Letter

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

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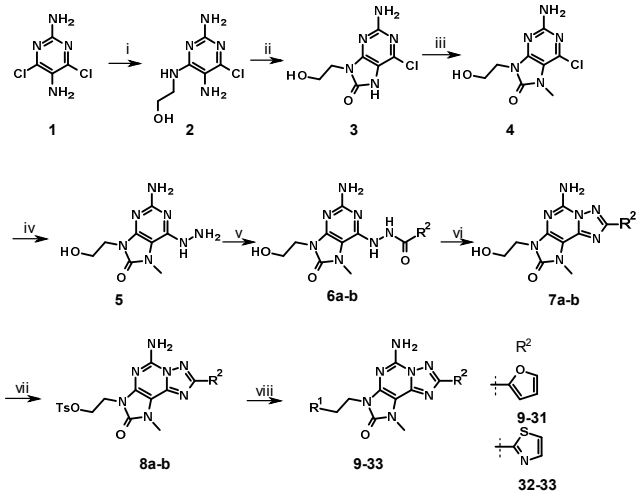
KEYWORDS: Parkinson's disease; Adenosine receptors; Rat liver microsomes; Pharmacokinetics.

ABSTRACT: Adenosine A_{2A} receptor (A_{2A}AdoR) antagonism is a nondopaminergic 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 antagonist. We herein described a novel series of [1,2,4]triazolo[5,1-f]purin-2-one derivatives that display functional antagonism of the A_{2A} receptor with high degree of selectivity over A_1, A_{2B} and A_3 receptors. Compounds from this new scaffold resulted to 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 model).

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 are predominantly located in the spiny neurons of striatum[3], where they are co-expressed 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, 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 and (TST) 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 non-xanthine 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 non-xanthine A_{2A}AdoR antagonists have been known in the literature and they are either monocyclic, bicyclic or tricyclic core-based antagonists.[9-16] In our preceding publication we have reported a series of novel non-xanthine compounds with moderate oral *in vivo* efficacy in 6-OHDA lesioned rat model of PD.[17] Based on these data, we have focused on identifying a new scaffold with high affinity, selective and robust orally efficacious antagonist of the 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, potency and selective A_{2A}AdoR antagonist with robust oral efficacy in an *in vivo* model of PD and depression without dyskinesia.

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 cyclisation 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 compound **7a-b**. Reaction of compound **7a-b** with *p*-toluene sulphonyl chloride yielded compound **8a-b**. Amination of **8a-b** with appropriate aryl piperazine yielded compounds **9-33**.

Scheme 1. Synthesis of 9-33[a]



[a] Reagents and conditions: (i) Ethanol amine, EtOH, 100-110 °C, 28 h; (ii) 4-nitrophenyl chloro formate, K_2CO_3, CH_3CN, rt, 24 h; (iii) MeI, CH_3CN, K_2CO_3, 60 °C, 16 h; (iv) $NH_2NH_2.H_2O$, EtOH, 100-110 °C, 16 h; (v) R^2-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) R^1 = aryl piperazine, DIPEA, DMF, 80 °C, 16 h.

A series of substituted aryl piperazines (**9-33,** Table-1) were synthesized and evaluated in *in vitro* assay as described in our earlier communication.[17-18] Our initial SAR study was focused on to impart 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 A_1AdoR. All these aryl piperazine derivatives showed extremely high selectivity over A_{2B}AdoR and A_3AdoR. 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 phenyl ring (**9-22**) showed sub-nanomolar binding affinity with *K*i in the range of 0.2-2.0 nM for A_{2A}AdoR. When tested in functional assay, compound **9-22** exhibited sub-nanomolar functional potency for both in human (K_i = 0.06-1.5 nM) and rat (rK_i = 0.06-3.9 nM) A_{2A}AdoR cAMP assay. Compound **13**, having extended ether at para position, showed excellent binding selectivity over A_1 (A_1/A_{2A} = 850 fold), A_{2B} (A_{2B}/A_{2A} > 3000 fold) and A_3AdoR (A_3/A_{2A} > 2000 fold; A_{2B} and A_3 selectivity data given in supplementary information, Table S1). It also showed excellent functional potency, both in human (*K*i = 0.42 nM) and 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, -CN, on phenyl ring exhibited sub-nanomolar 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 A_1, A_{2B} and A_3AdoR. 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 A_1, A_{2B} and A_3AdoR but it showed poor solubility at pH 7.4 (<2 μM). Compounds, **26-27** with larger electron withdrawing groups such as -$OCHF_2$,-CF_3, retained binding affinity with *K*i 1.9 and 3.1 nM respectively, for A_{2A}AdoR. Compound **26** exhibited sub-nanomolar functional potency in human with *K*i 0.27 nM. Replacement of phenyl with other heterocyles 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 (h*K*i in the range of 0.4-0.9 nM) for A_{2A}AdoR and selectivity over A_1AdoR. In general, the compounds described here were found to be metabolically stable (data given in supplementary information, Table S1) in rat liver microsomes (RLM) and human liver microsomes (HLM).

Next, we focused our attention towards 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 A_1AdoR and >3000-fold selective over A_{2B} and A_3AdoR (Table-2). It also possessed high aqueous solubility at acidic pH and good solubility at pH 7.4 (>2000 μM, 486 μM 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 Table 2 and 3. Compounds **33** exhibited high binding and functional selectivity in human A_{2A}AdoR over A_1, A_{2B} and A_3AdoR in *in vitro* assays (Table 2). Compound **33** (Table 3) displayed good metabolic stability in mouse, rat, dog and human liver microsomes and no significant inhibition of the major CYP450 enzymes, 3A4,1A2, 2C9, 2C19, 2D6 (IC_{50}: >10 μM), 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 cytototoxic 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** was evaluated in rat, is summarized in Table 3. Compound **33** exhibited high systemic plasma clearance in rat with elimination half-life 1.0 h. Moderate volume of distribution was observed. In oral PK, it exhibited rapid absorption (Tmax: ≤1 h). Moderate plasma concentration (AUC (0-t h) (μM.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 & 10 mg/kg, PO) showed significant effect on 1, 2 & 4h in 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 their 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 ED_{50}: 1.2 mg/kg,

PO. Compound **33** (10 & 30 mg/kg, PO) post 60 min showed significant effect on potentiation of L-DOPA induced contralateral rotations measured up to 2hr in 6-OHDA lesioned rats as compared with L-DOPA (4mg/kg, IP) alone (Figure1B). 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** tested in mouse model of FST (Figure 1C) and TST (Figure 1D), it significantly decreased immobility time. Compound **33** (0.3, 1, 3 & 10 mg/kg, PO) significantly decreased immobility time in FST with ED_{50} 5.29 mg/kg. In TST model, **33** (1, 3 & 10 mg/kg, PO) significantly decreased immobility time with ED_{50} 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. **33** displayed oral efficacy in two *in vivo* models of Parkinson diseases and depression. In 6-OHDA lesioned rats, **33** displayed robust oral efficacy with ED_{50}: 1.2 mg/kg, without dyskinesia. Further development around this series is in progress.

ASSOCIATED CONTENT

Supporting Information

Experimental procedures, analytical data for compounds **2-33,** selectivity data (for A_1, A_{2B} and A_3AdoR), metabolic stability data in HLM and RLM, protocol for catalepsy model, FST, TST.

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ABBREVIATIONS

EDCI, 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBT, Hydroxybenzotriazole; NMM, N-Methylmorpholine; HMDS, Hexamethyldisilazane, (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine)

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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.

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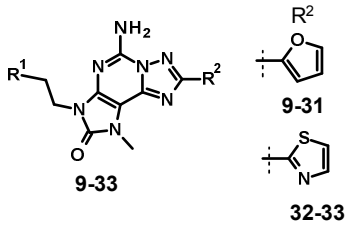
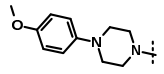
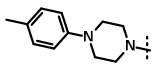
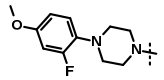
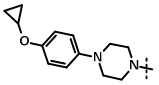
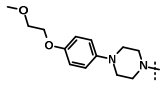
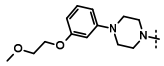
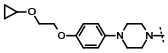
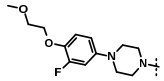
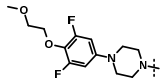
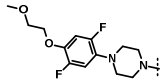
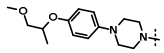
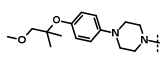
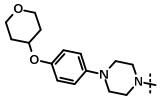
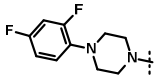
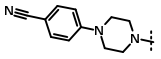
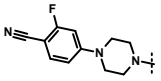
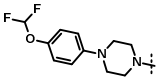
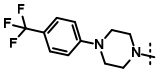
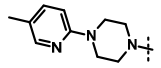
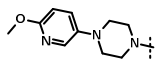
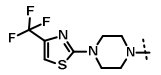
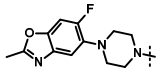
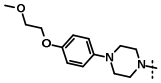
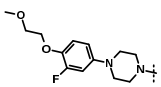
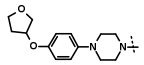
Table 1. In vitro profile of compounds 9-33



Cmpd	R[1]	Binding Ki (nM) hA2A [a]	% Inhibition at 1 µM or Ki (nM) hA1[a]	Functional Ki (nM) A2A[b]
9		1	58	0. 21 (h) 0.58 (r)
10		1.4	45	1.1 (h) 0.74 (r)
11		0.4	45	0.06 (h) 0.06 (r)
12		0.3	50	0.25 (h)
13		2.0	1700 nM	0.42 (h) 0.28 (r)
14		0.5	63	0.5 (h) 3.9 (r)
15		2.0	66	ND
16		0.7	68	ND
17		0.9	58	1.2 (h)
18		0.6	65	ND
19		0.4	56	1.5 (h) 0.42 (r)
20		0.5	36	2.1 (h) 0.72 (r)

Cmpd	R[1]	Binding Ki (nM) hA2A [a]	% Inhibition at 1 µM or Ki (nM) hA1[a]	Functional Ki (nM) A2A[b]
22		0.3	56	0.26 (h)
23		1	32	0.07 (h)
24		0.6	5000 nM	0.3 (h) 1.15 (r)
25		0.5	50	0.4(h)
26		1.9	34	0.27 (h) 1.07 (r)
27		3.1	57	ND
28		1.7	61	0.4(h) 0.65(r)
29		4.5	61	ND
30		1.2	58	0.9 (h)
31		0.4	48	0.15 (h) 0.05 (r)
32		6.4	19	1.2 (h) 11.1 (r)
33		1.5	1700 nM	0.4 (h) 4.4 (r)

21		0.2	49	0.22 (h)

ND- not determind. NR- no response at highest tested concentration 10 µM. [a]Binding 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, 8 concentrations IC_{50} curves were plotted. [b] The 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]

Table 2. Binding and functional selectivity profile of lead compound 33.

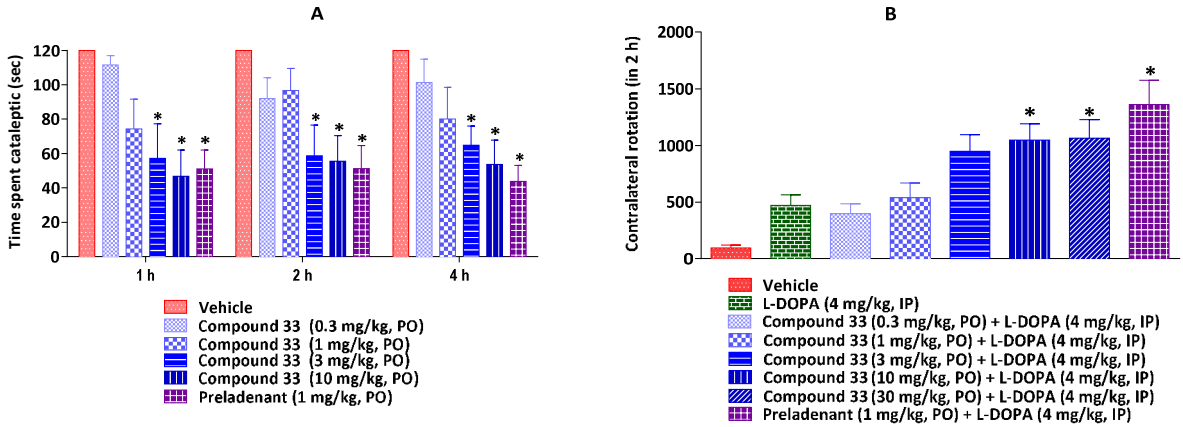
Cmpd	Binding *K*i (nM)				Functional *K*i (nM)[b]		
	hA_{2A}	hA_1	hA_{2B}	hA_3	hA_{2A}	hA_1	hA_{2B}
33	1.5	1700	5000	NR	0.4	NR	<50% inhibition at 10µM

NR- No inhibition observed at highest concentration 10 µM. [b] Evaluated at 12 concentrations, each data point in triplicates (n = 2). The mean *K*i from two independent experiments has been reported.

Table 3: DMPK properties of lead compound 33[a].

33 (in-vitro Parameters)		33 (in-vivo 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	iv	Po
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	Cmax (µM)	NA	0.6 ± 0.3
PPB (Mouse, Rat, Dog, Human)	51, 82, 78, 85	Tmax (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	Vss (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

[a]Values indicate mean for n = 4(rat) and 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



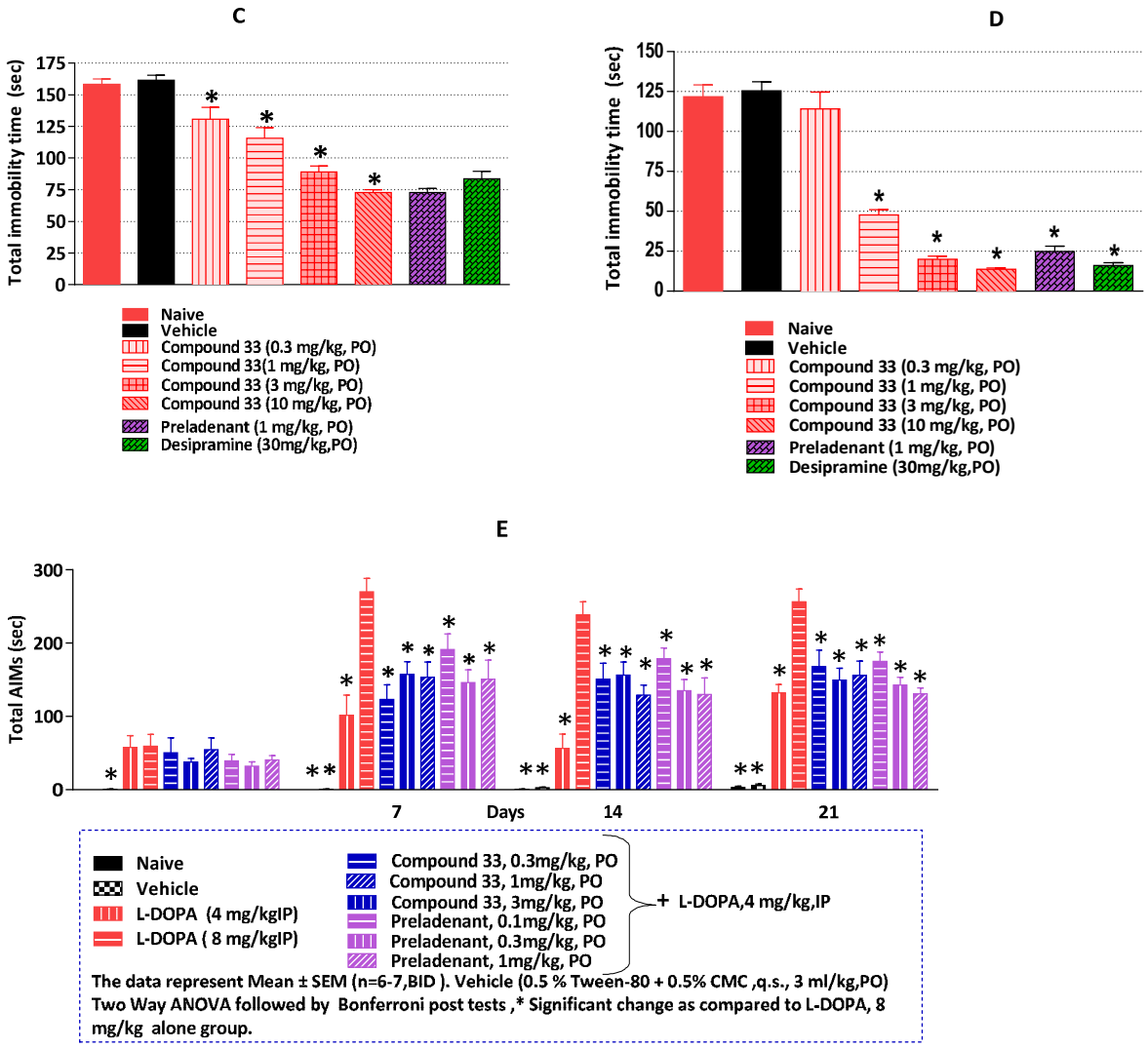


Figure 1: (**A**) Effect of compound **33** on Haloperidol induced catalepsy in rats; (**B**) Effect of compound **33** on Potentiation of Levodopa-Induced contralateral Rotations in 6-OHDA Lesioned Rats. *Significantly different as compared to L-DOPA, 4 mg/kg, IP alone group; (C) Effect of compound **33** in Forced Swim Test (FST); (**D**) Effect of compound **33** Tail Suspension Test (TST) in 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 ± SEM (n=6-7), * Significantly different as compared to Vehicle (* P<0.05); Vehicle: 0.5 % Tween-80+0.5% CMC,q.s.).

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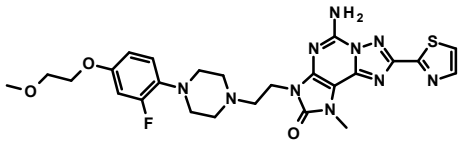
Discovery of Potent and Selective A_{2A} Antagonists with Efficacy in Animal Models of Parkinson's Disease and Depression.

Sujay Basu*, 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, Kasim A. Mookhtiar.



33

hA_{2A} binding K_i = 1.5 nM
hA_{2A} functional K_i = 0.4 nM
rA_{2A} functional K_i = 0.44 nM
Binding Selectivity
A_1/A_{2A} = > 1100 fold
A_{2B}/A_{2A} = >3000 fold
A_3/A_{2A} = >3000
Showed high functional selectivity over A_1 and A_{2B}AdoR
ED_{50} (in 6-OHDA lesioned rat, po) = 1.2 mg/kg
Showed efficacy in depression model (FST and TST)