Discovery of Potent and Selective A_{2A} Antagonists with Efficacy in Animal Models of Parkinson's Disease and Depression

Sujay Basu,*¹ Dinesh A. Barawkar, Vidya Ramdas, Minakshi Naykodi, Yogesh D. Shejul, Meena Patel, Sachin Thorat, Anil Panmand, K. Kashinath, Rajesh Bonagiri, Vandna Prasad, Ganesh Bhat, Azfar Quraishi, Sumit Chaudhary, Amol Magdum, Ashwinkumar V. Meru, Indraneel Ghosh, Ravi K. Bhamidipati, Amol A. Raje, Vamsi L. M. Madgula, Siddhartha De, Sreekanth R. Rouduri, Venkata P. Palle, Anita Chugh, Narayanan Hariharan, and Kasim A. Mookhtiar

Advinus Therapeutics Ltd., Drug Discovery Facility, Quantum Towers, Plot-9, Phase-I, Rajiv Gandhi Infotech Park, Hinjawadi, Pune 411 057, India

S Supporting Information

ABSTRACT: Adenosine A_{2A} receptor (A_{2A}AdoR) antagonism is a non-dopaminergic approach to Parkinson's disease treatment that is under development. Earlier we had reported the therapeutic potential of 7-methoxy-4-morpholino-benzothiazole derivatives as A_{2A}AdoR antagonists. We herein described a novel series of [1,2,4]triazolo[5,1-*f*]purin-2-one derivatives that displays functional antagonism of the A_{2A} receptor with a high degree of selectivity over A₁, A_{2B}, and A₃ receptors. Compounds from this new scaffold resulted in the discovery of highly potent, selective, stable, and moderate brain penetrating compound 33. Compound 33 endowed with satisfactory *in vitro* and *in vivo* pharmacokinetics properties. Compound 33 demonstrated robust oral efficacies in two commonly used models of Parkinson's disease (haloperidol-induced catalepsy and 6-OHDA lesioned rat models) and depression (TST and FST mice models).

KEYWORDS: Parkinson's disease, adenosine receptors, rat liver microsomes, pharmacokinetics

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder affecting approximately 1% population over the age of 65.¹ The number of patients affected by PD is expected to double by 2030 due to an aging population and increased life expectancy.² Adenosine A_{2A} receptor (A_{2A}AdoR) is a highly distributed receptor in the central nervous system and is expressed at high levels in the nigrostriatum. A_{2A}AdoR is predominantly located in the spiny neurons of striatum,³ where they are coexpressed with dopamine D2 receptors on GABAergic neurons on the indirect striatopallidal pathway.^{4,5} Inhibition of the striatopallidal neurons by A_{2A}AdoR antagonism likely reduces motor deficits caused by dopamine deficiency in PD. Unfortunately, current dopamine replacement therapies for PD suffer from poor long-term control and undesirable side effects, mainly dyskinesia (involuntary movements). Positive findings with A_{2A}AdoR antagonists have been observed in animal models of PD, ranging from the reversal of haloperidol-induced catalepsy in rodent and more disease relevant models like 6-hydroxydopamine (6-OHDA) lesioned rats models and MPTP-lesioned primates.⁶ PD patients also have high prevalence of depression, and current PD therapies do not treat depressive symptoms. A_{2A}AdoR knockout mice displayed reduction of immobility in functional assays *in vivo*, such as tail suspension (TST) and forced swim tests (FST), which are predictive of clinical antidepressant activity.⁷ Thus, interest in the use of A_{2A}AdoR antagonists in PD has increased

because it might improve over existing PD agents in the treatment of nonmotor PD symptoms like depression as well as improves motor function without causing dyskinesia. Numerous A_{2A}AdoR antagonists have reached phase I clinical trials and beyond. A_{2A}AdoR antagonists are classified as xanthine and nonxanthine derivatives. Among xanthine derivatives, Istradefylline (KW-6002, Kyowa Hakko Kirin Co Ltd.), with moderate receptor selectivity, was launched in Japan for PD.⁸ Several classes of nonxanthine A_{2A}AdoR antagonists have been known in the literature, and they are monocyclic, bicyclic, or tricyclic core-based antagonists.^{9–16} In our preceding publication, we reported a series of novel nonxanthine compounds with moderate oral *in vivo* efficacy in the 6-OHDA lesioned rat model of PD.¹⁷ Based on these data, we have focused on identifying a new scaffold with high affinity, the selective and robust orally efficacious antagonist of A_{2A}AdoR. Here, we report discovery and structure–activity relationship (SAR) studies on a novel structural class of 5-amino-[1,2,4]triazolo-[5,1-*f*]purin-2-one (9–33, Scheme 1) derivatives of high affinity and potency as selective A_{2A}AdoR antagonist with robust oral efficacy in an *in vivo* model of PD and depression without dyskinesia.

Received: April 22, 2017

Accepted: July 5, 2017

Published: July 5, 2017

Table 1. *In Vitro* Profile of Compounds 9–33

<div style="display: flex; align-items: center; justify-content: center;"> <div style="margin-left: 20px;"> </div> </div>									
Cmpd	R ¹	Binding K _i (nM) hA2A ^a	% Inhibi- tion at 1 μM or K _i (nM) hA1 ^a	Functional K _i (nM) A2A ^b	Cmpd	R ¹	Binding K _i (nM) hA2A ^a	% Inhibi- tion at 1 μM or K _i (nM) hA1 ^a	Functional K _i (nM) A2A ^b
9		1	58	0.21 (h) 0.58 (r)	22		0.3	56	0.26 (h)
10		1.4	45	1.1 (h) 0.74 (r)	23		1	32	0.07 (h)
11		0.4	45	0.06 (h) 0.06 (r)	24		0.6	5000 nM	0.3 (h) 1.15 (r)
12		0.3	50	0.25 (h)	25		0.5	50	0.4(h)
13		2.0	1700 nM	0.42 (h) 0.28 (r)	26		1.9	34	0.27 (h) 1.07 (r)
14		0.5	63	0.5 (h) 3.9 (r)	27		3.1	57	ND
15		2.0	66	ND	28		1.7	61	0.4(h) 0.65(r)
16		0.7	68	ND	29		4.5	61	ND
17		0.9	58	1.2 (h)	30		1.2	58	0.9 (h)
18		0.6	65	ND	31		0.4	48	0.15 (h) 0.05 (r)
19		0.4	56	1.5 (h) 0.42 (r)	32		6.4	19	1.2 (h) 11.1 (r)
20		0.5	36	2.1 (h) 0.72 (r)	33		1.5	1700 nM	0.4 (h) 4.4 (r)
21		0.2	49	0.22 (h)					

^aBinding affinities were determined by using membrane preparations from HEK-293 cells overexpressing the relevant human AdoR isoform.¹⁷ All data points were evaluated in triplicates. For K_i determination, eight concentration IC₅₀ curves were plotted. ^bThe functional activity of test compounds were determined using HTRF based cAMP assay. Each compound was evaluated in triplicates at 12 concentrations. The mean K_i from two independent experiments (*n* = 2) has been reported.¹⁷ ND: not determined.

Synthesis of the fused tricyclic core 7 began with the commercially available 4,6-dichloropyrimidine-2,5-diamine (Scheme 1). The compound 1 treated with ethanol amine and followed by cyclization and N-1 methylation afforded compound 4. Reaction of 4 with hydrazine gave intermediate 5, which, on reaction with appropriate acid derivatives, provided 6a–b. Dehydrative cyclization of 6a–b with *N,O*-bis(trimethylsilyl)-

acetamide (BSA) gave compounds 7a–b. Reaction of compounds 7a–b with *p*-toluene sulphonyl chloride yielded compounds 8a–b. Amination of 8a–b with appropriate aryl piperazine yielded compounds 9–33.

A series of substituted aryl piperazines (9–33, Table 1) were synthesized and evaluated in an *in vitro* assay as described in our earlier communication.^{17,18} Our initial SAR study was

Scheme 1. Synthesis of 9–33^a

^aReagents and conditions: (i) ethanol amine, EtOH, 100–110 °C, 28 h; (ii) 4-nitrophenyl chloroformate, K₂CO₃, CH₃CN, rt, 24 h; (iii) MeI, CH₃CN, K₂CO₃, 60 °C, 16 h; (iv) NH₂NH₂·H₂O, EtOH, 100–110 °C, 16 h; (v) R²-COOH, EDCI, HOBt, NMM, DMF, rt, 3 h; (vi) BSA, HMDS, 140–150 °C, 14 h; (vii) *p*-toluenesulfonyl chloride, pyridine, rt, 24 h; (viii) R¹ = aryl piperazine, DIPEA, DMF, 80 °C, 16 h.

Table 2. Binding and Functional Selectivity Profile of Lead Compound 33^a

Cmpd	binding K _i (nM)				functional K _i (nM) ^b		
	hA _{2A}	hA ₁	hA _{2B}	hA ₃	hA _{2A}	hA ₁	hA _{2B}
33	1.5	1700	5000	NR	0.4	NR	<50% inhibition at 10 μM

^aNR: No inhibition observed at highest concentration of 10 μM.

^bEvaluated at 12 concentrations, each data point in triplicates (*n* = 2). The mean K_i from two independent experiments has been reported.

focused on imparting high affinity to A_{2A}AdoR on to this novel scaffold. The majority of aryl piperazine derivatives 9–33 exhibited high human A_{2A}AdoR (hA_{2A}) binding affinity (K_i = 0.2–6.4 nM) with moderate to high selectivity over A₁AdoR. All these aryl piperazine derivatives showed extremely high selectivity over A_{2B}AdoR and A₃AdoR. Irrespective of the nature of the substitution, most of the compounds retained A_{2A}AdoR binding affinity. Compounds featuring electron releasing groups such as –OMe, –Me, cyclopropyloxy, and extended ether on the phenyl ring (9–22) showed subnanomolar binding affinity with K_i in the range of 0.2–2.0 nM for A_{2A}AdoR. When tested in a functional assay, compound 9–22 exhibited subnanomolar functional potency both in human (K_i = 0.06–1.5 nM) and in rat (K_i = 0.06–3.9 nM) A_{2A}AdoR

cAMP assay. Compound 13, having extended ether at the para position, showed excellent binding selectivity over A₁ (A₁/A_{2A} = 850-fold), A_{2B} (A_{2B}/A_{2A} > 3000-fold), and A₃AdoR (A₃/A_{2A} > 2000-fold; A_{2B} and A₃ selectivity data given in Supporting Information, Table S1). It also showed excellent functional potency, both in human (K_i = 0.42 nM) and in rat (K_i = 0.28 nM) A_{2A}AdoR cAMP assay. Compound 13 showed moderate solubility at pH 7.4 (<6 μM). Compounds 23–25 containing small electron withdrawing groups like –F and –CN on the phenyl ring exhibited subnanomolar to single digit nanomolar binding affinity with K_i in the range of 0.5–1.0 nM for A_{2A}AdoR and retained excellent selectivity over A₁, A_{2B}, and A₃AdoR. Compounds 23–24 showed excellent functional potency in human (K_i = 0.07, 0.3 nM, respectively) A_{2A}AdoR. To our delight, compound 24 was more than 8000-fold selective for human A_{2A}AdoR over A₁, A_{2B}, and A₃AdoR, but it showed poor solubility at pH 7.4 (<2 μM). Compounds 26–27 with larger electron withdrawing groups such as –OCHF₂ and –CF₃ retained binding affinity with K_i 1.9 and 3.1 nM, respectively, for A_{2A}AdoR. Compound 26 exhibited subnanomolar functional potency in human with K_i 0.27 nM. Replacement of phenyl with other heterocycles like pyridine (28–29), thiazole (30), and benzoxazole (31) retained binding affinity (K_i in the range of 0.4–4.5 nM) and functional potency (K_i in the range of 0.4–0.9 nM) for A_{2A}AdoR and selectivity over A₁AdoR. In general, the compounds described here were found to be metabolically stable (data given in Supporting Information, Table S1) in rat liver microsoms (RLM) and human liver microsoms (HLM).

Next, we focused our attention toward replacement of C-8 furanyl moiety with thiazole (32–33), and both compounds retained binding affinity (hA_{2A} K_i = 6.4 and 1.5 nM, respectively) and functional potency (hA_{2A} K_i = 1.2 and 0.4 nM, respectively; rA_{2A} K_i = 11.1 and 4.4 nM, respectively). Gratifyingly, compound 33 was >1100-fold selective for A_{2A}AdoR over A₁AdoR and >3000-fold selective over A_{2B} and A₃AdoR (Table 2). It also possessed high aqueous solubility at acidic pH and good solubility at pH 7.4 (>2000, 486, and 17 μM at pH 2.1, 4, and 7.4, respectively).

To identify a lead, compound 33 was shortlisted for further profiling as it achieved our predetermined targets for affinity, potency, selectivity, metabolic stability, and solubility.

In vitro pharmacology and *in vitro* DMPK (drug metabolism and pharmacokinetics)¹⁷ profiles of compound 33 have been summarized in Tables 2 and 3. Compound 33 exhibited high binding and functional selectivity in human A_{2A}AdoR over A₁, A_{2B}, and A₃AdoR in *in vitro* assays (Table 2). Compound 33 (Table 3) displayed good metabolic stability in mouse, rat, dog,

Table 3. DMPK Properties of Lead Compound 33^a

33 (<i>in vitro</i> parameters)		33 (<i>in vivo</i> parameters in male Wistar rats)		
MR (nmol/min/mg) (MLM, RLM, DLM, HLM) @ 0.125 mg/mL protein	0.08, 0.00, 0.04, 0.14	route of administration	<i>iv</i>	<i>po</i>
solubility (μM) at pH 2.0, 4.0, 7.4	>2000, 486, 17	dose (mg/kg)	3	10
Caco-2 permeability	A-B: 193, B-A: 423	C _{max} (μM)	NA	0.6 ± 0.3
PPB (mouse, rat, dog, human)	51, 82, 78, 85	T _{max} (h)	NA	0.8 ± 0.3
CYP: 3A4, 1A2, 2C9, 2C19, 2D6	IC ₅₀ : > 10 μM	AUC _{0–t} (μM·h)	3.6 ± 0.7	2.2 ± 0.7
cytotoxicity in HepG2 cell line	IC ₅₀ : > 30 μM	V _{ss} (L/kg)	3.6 ± 0.7	NA
PXR induction	EC ₅₀ : > 60 μM	CL (mL/min/kg)	45 ± 8	NA
hERG binding inhibition at 3, 10 μM	28%, 38%	t _{1/2} (h)	1.0 ± 0.1	NA
		F (%)	NA	32

^aValues indicate mean for *n* = 4 (rat). Vehicle: IV-NMP-10%, CrEL-10%, acetate buffer (pH 4.2) q.s.; PO-Tween 80–1%, 0.5% w/v NaCMC.¹⁷ NA: not applicable.

Figure 1. (A) Effect of compound 33 on haloperidol-induced catalepsy in rats. (B) Effect of compound 33 on potentiation of levodopa-induced contralateral rotations in 6-OHDA lesioned rats. *Significantly different as compared to L-DOPA, 4 mg/kg, IP alone group. (C) Effect of compound 33 in forced swim test (FST). (D) Effect of compound 33 in tail suspension test (TST) of mice. (E) Chronic effect of compound 33 in combination with L-DOPA or L-DOPA on abnormal involuntary movements (AIMs; dyskinesia) in 6-OHDA lesioned rats. All data represent mean $\pm$ SEM ($n = 6-7$). *Significantly different as compared to vehicle (* $P < 0.05$); vehicle: 0.5% Tween-80 + 0.5% CMC, q.s..

and human liver microsomes and no significant inhibition of the major CYP450 enzymes, 3A4, 1A2, 2C9, 2C19, 2D6 (IC_{50} : $>10 \mu M$), and no blocking of hERG channel was observed at the concentrations tested. No potential induction was observed in cytochrome P450 assay. In addition, it was not cytotoxic to HepG2 cells. It was moderately bound in mouse, rat, dog, and human plasma. It demonstrated high Caco-2 permeability (both active and passive) and was not a substrate for PGP drug efflux transporter. The *in vivo* pharmacokinetic (PK) properties of 33 evaluated in rat are summarized in Table 3. Compound 33 exhibited high systemic plasma clearance in rat with elimination half-life of 1.0 h. Moderate volume of distribution was observed. In oral PK, it exhibited rapid absorption (T_{max} : ≤ 1 h). Moderate plasma concentration (AUC_{0-t} ($\mu M \cdot h$) = 2.2) was observed for this compound. Compound 33 displayed 32% oral bioavailability in suspension

formulation. When tested for brain distribution study in rat (IV, 3 mpk), 33 showed moderate brain penetration with brain to plasma AUC_{0-4h} ratio of 0.38.

In Vivo Efficacy Study: Compound 33 was tested for efficacy in two *in vivo* rat models of PD; i.e., haloperidol-induced catalepsy in rat and potentiation of levodopa-induced contralateral rotations in 6-OHDA (6-hydroxydopamine) lesioned rats. In haloperidol-induced catalepsy in rat model (Figure 1A), 33 dose-dependently attenuated the cataleptic effects of haloperidol in 1, 2, and 4 h. Compound 33 (3 and 10 mg/kg, PO) showed significant effect at 1, 2 and 4 h in the catalepsy model. These data also suggest that, in this primary model, the duration of action for *in vivo* efficacy of 33 is at least 4 h following oral administration (3 and 10 mg/kg), and ED_{50} is 4.49 mg/kg. Compound 33 was then evaluated for its ability to potentiate L-DOPA induced rotational behavior in the 6-OHDA lesioned

rat (Figure 1B).¹⁷ Compound 33 dose-dependently potentiated L-DOPA-induced contralateral rotations with ED₅₀: 1.2 mg/kg, PO. Compound 33 (10 and 30 mg/kg, PO) post 60 min showed significant effect on potentiation of L-DOPA-induced contralateral rotations measured up to 2 h in 6-OHDA lesioned rats as compared to L-DOPA (4 mg/kg, IP) alone (Figure 1B). Given the high prevalence of depression in PD patients, the positive results in the FST and TST models of behavioral despair suggest the potential of A_{2A}AdoR antagonists to dramatically improve over existing PD agents in the treatment of nonmotor PD symptoms. When compound 33 was tested in mouse models of FST (Figure 1C) and TST (Figure 1D), it significantly decreased immobility time. Compound 33 (0.3, 1, 3, and 10 mg/kg, PO) significantly decreased immobility time in FST with ED₅₀ of 5.29 mg/kg. In TST model, 33 (1, 3, and 10 mg/kg, PO) significantly decreased immobility time with ED₅₀ of 0.70 mg/kg. In animal models of Parkinson's disease, compound 33 improves motor function without causing dyskinesia, and as an adjunct to levodopa, it improves motor function without worsening dyskinesia. Compound 33 (0.3–3 mg/kg) inhibited L-DOPA-induced behavioral sensitization after repeated daily administration, which suggests a reduced risk of the development of dyskinesias (Figure 1E).

In summary, substantial SAR was developed in a series of 5-amino-[1,2,4]triazolo-[5,1-f]purin-2-one, A_{2A}AdoR antagonists leading to identification of a lead compound 33. Compound 33 exhibited promising affinity, potency, and high selectivity. It also demonstrated a number of positive attributes with respect to *in vitro* DMPK perspectives. Compound 33 had acceptable PK properties with 32% oral bioavailability in rats. Compound 33 also displayed oral efficacy in two *in vivo* models of Parkinson's disease and depression. In 6-OHDA lesioned rats, 33 displayed robust oral efficacy with ED₅₀ of 1.2 mg/kg, without dyskinesia. Further development around this series is in progress.

■ ASSOCIATED CONTENT

■ Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsmedchemlett.7b00175.

Experimental procedures, analytical data for compounds 2–33, selectivity data (for A₁, A_{2B}, and A₃AdoR), metabolic stability data in HLM and RLM, protocol for catalepsy model, FST, and TST (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +91 20 66539600. Fax: +91 20 66539620. E-mail: sujay.basu@advinus.com.

ORCID

Sujay Basu: 0000-0001-7198-325X

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

Authors are grateful to the senior management of the Advinus Group for its support and encouragement. Analytical departments are being acknowledged for their help during this work. Advinus publication no. ADV-A-041.

■ ABBREVIATIONS

EDCI, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; HOBT, hydroxybenzotriazole; NMM, N-methylmorpholine; HMDS, hexamethyldisilazane (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

■ REFERENCES

- (1) Lozano, A. M.; Lang, A. E.; Hutchison, W. D.; Dostrovsky, J. O. New developments in understanding the etiology of Parkinson's disease and in its treatment. *Curr. Opin. Neurobiol.* **1998**, *8*, 783–790.
- (2) Dorsey, E. R.; Constantinescu, R.; Thompson, J. P.; Biglan, K. M.; Holloway, R. G.; Kieburtz, K.; Marshall, F. J.; Ravina, B. M.; Schifitto, G.; Siderowf, A.; Tanner, C. M. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. *Neurology* **2007**, *68*, 384–386.
- (3) Calon, F.; Dridi, M.; Hornykiewicz, O.; Bédard, P. J.; Rajput, A. H.; DiPaolo, T. Increased adenosine A_{2A} receptors in the brain of Parkinson's disease patients with dyskinesias. *Brain* **2004**, *127*, 1075–1084.
- (4) Cantor, C. R.; Stern, M. B. Dopamine agonists and sleep in Parkinson's disease. *Neurology* **2002**, *58*, 71–78.
- (5) Chou, K. L. Adverse events from the treatment of Parkinson's disease. *Neurol. Clin.* **2008**, *26*, 65–83.
- (6) Shah, U.; Hodgson, R. Recent progress in the discovery of adenosine A_{2A} receptor antagonists for the treatment of Parkinson's disease. *Curr. Opin. Drug Discovery Dev.* **2010**, *13*, 466–480.
- (7) Yacoubi, E. I.; Ledent, C.; Parmentier, M.; Bertorelli, R.; Ongini, E.; Costentin, J.; Vaugeois, J. M. *Br. J. Pharmacol.* **2001**, *134*, 68–77.
- (8) Approval for manufacturing and Marketing of NOURIAST tablets 20 mg. A novel Antiparkinsonian Agent. http://www.kyowa-kirin.com/news_releases/2013/e20130325_04.html.
- (9) Carlsson, J.; Tosh, D. K.; Phan, K.; Gao, Z. G.; Jacobson, K. A. Structure-activity relationships and molecular modeling of 1,2,4-triazoles as adenosine receptor antagonists. *ACS Med. Chem. Lett.* **2012**, *3*, 715–720.
- (10) Yang, Z.; Li, L.; Zheng, J.; Ma, H.; Tian, S.; Li, J.; Zhang, H.; Zhen, X.; Zhang, X. Identification of a New Series of Potent Adenosine A_{2A} Receptor Antagonists Based on 4-Amino-5-carbonitrilePyrimidine Template for the Treatment of Parkinson's Disease. *ACS Chem. Neurosci.* **2016**, *7*, 1575–1584.
- (11) Slee, D. H.; Chen, Y.; Zhang, X.; Moorjani, M.; Lanier, M. C.; Lin, E.; Rueter, J. K.; Williams, J. P.; Lechner, S. M.; Markis on, S.; Malany, S.; Santos, M.; Gross, R. S.; Jalali, K.; Sai, Y.; Zuo, Z.; Yang, C.; Castro-Palomino, J. C.; Crespo, M. I.; Prat, M.; Gual, S.; Diaz, J. L.; Saunders, J. 2-Amino-N-pyrimidin-4-ylacetamides as A_{2A} receptor antagonists: Structure-activity relationships and optimization of heterocyclic substituents. *J. Med. Chem.* **2008**, *51*, 1719–1729.
- (12) Vu, C. B.; Shields, P.; Peng, B.; Kumaravel, G.; Jin, X.; Phadke, D.; Wang, J.; Engber, T.; Ayyub, E.; Petter, R. C. Triamino derivatives of triazolotriazine and triazolopyrimidine as adenosine A_{2A} receptor antagonists. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4835–4838.
- (13) Zhou, G.; Aslanian, R.; Gallo, G.; Khan, T.; Kuang, R.; Purakkatt, B.; De Ruiz, M.; Stamford, A.; Ting, P.; Wu, H.; Wang, H.; Xiao, D.; Yu, T.; Zhang, Y.; Mullins, D.; Hodgson, R. Discovery of aminoquinazoline derivatives as human A(2A) adenosine receptor antagonists. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 1348–1354.
- (14) Burbiel, J. C.; Ghattas, W.; Küppers, P.; Köse, M.; Lacher, S.; Herzner, A. M.; Kombu, R. S.; Akkinapally, R. R.; Hockemeyer, J.; Müller, C. E. 2-Amino[1,2,4]triazolo[1,5-c]quinazolines and Derived Novel Heterocycles: Syntheses and Structure-Activity Relationships of Potent Adenosine Receptor Antagonists. *ChemMedChem* **2016**, *11*, 2361.
- (15) Baraldi, P. G.; Saponaro, G.; Aghazadeh Tabrizi, M.; Baraldi, S.; Romagnoli, R.; Moorman, A. R.; Varani, K.; Borea, P. A.; Preti, D. Pyrrolo- and pyrazolo-[3,4-e][1,2,4]triazolo[1,5-c]pyrimidines as adenosine receptor antagonists. *Bioorg. Med. Chem.* **2012**, *20*, 1046–59.

(16) Schering Corp. Neustadt, B. R.; Hao, J.; Liu, H.; Boyle, C. D.; Chackalamannil, S.; Shah, U. G.; Stamford, A.; Harris, J. M.. Preparation of pyrazolo-[4,3-e]-1,2,4-triazolo-[1,5-c]-pyrimidine adenosine A2A receptor antagonists. US-20050239795.

(17) Basu, S.; Barawkar, A. D.; Thorat, S.; Shejul, Y. D.; Patel, M.; Naykodi, M.; Jain, V.; Salve, Y.; Prasad, V.; Chaudhary, S.; Ghosh, I.; Bhat, G.; Quraishi, A.; Patil, H.; Ansari, S.; Menon, S.; Unadkat, V.; Thakare, R.; Seervi, M. S.; Meru, A. V.; De, S.; Bhamidipati, R.; Rouduri, S. R.; Palle, V. P.; Chug, A.; Mookhtiar, K. A. Design, Synthesis of Novel, Potent, Selective, Orally Bioavailable Adenosine A2A Receptor Antagonists and Their Biological Evaluation. *J. Med. Chem.* **2017**, *60*, 681–694.

(18) Barawkar, D.; Basu, S.; Ramdas, V.; Naykodi, M.; Patel, M.; Shejul, Y.; Thorat, S.; Panmand, A. Fused Tricyclic Compounds as Adenosine Receptor Antagonist. US-9006177B2, April 14, 2015.