaryl analogs **14-28** were prepared with different hydrophobic and hydrophilic substitutions at *meta* and *para* positions to understand detailed SAR for potency and selectivity against A_1R (**Table 3**). Compounds **14-16** with aryl rings substituted at *meta* positions with hydrophobic groups were found to be the most potent compounds in both A_1R binding (K_i = 12-23 nM) and functional assays (K_i = 2-13 nM). However, even though compounds **14** and **16** with 3,4-difluoro and 3-trifluoromethyl groups respectively were selective against other adenosine receptor subtypes, compound **15** with 3-methoxy group showed less selectivity against A_2B (70% inhibition at 1 µM) and A_3 (K_i = 430 nM) subtypes. Compounds **17**, **21** and **22** with *meta* or *para* substituted hydrophilic groups on aryl rings lost potency against A_1R (K_i > 1000 nM) though 3-hydroxyl substituted analog **28** was an exception. **28** was found to be potent (K_i = 10.5 nM) in A_1 binding assay but less selective against other adenosine receptor subtypes. Since this compound was expected to show high clearance *in vivo* due to Phase 2 metabolism, we shifted our focus to the indole bioisosteric replacements of phenol. Thus, compounds **20**, **24** and **25** with 5-, 7- and 4-indolyl moieties respectively were synthesized to understand the impact of different orientations of indole nitrogen on A_1 potency. All three compounds retained potency against A_1R and compound **24** with 7-indolyl substitution was identified as the most potent compound in A_1 binding and functional assays. Then our focus shifted to understand the effect of C-2 substituted pyridinoloxy groups on A_1R potency. Compounds with *para* substitutions with respect to either pyridine nitrogen (compound **26**, K_i > 1000 nM**)**) or point of attachment at C-2 (compound **18,** K_i = 630 nM) seemed to lose potency against A_1R. All other pyridine derivatives having *meta* or *ortho* substitutions with respect to pyridine nitrogen or point of attachment at C-2 were found to be potent against A_1R. The most potent compounds identified in this series were **23** [K_i (binding) = 6.6 nM, K_i (functional) = 0.28 nM)] and **27** (K_i = 3.6 nM) both of which had substitutions *ortho* to pyridine nitrogen and point of attachment at C-2. In binding assay, compound **23** was found to have less selectivity against A_{2B} (91% inhibition at 1 µM) though in functional assay, the compound showed about 2000 fold selectivity for A_1 over A_{2B}.

Table 3
In-vitro data of C-2 substituted O-aryl analogs



Compound	R_3	hA_1 K_i (nM)[a]	A_1 cAMP K_i (nM)	% Inhibition at 1 μM conc[a]		
				hA_{2B}	hA_{2A}	hA_3
14		23	13	IC_{50} > 10,000 nM	nr	IC_{50} > 10,000 nM
15		12.5	2.3	70%	36%	IC_{50}: 430 nM
16		19	5.9	0%	34%	35%
17		> 1,000	nt	nt	nt	nt
18		630	nt	nt	nt	nt
19		12	2.2	37%	22%	76%
20		36	nt	0%	0%	34%
21		> 1,000	nt	nt	nt	nt
22		> 1,000	nt	nt	nt	nt
23		6.6	0.28	91%[b]	33%	35%
24		5.5	5.6	42%	25%	40%

25		74	nt	nt	nt	nt
26		> 1,000	nt	nt	nt	nt
27		3.6	nt	nt	nt	nt
28		10.5	nt	53%	81%	69%

All data points were evaluated in triplicates. Each compound was evaluated at 8 concentrations with each data point in triplicates for Ki determination

[a]Binding affinities were determined by using membrane preparations from HEK-293 cells overexpressing the relevant human AdoR subtype.

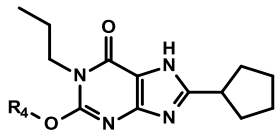
[b]% inhibition at 30 µM concentration.

nt - not tested.

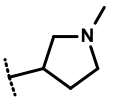
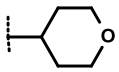
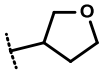
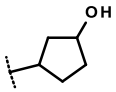
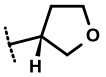
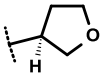
nr – no response at highest tested concentration.

In case of C-2 substituted O–alkyl analogs, similar trend in SAR was observed as seen in previous series (**Table 4**). All compounds with carbocyclic or heterocyclic substitutions at C-2 were found to be potent against A_1R though compound **30** with N-methyl pyrrolidine substitution was found to be less potent. In this series, compounds **29** [K_i (binding) = 14 nM; K_i (functional) = 13 nM] and **31** [K_i (binding) = 20 nM; K_i (functional) = 12 nM] were identified as the most potent compounds. However, compound **29** was found to be less selective against other three adenosine receptor subtypes while compound **31** was selective against A_3R. Racemic compound **32** with tetrahydrofuryl moiety at C-2 (K_i = 59 nM) and its two enantiomers, **34** (K_i = 85 nM) and **35** (K_i = 50 nM) retained similar potency against A_1R

Table 4
In-vitro data of C-2 substituted O-alkyl analogs



Compound	R_4	hA_1 K_i (nM)[a]	A_1 cAMP K_i (nM)	% Inhibition at 1 µM conc[a]		
				hA_{2B}	hA_{2A}	hA_3
29		14	13	92%	85%	79%

30		> 1,000	nt	nt	nt	nt
31		20	12	56%	82%	0%
32		59	nt	nt	nt	nt
33		57	9.6	35%	0%	37%
34		85	nt	nt	nt	nt
35		50	nt	nt	nt	nt

All data points were evaluated in triplicates. Each compound was evaluated at 8 concentrations with each data point in triplicates for Ki determination

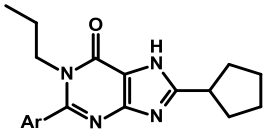
[a]Binding affinities were determined by using membrane preparations from HEK-293 cells overexpressing the relevant human AdoR subtype.

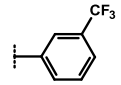
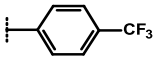
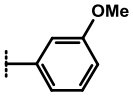
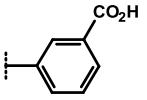
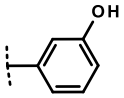
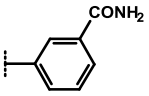
nt - not tested.

In case of C-2 substituted aryl analogs, compounds **36** and **38** with *meta* substituted aryl moieties were found to be potent against A_1R while compound **37** with *para* substitution lost A_1 potency to some extent. Compound **36** was also selective against other adenosine receptor subtypes. Compounds **39** and **41** with hydrophilic substitutions drastically lost potency against A_1. Compound **40** with *meta* hydroxyl group, however, retained very good A_1R potency both in binding (K_i = 1 nM) and functional (K_i = 0.2 nM) assays though it was found to be less selective against A_{2B} and A_{2A} receptor subtypes. This SAR confirms that hydrophilic carboxyl and amide functionalities are not well tolerated for A_1R activity across the series

Table 5

In-vitro data of C-2 aryl analogs



Compound	Ar	hA_1 K_i (nM) /% inhibition at 1μM conc[a]	A_1 cAMP K_i (nM)	Ki (nM)/% Inhibition at 1 μM conc[a]		
				hA_{2B}	hA_{2A}	hA_3
36		100%	12	14%	0%	0%
37		70%	nt	12%	0%	5%
38		16	15	1100	10,000	690
39		nr	< 50%[b]	nt	nt	nt
40		1	0.2	100%	74%	nt
41		> 1000	nt	nt	nt	nt

All data points were evaluated in triplicates. Each compound was evaluated at 8 concentrations with each data point in triplicates for Ki determination

[a]Binding affinities were determined by using membrane preparations from HEK-293 cells overexpressing the relevant human AdoR subtype.

[b]% inhibition at 30 μM concentration.

nt - not tested.

nr – no response at highest tested concentration.

Based on diverse SAR explored at C-2 position of hypoxanthine core (**Tables 1-5**), potent and selective representative compounds, **14**, **23**, **33** and **38** were selected for further profiling. The compounds were found to have good to moderate stability in liver microsomes and were free from any CYP liability. Compounds **14** and **38** did not show any hERG liability in binding assay (**Table 6**)

Table 6

In-vitro and ADME data of Compounds **14**, **23**, **33** and **38**

Parameter	**14**	**23**	**33**	**38**
A_1AdoR (binding) K_i (nM)	23	6.6	57	16
A_1AdoR cAMP	13	0.28	9.6	15

(functional) K_i (nM)				
Selectivity against other adenosine receptor subtypes (K_i/% inhibition at 1 µM)	> 10 µM	A_{2A}: 2000 nM A_{2B}: 49% A_{2B} cAMP K_i = 560 nM A_3 : 16%	A_{2A} :0% A_{2B}: 41% A_3 : 6%	A_{2A} : >10 µM A_{2B}: 1.1 µM A_3 : 650 nM
MR[a, b] nmol/min/mg protein (RLM/HLM); % remaining at 30 min (+NADPH)	0.08/0.05; 75/82	0.07/0.06; 78/79	0.10/0.12; 68/60	0.05/0.03; 82/97
hERG (% inhibition) @ 5, 25 uM concs	2, 4	nt	nt	9, 72
CYP 450 % Inhibition at 5 uM; 1A2, 2C9, 2C19, 2D6, 3A4	7, nt, 0, 10, 34	0, 4, 10, 0, 0	5, 4, 3, 0, 0	0, 7, nt, 1, 5
Plasma Protein % binding at 5 uM (Mouse/Rat)	99/99	nt / 77	nt / 80	95/95
Thermodynamic Solubility (µM) @ pH 2, 4, 7.4	77, 23, 17	2152, 43, 38	2589, 276, 182	614, 63, 54

nt- not tested.

[a]Metabolic Rate <0.1 nmol/min/mg is considered metabolically stable.

[b]Testosterone [MR (RLM) > 1.52, 2% remaining at 30 min (+NADPH); MR (HLM): 0.05, 84% remaining after 30 min (+NADPH)] and Verapamil [MR (RLM): 0.1, 68% remaining at 30 min (+NADPH); MR (HLM): 0.06, 78% remaining after 30 min (+NADPH)] have been used as positive controls in microsomal metabolic stability studies.

RLM: Rat liver microsomes.

HLM: Human liver microsomes.

2.3. *In-vivo* Pharmacokinetics in Male Wistar Rats

We next determined the pharmacokinetic (PK) profile of these four compounds, **14**, **23**, **33** and **38** in male Wistar rats. The results are summarized in **Table 7**. Compound **14**

showed reasonable half life of 2 hours with low clearance. Compounds **23** and **33** were found to have high clearance and very low half life. To address the high clearance of these compounds, PK studies of compounds **23** and **33** were carried out in presence and absence of 1-aminobenztriazole (ABT) which is a non-specific CYP inhibitor. In these studies **23** in presence of ABT was found to show 6 fold increase in exposure and 3 fold increase in half life with 6 fold decrease in clearance while in case of compound **33,** exposure and half life increased 3-4 fold and clearance was reduced by 4 fold suggesting that these compounds are undergoing CYP and non-CYP based Phase 1/2 metabolism. To find metabolic soft spots of these compounds, metabolite identification studies were carried out. Compound **23** appears to undergo hydroxylation at N-1 propyl and C-8 cyclopentyl ring followed by dehydration. Similarly, it was proposed that compound **33** undergoes N- oxidation and C-8 cyclopentyl ring hydroxylation.

Table 7

Pharmacokinetic (PK) profile[a] of **14**, **23**, **33** and **38** in male Wistar rats

Compound	**14**		**23**	**33**	**38**	
Route of administration	IV	PO	IV	IV	IV	PO
Dose (mg/kg)	3	5	3	3	3	5
C_{max} (µM)	NA	9.71 ± 1.8	NA	NA	NA	1.74 ± 0.8
T_{max} (h)	NA	1.63 ± 0.75	NA	NA	NA	0.3 ± 0.11
AUC_{0-t} (µM.h)	10.18 ± 3.53	3.19 ± 1.13	2.7 ± 0.9	3.0 ± 0.3	3.53 ± 0.33	1.6 ± 0.83
V_{ss} (L/kg)	1.73 ± 0.62	NA	2.0 ± 0.6	0.8 ± 0.2	1.2 ± 0.66	NA
CL (mL/min/kg)	13.61 ± 4.42	NA	55 ± 16	48 ± 5	39.22 ± 3.59	NA
$T_{1/2}$ (h)	2.06 ± 0.61	NA	0.5 ± 0.05	0.3 ± 0.04	0.49 ± 0.35	NA
%F	NA	27	NA	NA	NA	27

[a]Values indicate mean for n = 4.

NA: Not applicable.

Compound **14**: IV - NMP-10%,PEG-400 (20%), normal saline q.s pH adjusted to 12 with 1N NaOH; PO – NMP (10%), Labrasol (30%), citrate buffer q.s (pH 7.0).

Compounds **23 and 33**: NMP-5%, PEG-300 (10%), 0.1 M Citrate buffer q.s. (pH 5.5).

Compound **38**: NMP-10%, PEG-400 (20%), Citrate buffer q.s, pH 8.0 (pH 4.2).

2.3. *In-vivo* Brain Disposition of Compounds 14 and 38 in Male Wistar Rats

Since our objective was to identify selective A_1 receptor antagonists that do not cross blood brain barrier, we carried out *in-vivo* brain disposition studies of representative compounds **14** and **38** in male Wistar rats after oral administration of compounds at 5 mg/kg dose (**Table 8**). Compound **14** showed negligible (3-5% of plasma) concentrations in brain while as compound **38** showed brain concentrations below quantification limit.

Table 8

In-vivo Brain and Plasma Concentrations[a] of **14** and **38** in male Wistar Rats.

Compound	**14**			**38**		
Dose	5 mg/kg, PO					
Time (h)	1	2	4	1	2	4
Concentrations in Plasma (µM)	1.64	0.94	1.81	0.67	0.54	0.04
Concentrations in Brain (µM)	0.09	0.05	0.08	BQL	BQL	BQL
Brain/Plasma Ratio	0.05	0.05	0.05	NA	NA	NA

[a]Values indicate mean for n = 2 per time point

BQL: Below Quantification level (10 ng/mL)

NA: Not applicable

Compound **14**: NMP (10%) v/v, Labrasol- 30% v/v, citrate buffer pH 8.5

Compound **38**: NMP (10%) v/v, polyethylene glycol-400 (PEG-400) - 20% v/v, citrate buffer pH 8.5

2.4. *In-vivo* Pharmacodynamics of Compound 14 in Acute Diuretic Model

Since compound **14** showed negligible brain concentrations and longer half life as well as better exposure in PK studies, this compound was selected for assessment of oral activity in an acute rat diuresis model at 3, 10 and 30 mg/kg doses. Compound **14** showed significant diuresis at 10 and 30 mg/kg doses and natriuresis at all the three doses as compared to the vehicle treated group (**Figure 3**). These results were comparable with those obtained with Rolofylline treated animals at 0.1 mg/kg dose. There was no significant kaliuresis observed with compound **14** treated groups compared to vehicle. Also there was no significant difference in serum Na^+ and K^+ levels as compared to vehicle group.

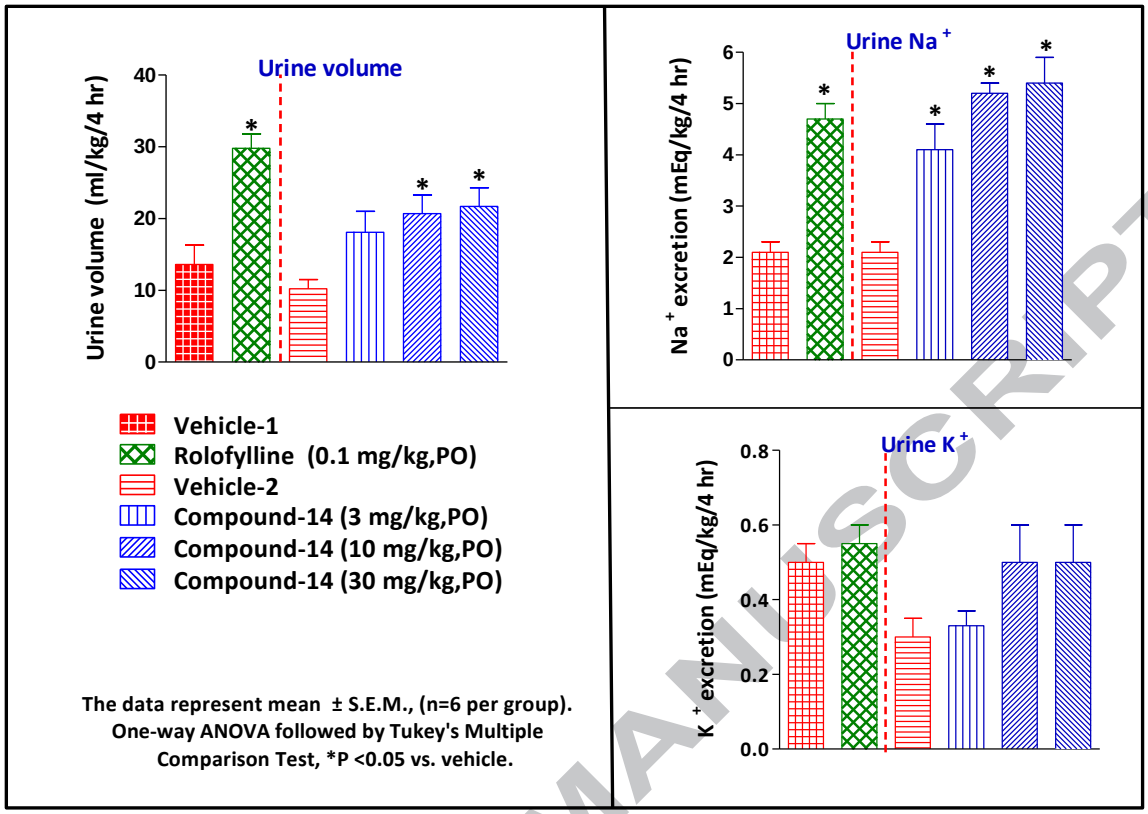


Figure 3. Effect of compound **14** on acute diuretic activity in Wistar Rats.

3. Conclusions

In summary, we have reported herein the discovery of a series of novel hypoxanthine based antagonists which demonstrated good *in-vitro* A_1R antagonism and acceptable selectivity against other adenosine receptor subtypes. Four compounds **14, 23**, **33** and **38** were evaluated for their pharmacokinetic properties in Wistar rats. Compounds **14** and **38** were further profiled in brain disposition study**.** Compound **14** showed reasonable exposure, half life and negligible brain exposure. This warranted the evaluation of this compound in rat PD model in which **14** exhibited significant diuretic activity at 10 mg/kg dose.

4. Materials and methods

The purity of compounds was determined by HPLC and all compounds had purities of >95% unless otherwise stated. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded in the deuterated solvents specified on a Varian 400 spectrometer operating at 400 MHz. The signal of the deuterated solvent was used as internal reference. Chemical

shifts (δ) are given in ppm and are referenced to residual not fully deuterated solvent signal. Coupling constants (*J*) are given in Hz. Chemical shifts are reported in parts per million (δ) from the tetramethylsilane resonance in the indicated solvent (TMS: 0.0 ppm). Data are reported as follows: chemical shift, multiplicity (br=broad, s=singlet, d=doublet, t=triplet, q=quartet, quint=quintet, m=multiplet) and integration. Mass spectra were determined by using Agilent MSD/VL spectrometer or Agilent 1200SL-6110 LC/MS (ESI) system using positive-negative switching.

4.1. Chemical synthesis

4.1.1. N-(6-amino-2, 4-dioxo-3-propyl-1H-pyrimidin-5-yl) cyclopentanecarboxamide (II)

To a solution of 5, 6-diamino-3-propyI-lH-pyrimidine-2, 4-dione (0.6 g, 2.72 mmol) in methanol (50 mL) was added cyclopentane carboxylic acid (0.31 g, 2.72 mmol). The reaction mixture was cooled to 0°C and then l-ethyl-3(3'-dimethylaminopropyl) carbodiimide hydrochloride (EDCI.HC1) (0.78 g, 4.1 mmol) was added. The resulting reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated under reduced pressure. The residue was suspended in water. The solid was filtered and washed thoroughly with water followed by diethyl ether. The product obtained was dried under high vacuum. The crude product **II** (0.40 g) was used for the next step without further purification.

4.1.2. 2-chloro-8-cyclopentyl-1-propyl-7H-purin-6-one (III)

To a suspension of cyclopentane carboxylic acid (6-amino-2,4-dioxo-3-propyl- 1,2,3,4-tetrahydro-pyrimidin-5-yl)-amide (0.40 g, crude) obtained from step 1 in phosphorus oxychloride (25 ml) was added phosphorus pentachloride (0.10 g) and the resulting reaction mixture was refluxed overnight. Phosphorus oxychloride was evaporated under reduced pressure. The residue was slowly quenched with water. Ethyl acetate was added and the organic layer was separated and washed thoroughly with water followed by brine. The ethyl acetate layer was dried over anhydrous sodium sulphate and concentrated under vacuum. The crude product was purified by preparative TLC using dichloromethane/ methanol (9:1) as the solvent system to give 0.075 g (19% over two steps) of **III** as a white solid. **III**: mp: 204-206 ^{o}C; LCMS: *m/z* 281.3 [M^{+}+1]; ^{1}HNMR (400 MHz, DMSO-

d6) δ 0.9 (t, J = 8 Hz, 3H), 1.59-1.82 (m, 8H), 1.99 (m, 2H), 3.15 (t, J = 8 Hz, 1H), 4.12 (t, J = 8 Hz, 2H)

4.1.3. General Synthesis of Compounds 4-8a, 9a

To a solution of **III** (1 equiv., 0.10 g) in N-methyl-2-pyrrolidone (10 equiv., 0.3 mL) was added corresponding amine (1.2 equiv.) followed by diisopropylethylamine (3 equiv., 0.2 mL) and the reaction mixture was heated at 130 °C for 2 hours. The reaction mixture was then diluted with water and extracted with ethyl acetate. Ethyl acetate layer was dried over anhydrous sodium sulphate and concentrated under vacuum. The crude products so obtained were purified by preparative TLC using varying proportions of methanol in dichloromethane as the solvent systems to give the desired products compounds **4-8a** and **9a** in moderate to good yields

4.1.3.1. 8-cyclopentyl-1-propyl-2-[[4-(trifluoromethyl) phenyl] methylamino]-7H-purin-6-one (4*)*

Yield: 0.091 g, 61%; mp: 235-237 ^{o}C; LC-MS: *m/z* 420.0 [M^{+} +1]; HPLC R_T: 19.781 min; Purity: 94%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.92 (t, J = 8 Hz, 3H), 1.58-1.74 (m, 8H), 1.89 (br. s, 2H), 2.96-3.04 (m, 1H), 3.98 (br. s, 2H), 4.64 (br. s, 2H), 7.52 (s, 2H), 7.64-7.69 (m, 2H), 12.1 (br. s, 1H)

4.1.3.2. 8-Cyclopentyl-2-(4-methyl-benzylamino)-1-propyl-1,7-dihydro-purin-6-one (5)

Yield: 0.077 g, 59%; mp: 228-230 ^{o}C; LC-MS: *m/z* 366.1 [M^{+} +1]; HPLC R_T: 18.002 min; Purity: 98%; ^{1}HNMR (400 MHz, CD_3OD) δ 0.90 (t, J = 8 Hz, 3H), 1.56-1.58 (m, 4H), 1.69-1.75 (m, 4H), 1.91 (br.s, 2H), 2.26 (s, 3H), 2.99 (m, 1H), 3.97 (br.s, 2H), 4.51 (br.s, 2H), 7.11 (d, J = 8 Hz, 2H), 7.18 (d, J = 8Hz, 2H), 12.41 (br.s, 1H)

4.1.3.3. 8-Cyclopentyl-2-(2-hydroxy-ethylamino)-1-propyl-1,7-dihydro-purin-6-one (6):

Yield: 0.051 g, 47%; mp: 257-259 ^{o}C; LC-MS: *m/z* 306.3 [M^{+} +1]; HPLC R_T: 10.740 min; Purity: 96%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.88 (br.s, 3H), 1.54-1.77 (br.s, 8H), 1.93 (br.s, 2H), 3.14 (br.s, 1H), 3.55 (br.s, 3H), 3.90 (br.s, 2H), 4.72 (br.s, 1H), 6.86 (br.s, 1H), 12.15 (br.s, 1H)

4.1.3.4. 2-(3-Chloro-benzylamino)-8-cyclopentyl-1-propyl-1, 7-dihydro-purin-6-one (7)

Yield: 0.072 g, 51%; mp: 163-165 ^{o}C; LC-MS: *m/z* 387.9 [M^{+} +2]; HPLC R_T: 18.540 min; Purity: 96.9%; ^{1}HNMR (400 MHz, CD_3OD) δ 1.00 (t, *J* = 8 Hz, 3H), 1.69-1.81 (m, 8H), 2.05 (br.s, 2H), 3.01 (m, 1H), 4.04 (t, *J* = 8 Hz, 2H), 4.55 (br.s, 2H), 7.21-7.36 (m, 4H)

4.1.3.5. 4-[(8-Cyclopentyl-6-oxo-1-propyl-6,7-dihydro-1H-purin-2-ylamino)-methyl]-benzoic acid methyl ester (8a)

Yield: 0.087 g, 60%; mp: 161-163 ^{o}C; LC-MS: *m/z* 410.0 [M^{+} +1]; HPLC R_T: 16.50 min; Purity: 96%; ^{1}HNMR (400 MHz, CD_3OD) δ 1.01 (t, *J* = 8 Hz, 3H), 1.68-1.83 (m, 8H), 2.04-2.08 (m, 2H), 3.14 (t, *J* = 8 Hz, 1H), 3.89 (s, 3H), 4.07 (t, *J* = 8 Hz, 2H), 4.73 (s, 2H), 7.47 (d, *J* = 8 Hz, 2H), 7.83 (d, *J* = 8 Hz, 2H)

4.1.3.6. (8-Cyclopentyl-6-oxo-1-propyl-6,7-dihydro-1H-purin-2-ylamino)-acetic acid ethyl ester (9a)

Yield: 0.068 g, 55%; mp: 238-240 ^{o}C; LC-MS: *m/z* 348.1 [M^{+} +1]; HPLC R_T: 13.783 min; Purity: 93%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.87 (t, *J* = 8 Hz, 3H), 1.19 (t, *J* = 8 Hz, 3H), 1.59 (br. s, 4H), 1.70-1.79 (m, 4H), 1.93 (br. s, 2H), 3.33 (br. s, 1H), 3.93 (s, 2H), 4.03 (t, *J* = 8 Hz, 2H), 4.1 (q, *J* = 8 Hz, 2H), 7.4 (br. s, 1H), 12.2 (br. s, 1H)

4.1.4. General synthesis of Compounds 8 and 9

To a solution of ester **8a** or **9a** (1 equiv., 0.05 g) in a mixture of MeOH/H_2O/THF (1:1:1) was added NaOH (3 equiv.) and the resulting reaction mixture was refluxed overnight. The reaction mixture was concentrated under reduced pressure. The residue was dissolved in water and neutralized with 1 N HCl and then extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulphate and concentrated under vacuum. The crude product was purified by washing repeatedly with diethyl ether to give the products in moderate to good yield

4.1.4.1. 4-[(8-cyclopentyl-6-oxo-1-propyl-6,7-dihydro-1H-purin-2-ylamino)-methyl]-benzoic acid (8)

Yield: 0.015 g, 32%; mp: 204-206 ^{o}C; LC-MS: *m/z* 396.0 [M^{+} +1]; HPLC R_T: 13.60 min; Purity: 98%; ^{1}HNMR (400 MHz, CD_3OD) δ 1.00 (t, *J* = 8 Hz, 3H), 1.68-1.81 (m, 7H), 1.99 (s, 1H), 2.06-2.10 (m, 2H), 3.16 (t, *J* = 8 Hz, 1H), 4.06 (t, *J* = 8 Hz, 2H), 4.73 (s, 2H), 7.44 (d, *J* = 8 Hz, 2H), 7.95 (d, *J* = 8 Hz, 2H); ^{13}CNMR (100 MHz, DMSO-d6) δ

11.00, 20.64, 25.01, 31.52, 41.41, 44.48, 126.65, 129.09, 129.36, 145.22, 151.43, 156.32, 159.45, 167.23

4.1.4.2. (8-Cyclopentyl-6-oxo-1-propyl-6,7-dihydro-1H-purin-2-ylamino)-acetic acid (9)

Yield: 0.022 g, 48%; mp: 243-245 ^{o}C; LC-MS: *m/z* 320.1 [M^{+} +1]; HPLC R_T: 11.046 min; Purity: 92%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.90 (t, *J* = 8 Hz, 3H), 1.56-1.73 (m, 4H), 1.74-1.88 (m, 4H), 1.89-2.01 (br. s, 2H), 3.01-3.10 (m, 1H), 3.2 (s, 1H), 3.93 (br. s, 3H), 7.25 (br. s, 1H), 12.2 (s, 1H)

4.1.5. General Synthesis of Compounds 10-13

To a solution of **III** (1 equiv., 0.10 g) in N-methyl-pyrrolidone (10 equiv., 0.3 mL) was added aniline (10 equiv., 0.3 mL) and the resulting reaction mixture was heated at 150 °C for 12 hours. After completion of reaction, the reaction mixture was cooled down to room temperature, diluted with water and the product was extracted with ethyl acetate. The ethyl acetate layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude products so obtained were purified by preparative TLC using methanol in dichloromethane as solvent system to give the target compounds **10-13** in good yields.

4.1.5.1. 2-anilino-8-cyclopentyl-1-propyl-7H-purin-6-one, (10)

Yield: 0.076 g, 63%; mp: 313-315 ^{o}C; LC-MS: *m/z* 338.0 [M^{+} +1]; HPLC R_T: 16.478 min; Purity: 97.8%; ^{1}HNMR (400 MHz, CD_3OD) δ 1.01 (s, *J* = 8 Hz, 3H), 1.68 (br. s, 2H), 1.73-1.83 (m, 6H), 2.04-2.10 (m, 2H), 3.15 (t, *J* = 8 Hz, 1H), 4.22 (t, *J* = 8 Hz, 2H), 7.13 (t, *J* = 8 Hz, 1H), 7.32 (t, *J* = 8 Hz, 2H), 7.49 (d, *J* = 8 Hz, 2H); ^{13}CNMR (100 MHz, DMSO) δ 11.02, 20.93, 25.03, 31.54, 41.99, 116.38, 123.77, 128.40, 139.63, 149.47, 152.39, 156.25, 158.93

4.1.5.2. 8-cyclopentyl-2-(4-fluoroanilino)-1-propyl-7H-purin-6-one (11)

Yield: 0.066 g, 52%; mp: 327-329 ^{o}C; LC-MS: *m/z* 356.0 [M^{+} +1]; HPLC R_T: 17.143 min; Purity: 99.2%; ^{1}HNMR (400 MHz, CD_3OD) δ 1.01 (t, *J* = 8 Hz, 3H), 1.67-1.68 (m, 2H), 1.73-1.82 (m, 6H), 2.03-2.08 (m, 2H), 3.14 (t, *J* = 8 Hz, 2H), 4.19 (t, *J* = 8 Hz, 2H), 7.06 (t, *J* = 8 Hz, 2H), 7.45-7.49 (m, 2H)

4.1.5.3. 8-cyclopentyl-1-propyl-2-[3-(trifluoromethyl) anilino]-7H-purin-6-one (12)

Yield: 0.071 g, 49%; mp: 312-314 ^{o}C; LC-MS: *m/z* 406.0 [M^{+} + 1]; HPLC R_T: 11.776

min; Purity: 92%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.89 (t, J = 7.2 Hz, 3H), 1.58-1.78 (m, 8H), 1.93 (br s, 1H), 2.98-3.05 (m, 1H), 4.09-4.16 (m, 2H), 7.97-7.44 (m, 1H), 7.55 (t, J = 8.0 Hz, 1H), 7.81-7.88 (m, 2H); ^{13}CNMR (100 MHz, DMSO-d6) δ 11.00, 20.96, 25.05, 31.63, 41.81, 119.42, 122.91, 125.62, 129.35, 129.49, 140.70, 148.82, 152.77, 155.89, 158.72

4.1.5.4. 8-cyclopentyl-2-(3-fluoroanilino)-1-propyl-7H-purin-6-one (13)

Yield: 0.078 g, 62%; mp: 300-302 ^{o}C; LCMS: *m/z* 356.0 [M^{+} +1]; HPLC R_T: 10.265 min; Purity: 97%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.87 (t, J = 7.6 Hz, 3H), 1.55-1.80 (m, 8H), 1.92-1.97 (m, 2H), 3.03-3.07 (m, 1H), 4.13-4.17 (m, 2H), 6.83-6.87 (m, 1H), 7.30-7.35 (m, 2H), 7.47-7.49 (m, 1H), 8.6 (br. s, 1H), 12.4 (br. s, 1H)

4.1.6. General Synthesis of Compounds 14-28

To a solution of **III** (1 equiv., 0.10 g) in N-methyl-2-pyrrolidone (10 equiv., 0.3 mL) was added K_2CO_3 (1.5 equiv., 0.074 g) followed by the corresponding phenol (1.1 equiv.) and the reaction mixture was heated at 130 ^{0}C for 12 hours. The reaction mixture was diluted with ethyl acetate and water. The layers were separated and ethyl acetate layer was washed with water. The ethyl acetate layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude product was purified by preparative TLC using methanol in dichloromethane as solvent system to give the desired compounds **14-28** in good to moderate yields.

4.1.6.1. 8-cyclopentyl-2-(3,4-difluorophenoxy)-1-propyl-7H-purin-6-one (14)

Yield: 0.095 g, 71%; mp: 237-239 ^{o}C; LC-MS: *m/z* 375.3 [M^{+} +1]; HPLC R_T: 15.511 min; Purity: 100%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.94 (t, J = 8 Hz, 3H), 1.59-1.74 (m, 6H), 1.94 (br.s, 2H), 3.12 (m, 2H), 4.09 (br. s, 2H), 7.21 (d, J = 8 Hz, 1H), 7.53-7.65 (m, 2H), 12.74 (br.s, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 11.22, 21.52, 25.03, 31.82, 42.94, 112.44, 112.64, 117.69, 117.86, 119.06, 147.83, 148.02, 148.70, 150.47, 152.87, 153.55

4.1.6.2. 8-cyclopentyl-2-(3-methoxyphenoxy)-1-propyl-7H-purin-6-one (15)

Yield: 0.091 g, 69%; mp: 197-199 ^{o}C; LC-MS: *m/z* 369.0 [M^{+} +1]; HPLC R_T: 22.274 min; Purity: 96%; ^{1}HNMR (400 MHz, CD_3OD) δ 1.03-1.07 (t, J = 8 Hz, 3H), 1.69-1.72 (m, 2H), 1.77-1.89 (m, 6H), 2.05-2.12 (m, 2H), 3.15-3.21 (m, 1H), 3.82 (s, 3H), 4.25 (t, J = 8 Hz, 3H), 6.81-6.89 (m, 3H); ^{13}CNMR (100 MHz, DMSO-d6) δ 11.15, 21.52, 25.02,

31.35, 31.82, 42.83, 55.46, 107.99, 110.73, 111.58, 113.95, 130.02, 152.98, 153.32, 154.02, 159.38, 160.34

4.1.6.3. 8-cyclopentyl-1-propyl-2-[3-(trifluoromethyl)phenoxy]-7H-purin-6-one (16)

Yield: 0.075 g, 55%; mp: 222-224 ^{o}C; LC-MS: *m/z* 382.1 [M^{+} +1]; HPLC R_T: 15.511 min; Purity: 100%; ^{1}HNMR (400 MHz, CD_3OD) δ 1.04 (t, *J* = 8 Hz, 3H), 1.66-1.71 (m, 2H), 1.79-1.87 (m, 6H), 2.03-2.08 (m, 2H), 3.16 (m, 1H), 4.23-4.27 (m, 2H), 7.55 (d, *J* = 8 Hz, 1H), 7.61-7.67 (m, 3H)

4.1.6.4. 4-[(8-cyclopentyl-6-oxo-1-propyl-7H-purin-2-yl)oxy]benzamide (17)

Yield: 0.041 g, 30%; mp: 302-304 ^{o}C; LC-MS: *m/z* 382.1 [M^{+} +1]; HPLC R_T: 15.51 min; Purity: 100%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.95 (t, *J* = 7.2 Hz, 3H), 1.58-1.76 (m, 9H), 1.94 (br.s, 3H), 4.12 (br.s, 2H), 7.35-7.44 (m, 3H), 7.96-8.04 (m, 3H)

4.1.6.5. 8-cyclopentyl-2-[(6-methyl-3-pyridyl)oxy]-1-propyl-7H-purin-6-one (18)

Yield: 0.053 g, 42%; mp: 193-195 ^{o}C; LC-MS: *m/z* 354.1 [M^{+} +1]; HPLC R_T: 16.54 min; Purity: 98.98%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.93 (t, *J* = 8 Hz, 3H), 1.55-1.57 (m, 2H), 1.65-1.74 (m, 6H), 1.90-1.95 (m, 2H), 3.09-3.11 (m, 2H), 3.32 (s, 3H), 4.10 (br. s, 2H), 7.36 (d, *J* = 8 Hz, 1H), 7.63-7.69 (m, 1H), 8.38-8.43 (dd, *J* = 8 Hz, 1H)

4.1.6.6. 2-[(5-chloro-3-pyridyl)oxy]-8-cyclopentyl-1-propyl-7H-purin-6-one (19)

Yield: 0.077 g, 58%; mp: 202-204 ^{o}C; LC-MS: *m/z* 374.0 [M^{+} +1]; HPLC R_T: 21.179 min; Purity: 100%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.93 (t, *J* = 8 Hz, 3H), 1.55-1.61 (m, 6H), 1.66-1.73 (m, 2H), 1.90-1.95 (m, 2H), 3.09-3.11 (m, 2H), 4.10 (t, *J* = 5.6 Hz, 2H), 8.14-8.21 (m, 1H), 8.58-8.63 (m, 2H)

4.1.6.7. 8-cyclopentyl-2-(1H-indol-5-yloxy)-1-propyl-7H-purin-6-one (20)

Yield: 0.033 g, 25%; mp: 352-354 ^{o}C; LC-MS: *m/z* 378.1 [M^{+} +1]; HPLC R_T: 20.37 min; Purity: 99.3%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.97 (t, *J* = 8 Hz, 3H), 1.57-1.581 (m, 8H), 1.90-1.95 (m, 2H), 3.00-3.10 (m, 1H), 4.12-4.17 (m, 2H), 6.45 (br.s, 1H), (td, *J* = 8 Hz, 4 Hz, 1H), 7.38 (br.s, 1H), 7.41-7.44 (m, 2H)

4.1.6.8. 4-[(8-cyclopentyl-6-oxo-1-propyl-7H-purin-2-yl) oxy] benzenesulfonamide (21)

Yield: 0.033 g, 22%; mp: 268-270 ^{o}C; LC-MS: *m/z* 418.0 [M^{+} +1]; HPLC R_T: 9.665 min; Purity: 99.2%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.95 (t, *J* = 8 Hz, 3H), 1.58 (m, 2H),

1.75 (m, 6H), 1.94 (m, 2H), 3.02-3.14 (m, 1H), 4.12 (m, 2H), 7.42-7.54 (m, 4H), 7.91-7.93 (m, 2H)

4.1.6.9. 2-[3-[(8-cyclopentyl-6-oxo-1-propyl-7H-purin-2-yl) oxy] phenyl] acetic acid (22)

Yield: 0.049 g, 35%; mp: 217-219 ^{o}C; LC-MS: *m/z* 397.0 [M^{+} +1]; HPLC R_T: 11.052 min; Purity: 97%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.95 (t, J = 8 Hz, 3H), 1.58-1.74 (m, 8H), 1.94 (d, J = 8 Hz, 2H), 3.01-3.11 (m, 1H), 3.63 (s, 2H), 4.11 (m, 2H), 7.15-7.21 (m, 3H), 7.39-7.43 (m, 1H), 12.96 (br.s, 1H)

4.1.7.0. 8-cyclopentyl-2-[(2-methyl-3-pyridyl) oxy]-1-propyl-7H-purin-6-one (23)

Yield: 0.067 g, 53%; mp: 230-232 ^{o}C; LC-MS: *m/z* 354.2 [M^{+} +1]; HPLC R_T: 10.093 min; Purity: 96.4%; ^{1}HNMR (400 MHz, DMSO-d6) δ 0.68 (t, J = 8 Hz, 3H), 1.30 (br.s, 2H), 1.40-1.50 (m, 6H), 1.65 (br.s, 2H), 2.08 (s, 3H), 2.80 (br.s, 1H), 3.87 (t, J = 8 Hz, 3H), 7.09 (t, 1H), 7.42 (br.s, 1H), 8.13 (br.s, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 11.17, 19.06, 21.57, 25.01, 31.66, 43.02, 122.81, 130.45, 146.51, 146.85, 150.98, 152.74, 154.20, 157.03

4.1.7.1. 8-cyclopentyl-2-(1H-indol-7-yloxy)-1-propyl-7H-purin-6-one (24)

Yield: 0.039 g, 29%; mp: 285-287 ^{o}C; LC-MS: *m/z* 378.2 [M^{+} +1]; HPLC R_T: 12.441 min; Purity: 100%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.99 (t, J = 7.2 Hz, 3H), 1.56-1.91 (m, 10H), 2.99-3.11 (m, 1H), 4.17-4.22 (m, 2H), 6.50-6.53 (m, 1H), 6.94-7.05 (m, 2H), 7.35-7.37 (m, 1H), 7.48-7.51 (m, 1H), 11.25 (br. s, 1H), 12.78 (br. s, 1H)

4.1.7.2. 8-cyclopentyl-2-(1H-indol-4-yloxy)-1-propyl-7H-purin-6-one (25)

Yield: 0.037 g, 28%; mp: > 300 ^{o}C (d); LC-MS: *m/z* 378.2 [M^{+} +1]; HPLC R_T: 11.224 min; Purity: 100%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.97 (t, J =7.2 Hz, 3H), 1.52-1.92 (m, 10H), 2.96-3.09 (m, 1H), 4.16-4.20 (m, 2H), 6.19-6.23 (m, 1H), 6.85-6.90 (m, 2H), 7.09-7.13 (m, 1H), 7.31-7.35 (m, 1H), 11.35 (br. s, 1H), 12.77 (br. s, 1H)

4.1.7.3. 8-cyclopentyl-2-[(4-methyl-2-pyridyl) oxy]-1-propyl-7H-purin-6-one (26)

Yield: 0.069 g, 55%; mp: 287-289 ^{o}C; LC-MS: *m/z* 354.0 [M^{+} +1]; HPLC R_T: 19.865 min; Purity: 95.3%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 7.42 (t, J = 8 Hz, 3H), 1.45-1.52 (m, 2H), 1.63-1.67 (m, 2H), 1.74-1.1.85 (m, 4H), 2.01 (m, 2H), 2.23 (s, 3H), 3.22 (t, J = 8 Hz, 2H), 6.32 (d, J = 8 Hz, 1H), 6.37 (s, 1H), 7.68-7.78 (m, 1H)

4.1.7.4. 2-[(2-chloro-3-pyridyl) oxy]-8-cyclopentyl-1-propyl-7H-purin-6-one (27)

Yield: 0.064 g, 48%; mp: 259-261 ^{o}C; LC-MS: *m/z* 376.0 [M^{+} +2]; HPLC R_T: 13.538 min; Purity: 100%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.96 (t, *J* = 8 Hz, 3H), 1.57-1.63 (m, 2H), 1.67-1.84 (m, 6H), 1.92-1.99 (m, 2H), 3.02-3.14 (m, 1H), 4.15 (t, *J* = 8 Hz, 2H), 7.60-7.64 (m, 1H), 8.02-8.09 (m, 1H), 8.41-8.43 (t, *J* = 4 Hz, 1H)

4.1.7.5. 8-cyclopentyl-2-(3-hydroxyphenoxy)-1-propyl-7H-purin-6-one (28)

Yield: 0.042 g, 33%; mp: 264-266 ^{o}C; LC-MS: *m/z* 355.1 [M^{+} +1]; HPLC R_T: 9.892 min; Purity: 99.4%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.93 (t, *J* = 8 Hz, 3H), 1.57-1.60 (m, 2H), 1.67- 1.78 (m, 6H), 1.94 (m, 2H), 3.09 (m, 1H), 4.09 (t, *J* = 8 Hz, 2H), 6.65-6.72 (m, 3H), 7.21-7.25 (m, 1H), 9.95 (br. s, 1H), 12.93 (br. s, 1H)

4.1.8. General synthesis of Compounds 29-35

To a solution of **III** (1 equiv., 0.100 g) in corresponding alcohol (10 equiv.) was added NaH (60% dispersion in mineral oil, 10 equiv., 0.51 g) under nitrogen atmosphere and the reaction mixture was refluxed for 3 hours. After cooling to room temperature, the reaction mixture was concentrated under reduced pressure. Water was added to the residue and the products were extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulphate and concentrated under reduced pressure. The crude products were purified by preparative TLC using methanol in dichloromethane as solvent system to give the desired compounds **29-35** in good to moderate yields

4.1.8.1. 2-(cyclopentoxy)-8-cyclopentyl-1-propyl-7H-purin-6-one (29)

Yield: 0.073 g, 62%; mp: 245-247 ^{o}C; LC-MS: *m/z* 331.0 [M^{+} +1]; HPLC R_T: 21.933 min; Purity: 99.6%; ^{1}HNMR (400 MHz. CD_3OD) δ 0.94 (t, *J* = 8 Hz, 3H), 1.64-2.11 (m, 18H), 3.12-3.35 (m, 2H), 3.99-4.03 (m, 2H)

4.1.8.2. 8-cyclopentyl-2-(1-methylpyrrolidin-3-yl)oxy-1-propyl-7H-purin-6-one (30)

Yield: 0.050 g, 41%; mp: 232-234 ^{o}C; LC-MS: *m/z* 346.0 [M^{+} +1]; HPLC R_T: 8.28 min; Purity: 97.7%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.92 (t, *J* = 7.2 Hz, 3H), 1.58-1.85 (m, 9H), 1.94-2.02 (m, 3H), 2.39-2.42 (m, 1H), 2.77 (s, 3H), 3.12-3.18 (m, 1H), 3.34-3.53 (m, 3H), 4.15-4.23 (m, 3H), 12.9 (br. s, 1H)

4.1.8.3. 8-cyclopentyl-1-propyl-2-tetrahydropyran-4-yloxy-7H-purin-6-one (31)

Yield: 0.073 g, 59%; mp: 236-238 ^{o}C; LC-MS: *m/z* 347.3 [M^{+} +1]; HPLC R_T: 10.256 min; Purity: 100%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.86 (t, *J* = 7.6 Hz, 3H), 1.21-1.81

(m, 10H), 1.93-2.03 (m, 4H), 3.05-3.11 (m, 1H), 3.51-3.56 (m, 2H), 3.77-3.82 (m, 2H), 3.93 (t, *J* = 6.8 Hz, 2H), 5.20-5.22 (m, 1H), 12.8 (br. s, 1H)

4.1.8.4. 8-cyclopentyl-1-propyl-2-tetrahydrofuran-3-yloxy-7H-purin-6-one (32)

Yield: 0.064 g, 54%; mp: 243-245 ^{o}C; LC-MS: *m/z* 333.0 [M^{+} +1]; HPLC R_T: 9.99 min; Purity: 99.8%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.83 (t, *J* = 7.2 Hz, 3H), 1.52-1.59 (m, 4H), 1.69-1.78 (m, 4H), 1.94-1.96 (m, 2H), 2.05-2.12 (m, 1H), 2.17-2.25 (m, 1H), 3.04-3.15 (m, 1H), 3.78-3.92 (m, 6H), 5.51-5.52 (m, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 11.12, 21.37, 24.97, 25.12, 31.44, 31.91, 32.39, 42.17, 66.40, 72.40, 78.72, 147.7, 152.48, 153.21, 154.83, 159.05

4.1.8.5. 8-cyclopentyl-2-(3-hydroxycyclopentoxy)-1-propyl-7H-purin-6-one (33)

Yield: 0.043 g, 35%; mp: 238-240 ^{o}C; LC-MS: *m/z* 347.0 [M^{+} +1]; HPLC R_T: 9.625 min; Purity: 97.6%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.83 (t, *J* = 7.2 Hz, 3H), 1.21 (s, 2H), 1.51-1.61 (m, 4H), 1.70-1.81 (m, 4H), 1.87-1.96 (m, 4H), 2.12-2.20 (m, 1H), 3.02-3.12 (m, 1H), 3.84-3.93 (m, 2H), 4.26-4.28 (m, 1H), 4.65-4.69 (m, 1H), 5.40-5.44 (m, 1H), 12.70 (m, 1H)

4.1.8.6. 8-cyclopentyl-1-propyl-2-[(3S)-tetrahydrofuran-3-yl] oxy-7H-purin-6-one (34)

Yield: 0.059 g, 50%; mp: 230-232 ^{o}C; LC-MS: *m/z* 333.2 [M^{+} +1]; HPLC R_T: 10.315 min; Purity: 100%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.83 (t, *J* = 8 Hz, 3H), 1.53-1.80 (m, 8H), 1.94-2.48 (m, 5H), 3.07-3.11 (m, 1H), 3.78-3.91 (m, 5H), 5.51 (br. s, 1H)

4.1.8.7. 8-cyclopentyl-1-propyl-2-[(3R)-tetrahydrofuran-3-yl] oxy-7H-purin-6-one (35)

Yield: 0.061 g, 52%; mp: 228-230 ^{o}C; LC-MS: *m/z* 333.2 [M^{+} +1]; HPLC R_T: 9.639 min; Purity: 98.4%; ^{1}HNMR (400 MHz, CD_3OD) δ 0.85 (t, *J* = 8 Hz, 3H), 1.22-1.25 (m, 3H), 1.55-1.64 (m, 3H), 1.70-1.82 (m, 3H), 1.93-2.00 (m, 2H), 2.08-2.13 (m, 1H), 2.21-2.27 (m, 1H), 3.11 (m, 1H), 3.79-3.93 (m, 5H), 5.52-5.54 (m, 1H)

4.1.9. General synthesis of Compounds 36-41

To a solution of **III** (1 equiv., 0.100 g) in THF was added sodium bicarbonate (4 equiv., 0.12 g) dissolved in minimum amount of water followed by the corresponding boronic acid (1.5 equiv.). The resulting reaction mixture was degassed for 10 minutes by purging argon and then Pd $[PPh_3]_4$ (0.1 equiv., 0.041 g) was added. The reaction mixture was

refluxed for 12 hours. After completion of reaction, the reaction mixture was concentrated under reduced pressure. The product was extracted with ethyl acetate, the organic layer was washed with water followed by brine and dried over anhydrous sodium sulphate. The organic layer was concentrated under reduced pressure to give the crude products that were purified by preparative TLC using methanol in dichloromethane as solvent system to afford desired products **36-41** in moderate to good yields

4.1.9.1. 8-cyclopentyl-1-propyl-2-[3-(trifluoromethyl) phenyl]-7H-purin-6-one (36)

Yield: 0.074 g, 53%; mp: 220-222 ^{o}C; LCMS: *m/z* 391.0 [M^{+} +1]; HPLC R_T: 23.020 min; Purity: 99%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.63 (t, *J* = 7.6 Hz, 3H), 1.45-1.49 (m, 2H), 1.63 (br. s, 2H), 1.74 (br. s, 2H), 1.84 (br. s, 2H), 2.00 (br. s, 2H), 3.16-3.33 (m, 2H), 3.79 (t, *J* = 7.2 Hz, 2H), 7.77 (t, *J* = 7.6 Hz, 1H), 7.89-7.93 (m, 2H), 7.98 (s, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 10.84, 21.68, 25.07, 31.80, 46.59, 122.55, 125.05, 125.25, 126.08, 129.01, 129.33, 129.64, 132.29, 136.95, 153.60

4.1.9.2. 8-cyclopentyl-1-propyl-2-[4-(trifluoromethyl) phenyl]-7H-purin-6-one (37)

Yield: 0.077 g, 55%; mp: 253-255 ^{o}C; LCMS: *m/z* 391.0 [M^{+} +1]; HPLC R_T: 23.224 min; Purity: 97%; ^{1}HNMR (400 MHz, DMSO-*d6*) δ 0.63 (t, *J* = 7.2 Hz, 3H), 1.44-1.50 (m, 2H), 1.63 (br. s, 2H), 1.74 (br. s, 2H), 1.80-1.87 (m, 2H), 2.00 (br. s, 2H), 3.18-3.33 (m, 1H), 3.81 (t, *J* = 6.8 Hz, 2H), 7.86 (dd, *J* = 7.6 Hz, 8 Hz, 4H)

4.1.9.3. 8-cyclopentyl-2-(3-methoxyphenyl)-1-propyl-7H-purin-6-one (38)

Yield: 0.061 g, 49%; mp: 195-197 ^{o}C; LC-MS: *m/z* 353.0 [M^{+} +1]; HPLC R_T: 19.958 min; Purity: 97.8%; ^{1}HNMR (400 MHz, CD_3OD) δ 0.74 (t, *J* = 7.6 Hz, 3H), 1.28 (m, 1H), 1.59-1.65 (m, 2H), 1.72-1.74 (m, 2H), 1.87-1.93 (m, 4H), 2.14 (d, *J* = 8 Hz, 2H), 3.85 (s, 3H), 3.93-3.97 (m, 2H), 7.07-7.13 (m, 3H), 7.44 (t, *J* = 8 Hz, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 10.97, 21.79, 25.09, 31.94, 46.52, 55.32, 113.66, 114.98, 120.16, 129.59, 137.26, 153.62, 154.57, 155.19, 156.31, 158.94, 159.77

4.1.9.4. 3-(8-cyclopentyl-6-oxo-1-propyl-7H-purin-2-yl) benzoic acid (39)

Yield: 0.034 g, 26%; mp: 305-307 ^{o}C; LC-MS: *m/z* 367.0 [M^{+} +1]; HPLC R_T: 16.234 min; Purity: 99.2%; ^{1}HNMR (400 MHz, CD_3OD) δ 0.74 (t, *J* = 7.2 Hz, 3H), 1.57-1.66 (m, 3H), 1.72-1.79 (m, 2H), 1.87-1.92 (m, 4H), 1.94-2.20 (m, 2H), 3.95 (t, *J* = 7.6 Hz, 2H), 7.66 (t, *J* = 7.6 Hz, 1H), 7.79 (d, *J* = 7.6 Hz, 1H), 8.18-8.22 (m, 2H)

4.1.9.5. 8-cyclopentyl-2-(3-hydroxyphenyl)-1-propyl-7H-purin-6-one (40)

Yield: 0.045 g, 37%; mp: 326-328 ^{o}C; LC-MS: *m/z* 339.0 [M^{+} +1]; HPLC R_T: 16.728 min; Purity: 99.8%; ^{1}HNMR (400 MHz, CD_3OD) δ 0.75 (t, *J* = 7.6 Hz, 3H), 1.59-1.65 (m, 2H), 1.71-1.74 (m, 3H), 1.87 (br. s, 4H), 2.13-2.15 (m, 2H), 3.94-3.98 (m, 2H), 6.90-6.97 (m, 3H), 7.34 (t, *J* = 7.6 Hz, 1H); ^{13}CNMR (100 MHz, DMSO-d6) δ 10.99, 21.84, 25.14, 31.98, 46.51, 113.71, 114.98, 116.28, 118.59, 129.59, 136.99, 153.69, 154.87 155.23, 157.20, 159.77

4.1.9.6. 3-(8-cyclopentyl-6-oxo-1-propyl-7H-purin-2-yl)benzamide (41)

Yield: 0.043 g, 33%; mp: 332-334 ^{o}C; LC-MS: *m/z* 366.0 [M^{+} +1]; HPLC R_T: 14.39 min; Purity: 95%; ^{1}HNMR (400 MHz, CD_3OD) δ 0.73 (t, *J* = 7.2 Hz), 1.28-1.33 (m, 3H), 1.35-1.39 (m, 1H), 1.57-1.63 (m, 2H), 1.93 (br. s, 3H), 2.14-2.18 (m, 1H), 3.96 (t, *J* = 8 Hz, 2H), 7.65 (t, *J* = 7.6 Hz, 1H), 7.75 (d, *J* = 7.6 Hz, 1H), 8.07 (d, *J* = 9.2 Hz, 2H)

4.2. Biology

4.2.1. Radioligand Binding for Adenosine Receptors A_1, A_{2A}, A_{2B} and A_3

Human adenosine receptor (A_1, A_{2A}, A_{2B} and A_3) cDNA was stably transfected into HEK-293 cells (referred to as HEK-A_1, HEK-A_{2A}, HEK-A_{2B}, HEK-A_3 cells). The HEK-293 cell was obtained from ATCC. The cells monolayer was washed with PBS once and harvested in a buffer containing 150 mM NaCl, 1 mM EDTA, 50 mM Tris, pH 7.4 (10mM EDTA, 10mM HEPES, pH-7.4 for HEK- A_3) at 1500 rpm for 5 minutes at room temperature. The cell pellet was incubated in sonication buffer containing 1 mM EDTA, 5 mM Tris, pH 7.4 (1mM EDTA, 10mM HEPES, pH-7.4 for HEK-A_3) for 10 mins at 4^{o}C followed by sonication on ice for 6 minutes. The lysate was centrifuged at 1000 x g for 10 minutes at 4^{o}C and the pellet was discarded. The supernatant was centrifuged at 49,000 x g for 45 minutes at 4^{o}C. The resultant protein pellet was resuspended in the sonication buffer supplemented with 1 U/ml adenosine deaminase (ADA, Roche) and incubated for 30 minutes at room temperature with constant mixing. The protein was washed twice with same buffer at 49,000 x g for 45 minutes at 4^{o}C and the final protein was stored in 50 mM Tris, pH 7.4 supplemented with 1 U/mL ADA and 10 % sucrose (1 mM EDTA, 5 mM Tris, pH 7.4, 1 U/ml ADA and 10% sucrose for HEK-A3). The protein concentration was estimated by Bradford assay and aliquots were stored at -80^{o}C.

The binding affinity and selectivity of test compounds was determined using radioligand binding assays. DPCPX, Preladenant, CVT-6883 and MRS-1191 were used as internal standards. Competition radioligand binding assays were started by mixing 1 nM ^{3}H-DPCPX (for HEK-A_1), 1 nM ^{3}H-ZM241385 (HEK-A_{2A}), 1.6 nM ^{3}H-MRS-1754 (HEK-A_{2B}) or 2 nM ^{3}H-HEMADO (HEK-A_3) with various concentrations of test compounds and the respective membranes in assay buffer containing 50 mM Tris pH 7.4, 1 mM EDTA (HEK-A_1), 50 mM Tris, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA, 1 U/ml ADA (HEK-A_{2A}, HEK-A_3) or 50 mM Tris pH 6.5, 5 mM $MgCl_2$, 1 mM EDTA (HEK-A_{2B}) supplemented with 1 unit/ml ADA. The assays were incubated at room temperature for 90 min with gentle agitation, stopped by filtration using a Harvester (Molecular Devices), and washed four times with ice-cold 50mM Tris (pH 7.4). Nonspecific binding was determined in the presence of 100 μM NECA. The affinities of compounds (i.e., K_i values) were calculated using GraphPad software.

4.2.2. cAMP Assay for Adenosine Receptor

The functional activity of test compounds on A_1 adenosine receptor and selectivity against adenosine receptor subtype A_{2B} was determined using HTRF based cAMP assay (Cisbio). Briefly, overnight seeded cultures (HEK-A_1, HEK-A_{2B}) were treated with 1U/ml ADA for 90 minutes at 37^{o}C and 5% CO_2. Cell suspensions were treated with increasing concentrations of test compounds for 15 minutes followed by treatment with agonists for 15 minutes (1 nM CPA for HEK-A_1 and 70 nM NECA for HEK-A_{2B}) at room temperature with continuous mixing in incomplete DMEM supplemented with 1U/ml ADA. Rolipram (20 μM) was included in the assay for A_1 adenosine receptor. For functional activity of HEK-A_1, cell suspensions were further treated with forskolin for 30 minutes at room temperature with constant mixing. cAMP levels were quantified using a Flex Station III (Molecular Devices) at an excitation maximum of 313 nm and emission maxima of 620 nm and 665 nm. Data was analyzed using GraphPad Prism to generate IC_{50} and K_i.

4.3. Pharmacokinetics

4.3.1. General

Male Wistar rats (weighing 230 ± 15g) were obtained from in-house breeding facility, Advinus Therapeutics Ltd., Pune, INDIA. The rats were grouped and housed in polycarbonate cages with not more than 3 rats per cage and maintained under standard

laboratory conditions (temperature 25 ± 2°C) with dark/ light cycle (12 h). Rats were maintained on T.2014C Global 14% protein rodent maintenance diets (Harlan, Teklad diet, USA) and water ad libitum. All procedures described were reviewed and approved by the Institutional Animal Ethics committee (IAEC) constituted by Committee for the Purpose of Control and Supervision on Experiments (CPCSEA) on Animals, Govt. of India.

Pharmacokinetic experiments were carried-out in male Wistar rats following Intravenous (IV) and Per oral (PO) administration. The animals were fasted overnight before the start of experimentation but had free access to water. For IV dosing, tested compounds were dissolved in different vehicles and solution formulation (dose volume: 5 mL/kg and formulation strength: 0.6 mg/mL) was administered to each rat at a dose of 3 mg/kg via tail vein. For PO suspension formulation was administered to each rat at a dose of 5 mg/kg via oral gavage using oral feeding tube.

Study used serial sampling design (n=3/time point) with blood samples collected at 0.008 (IV only), 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h post dose into labeled micro-centrifuge tubes contained K_2EDTA as an anticoagulant. Tissue samples were washed with phosphate buffer saline (pH 7.4), blotted dry and weighed. All samples were stored below -70°C until bioanalysis. Blood samples were immediately centrifuged at 6000 RPM for 5 minutes to separate the plasma.

Tissue samples were homogenized in phosphate buffer saline by homogenizer (Polytron PT 3100, Kinematica). Aliquot of plasma (100 µL) or tissue sample (300 µL) was precipitated with 300 µL of acetonitrile containing internal standard was added to all the samples and vortex mixed for 1 min on multiple vortex mixer and centrifuged at 8000 RPM for 8 minutes. Finally, supernatant (100 µL) was collected from each test sample microcentrifuge tube and transferred into HPLC vials for LC/MS/MS to determine the concentrations in plasma. Calibration standards and quality control (separate weighing) samples were prepared in blank rat plasma or brain homogenates.

4.3.2. Data analysis

Plasma pharmacokinetic parameters were calculated using the non-compartmental analysis tool of WinNonlin Professional software (Version 5.2.1). Pharmacokinetic parameters were determined from individual animals in each group. The area under the plasma concentration-time curve (AUC_{0-t} & AUC_{0-inf}), elimination half-life ($T_{1/2}$), clearance

(CL) and volume of distribution (V_{ss}) were calculated from intravenous group. The peak plasma concentration (C_{max}), time to achieve C_{max} (T_{max}), area under the plasma concentration-time curve (AUC_{0-t} & AUC_{0-inf}), and oral bioavailability were calculated from the oral groups.

4.3.3. *In-vivo* disposition study in brain

Rats were divided into two groups each consisting of six rats. The rats were fasted overnight prior to dose administration and for 2 hours post dose but had free access to water. Group 1 rats received oral solution administration of Compound **14** and group 2 received oral solution administration of Compound **38** at dose of 5 mg/kg (formulation strength: 0.5 mg/mL). Oral solution formulation of **14** comprised of N-methyl-pyrrolidinone (NMP) -10% v/v, Labrasol- 30% v/v, citrate buffer pH 8.5 quantity sufficient. Oral solution formulation of **38** comprised of NMP -10% v/v, polyethylene glycol-400 (PEG-400) - 20% v/v, citrate buffer pH 8.5 quantity sufficient. Study used discrete sample design. Blood and brain were collected from two rats at 1, 2 and 8 h post dose administration. Brain samples were washed with phosphate buffer saline (pH 7.4), blotted dry and weighed. All samples were stored below -70°C until bioanalysis. Blood samples were centrifuged at 6000 rpm for 5 min to obtain the plasma. Plasma and brain samples were stored below -70 °C until bioanalysis.

Brain samples were transferred to the pre-labeled tubes, phosphate buffer saline was then added into tubes in a ratio of 1:2 (for each 1 g of brain, 2 mL of phosphate buffer saline was added). Samples were then homogenized by using homogenizer (polytron PT 3100, Kinematica). Plasma samples were thawed at room temperature. Plasma and brain homogenate samples were analyzed following protein precipitation with acetonitrile containing internal standard. Quantitative bioanalysis of **14** and **38** in the plasma and brain samples was performed using a fit for purpose liquid chromatography tandem mass spectrometry (LC-MS/MS). The lower limit of quantification of both the compounds was 10 ng/mL (0.02 µM) in both the matrices.

4.4. Rat Oral Efficacy Screen

Male Wistar rats (n = 6; dose volume: 5mL/kg) with 250-300 g body weight were treated with vehicle for Rolofylline (**1**) [DMAC (5%) + 1% 1N NaOH + Normal saline (qs)], Rolofylline (0.1 mg/kg, PO), vehicle for compound **14** [NMP (10%), PEG -400 (20%),

normal saline q.s. (pH 12.0)] and compound **14** (3, 10 and 30 mg/kg, PO). After the vehicle /test compound administration, rats were placed individually in metabolic cages without food and water for 4 hours and then acute diuretic activity was measured by measuring the effect on urine volume, natriuresis and kaliuresis using Selectra Junior Biochemist Analyser

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Abbreviations: AR, Adenosine receptor; ADME, absorption, distribution, metabolism and excretion; hERG, Human ether-a-go-go-related gene; CYPs, Cytochromes P450; NMP, N-methyl Pyrrolidine; R_T, Retention time; ZM241385, 4-(2-((7-amino-2-(furan-2-yl)-[1,2,4]triazolo[1,5-a]-[1,3,5]triazin-5-yl)amino)ethyl)-phenol, DPCPX, 8-Cyclopentyl-1,3-dipropylxanthine; MRS 1191, 3-Ethyl-5-benzyl-2-methyl-4-phenylethynyl-6-phenyl-1,4-(±)-dihydropyridine-3,5-dicarboxylate; MRS-1754, N-(4-cyanophenyl)-2-[4-(2,6-dioxo-1,3-dipropyl-7H-purin-8-yl)phenoxy]acetamide; HEMADO, 2-(1-Hexynyl)-N-methyladenosine; AUC, area under the curve.

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