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Synthesis of novel 7-imino-2-thioxo-3,7-dihydro-2*H*-thiazolo [4,5-*d*] pyrimidine derivatives as adenosine A_{2A} receptor antagonists

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ABSTRACT

Novel bicyclic thiazolopyrimidine compounds (**15**–**26**) were synthesized to develop adenosine A_{2A} receptor ($A_{2A}R$) antagonist for the treatment of Parkinson's disease (PD). The binding affinity of the compounds (**15**–**26**) with $A_{2A}R$ was evaluated using radioligand binding assay on isolated membranes from stably transfected HEK293 cells. Selectivity of the compounds towards $A_{2A}R$ was assessed by comparing their binding affinities with A_1 receptors (A_1R). cAMP concentrations were measured from HEK293 cells treated with compounds (**15**–**26**) as compared to NECA ($A_{2A}R$ agonist). The compound (**16**) possessed strongest $A_{2A}R$ binding affinity (K_i value = 0.0038 nM) and selectivity (737-fold) versus A_1R. Decrease in $A_{2A}R$-coupled release of endogenous cAMP from HEK293 cells treated with compounds (**15**–**26**) is evocative of their potential as $A_{2A}R$ antagonist.

Parkinson's disease (PD) is a neurodegenerative disorder caused by the degeneration of dopaminergic neurons in nigrostriatal region of the brain and characterized by loss of motor coordination manifested as tremor and rigidity of the limbs and trunk.[1] Dopamine depletion due to degeneration of dopaminergic neurons can be supplemented by administration of the dopamine precursor L-DOPA, one of the major treatments for PD.[2] However, undesirable side effects such as dyskinesia (abnormal involuntary movements) induced by L-dopa therapy are one of the major challenges for the treatment of PD.[3] Other available treatments (dopaminergic therapy) of PD include dopamine agonists and inhibitors of endogenous dopamine degradation by monoamine oxidase [MAO]B and catechol-*O*-methyltransferase[COMT]), and anticholinergics. As an alternative therapeutic approach, PD therapeutics involving $A_{2A}Rs$ antagonists are gaining importance due to their capability to give partial symptomatic relief.[4–6] Blockade of the $A_{2A}Rs$ in striatopallidal neurons diminished postsynaptic effects of dopamine depletion, and in turn reduced the motor deficits of PD.[7] $A_{2A}R$ antagonists might partially improve not only the symptoms of PD but also its course, by lessening the prime neurodegeneration and reducing the maladaptive neuroplasticity[3] $A_{2A}R$ belongs to the family of seven trans-membrane G-protein coupled receptors (GPCRs) and is highly expressed in the nigrostriatum (basal ganglia) co-localized with dopamine D_2 receptors on striatopallidal output neurons.[8] $A_{2A}R$ antagonist activated nigrostriatal pathway via D_2 receptor leading to antiparkinsonian effect. $A_{2A}R$ and D_2R interact antagonistically in striatopallidal neurons. The effect of $A_{2A}R$ activated adenylyl cyclase by persistent activation of D_2 receptor was antagonized at the level of the striatopallidal GABA neurons suggesting D_2 receptor mediated anti-parkinsonian role of $A_{2A}R$ antagonists.[9] Besides providing symptomatic relief in PD, $A_{2A}R$ antagonists have also been shown to be neuroprotective in animal models of PD.[10]

Several xanthine and non-xanthine derivatives were synthesized in last decade in search of potent $A_{2A}R$ antagonist as novel antiparkinsonian agent (Fig. 1), however, not a single drug could be accomplished for the treatment of PD. Xanthine-based $A_{2A}R$ antagonists such as KW6002 showed efficacy in models of PD without inducing hyperactivity or inducing dyskinesia but failed in clinical trials.[11–15] Non-xanthine compounds such as ZM241385 and SCH58261 possessed strong $A_{2A}R$ antagonist activity.[16–19] However, suffered from several drawbacks including lower selectivity, poor solubility and poor pharmacokinetic profile.[20] The discovery of selective and potent $A_{2A}R$ antagonist still remains a challenge.

Thiazoles have emerged as an important class of compounds due to their anti-oxidant,[21] anti-inflammatory,[22] and neuroprotective effect.[23] A series of aryl/heteroaryl urea bearing thiazole compounds possessed selective cycline dependent kinase inhibiting activity.[24] Recently, thiazole derivatives with urea moiety have demonstrated $A_{2A}R$ antagonist activity.[25] ECLiPS™ (Encoded

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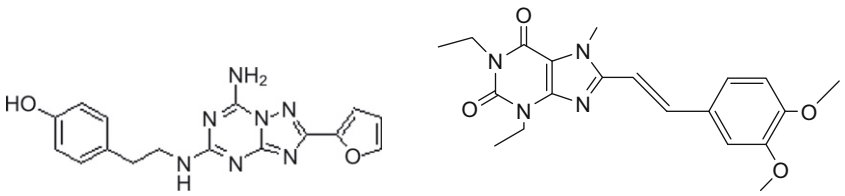


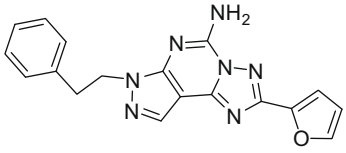


Figure 1. Structures of $A_{2A}R$ antagonists ZM241385, KW6002, and SCH58261. Binding affinities are as follows: (a) ZM241385 (K_i value; $A_{2A}R$ = 0.8 nM, A_1R = 260 nM, and hA_1/hA_2 = 325)[33] (b) KW6002 (K_i value; $A_{2A}R$ = 2.2 nM, A_1R = 150 nM, and hA_1/hA_2 = 68),[34] and (c) SCH58261 (K_i value; $A_{2A}R$ = 1.23 nM, A_1R = 594.1 nM, and hA_1/hA_2 = 483).

combinatorial libraries on Polymeric Support) libraries showed aminothiazole core structure as active $A_{2A}R$ antagonists[26] Several pyrazolo-triazolo-pyrimidine derivatives were synthesized as potent $A_{2A}R$ antagonist such as SCH58261.[16] In our efforts to develop potent $A_{2A}R$ antagonist, pyrazolo-triazolo-pyrimidine pharmacophore of known $A_{2A}R$ antagonists (SCH58261) was replaced by thiazolopyrimidine pharmacophore, considered as thiazolo-analog of natural purine base along with open triazole ring to obtain 7-imino-2-thioxo-3,7-dihydro-2*H*-thiazolo [4,5-*d*] pyrimidine pharmacophore with urea and furonamide moiety. Novel thiazolopyrimidine pharmacophore with urea moiety possessing aliphatic flexible groups, and aromatic planar structure was synthesized to give the compounds (**15**–**21**). Furan ring has been found essential for the activity of several non-xanthine derivatives to hold the molecule in active site cavity of $A_{2A}R$,[27] therefore, amino group of urea was replaced with furan ring to give furonamide series of the compounds (**22**–**26**). Since, non-xanthine compounds showed considerable selectivity for $A_{2A}R$ subtype, but suffered from low water solubility. We thought that thiazolopyrimidine ring possessing urea and furonamide derivatives would increase water solubility due to flexible polar interactions.

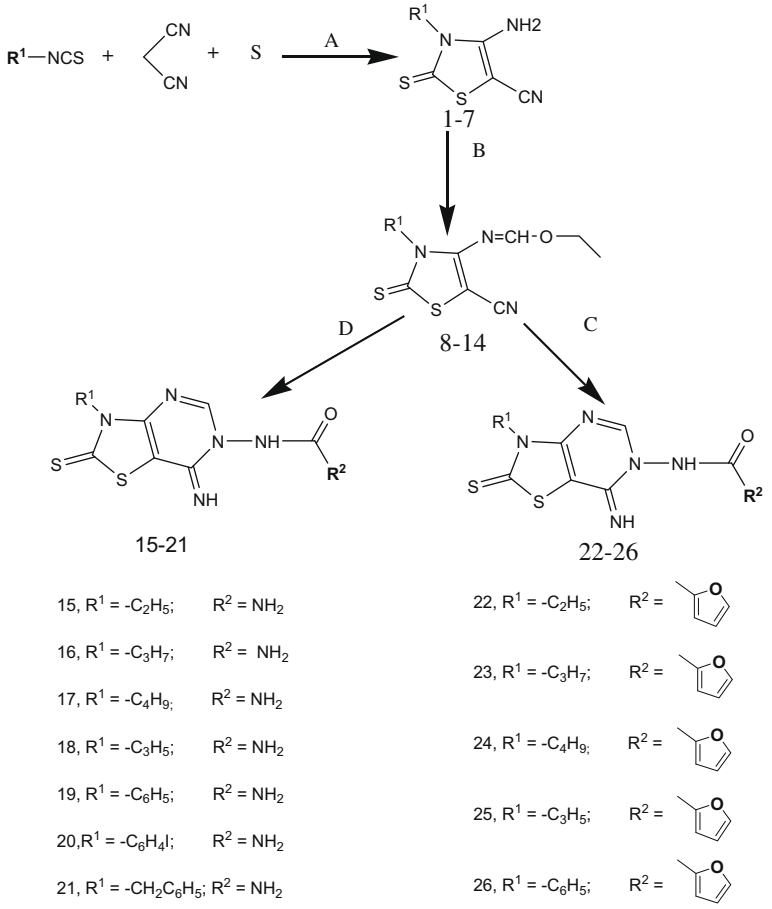
The binding affinity of the compounds (**15**–**26**) with human $A_{2A}R$ was evaluated in vitro using competitive binding with [^{3}H]ZM241385. The $A_{2A}R$ selectivity of the compounds was determined using the A_1R selective antagonist [^{3}H]DPCPX. $A_{2A}R$-coupled cAMP concentrations were measured in HEK293 cells treated with compounds (**15**–**26**).[28]

Synthesis of the compounds (**15**–**26**) was carried out according to scheme 1.[29] An equimolar mixture of isothiocyanate, malononitrile and sulfur powder in DMF was stirred in ice bath. After 15 min, triethylamine was added drop-wise to the mixture, and the reaction was continued for 4 h to give 4-amino-3-substituted-2-thioxo-2,3-dihydro-thiazole-5-carbonitrile derivatives (**1**–**7**). The carbonitrile compounds (**1**–**7**) were refluxed in toluene with triethylorthoformate (equimolar ratio) and *p*-toluene sulfonic acid (catalytic amount) for 6 h to yield imino-ether derivatives (**8**–**14**). The disappearance from IR spectra of an intense absorption band at 3230–3369 cm^{-1} (free amino group), and the appearance of a singlet at 8.1–8.4 ppm (N=CH of methylene amine) in the 1H spectra validated the formation of Schiff's bases.

Mixture of imino-ether derivatives (**8**–**14**), semicarbazide hydrochloride/furoic acid hydrazide (equal mol) and TEA (catalyst) in ethanol was stirred at rt for 12 h to give the compounds (**15**–**26**). The disappearance from IR spectra of an intense absorption band at 2205 cm^{-1}, and the appearance of a singlet at 8.1–8.8 ppm (N=CH of pyrimidine ring) in the 1H spectra confirmed the formation of bicyclic compounds. All the compounds have been fully characterized and their purity was checked by HPLC (Ref. 29 and Supplementary data).

The result of $A_{2A}R$ binding assay are expressed as inhibition constants (K_i in nM). The $A_1R/A_{2A}R$ describes their selectivity over A_1R.[30] In the set of thiazolopyrimidine urea derivatives (**15**–**21**, $R^2 = NH_2$), ethyl substitution (**15**, $R^1 = -C_2H_5$) exhibited significantly higher binding affinity with A_1R (0.00016 ± 0.007 nM) as compared to $A_{2A}R$ (0.09 ± 0.01 nM). Homologation of one carbon in compound **15** gave the propyl derivative of thiazolo pyrimidine urea (**16**, $R^1 = -C_3H_7$). In competitive radioligand binding assay, the displacement of [^{3}H]ZM241385 with **16** was significantly increased (~25 times) as compared to **15**, and with very high selectivity for $A_{2A}R$ (737-fold selectivity over A_1R), and was better than the known antagonist SCH58261 (K_i = 1.23 ± 0.016, hA_1/hA_2 = 483).[31] However 3-carbon chain with π-overlap in allyl derivative (**18**, $R^1 = -C_3H_5$) displayed good binding affinity (K_i = 0.092 ± 0.01) but reduced selectivity (hA_1/hA_2 = 5). Further extending the alkyl chain to give butyl derivative of thiazolopyrimidine urea (**17**, $R^1 = -C_4H_9$) resulted in decreased selectivity over A_1R. Incorporation of aromatic ring (phenyl) substituent in thiazolopyrimidine urea (**19**, $R^1 = -C_6H_5$) gave enhanced binding affinity and selectivity. Moreover, *p*-iodophenyl substitution (**20**, $R^1 = -C_6H_4I$) on the pharmacophore improved both binding affinity and selectivity (144-fold). Insertion of one carbon homologation in planar aromatic ring of thiazolopyrimidine urea (**21**, $R^1 = -CH_2C_6H_5$) led decreased selectivity. We can conclude that both **19** and **20** possess promising activity, yet the compound (**16**) is the most active among all thiazolopyrimidine urea derivatives. The amino (NH_2) group of urea moiety of thiazolopyrimidine pharmacophore was replaced by furan ring to give another set of compounds (**22**–**26**, R^2 = –furyl). Overall substituent effects to binding affinity (propyl > butyl > allyl > aryl > ethyl) and selectivity (propyl > allyl > butyl > aryl > ethyl) profile of thiazolopyrimidine furonamide (**22**–**26**) decreased, however in the set of compound (**22**–**26**) propyl derivative (**23**, $R^1 = -C_3H_7$) showed maximum binding and selectivity to $A_{2A}R$.

The finding clearly demonstrated that bicyclic thiazolopyrimidine urea derivatives (**15**–**21**) were more potent and selective than the corresponding bicyclic thiazolopyrimidine furonamide



Scheme 1. Reagents and conditions: (A) triethyl amine (TEA), rt; (B) triethyl orthoformate, *p*-toluene sulfonic acid (PTSA), reflux; (C) furoic acid hydrazide, TEA, rt; and (D) semi-carbazide HCl, TEA, rt.

derivatives (**22**–**26**) suggesting that one H-bond acceptor and three H-bond donors are required for high affinity of $A_{2A}R$. The low affinity and selectivity of furonamide derivatives suggested that basic amino group of urea at the active site is essentially required (Fig. 2).

$A_{2A}R$ mediate cellular response through multiple signal transduction pathways and a generic functional assay for G_i- and G_s-protein-coupled receptor directly measures adenosine 3′,5′-cyclic monophosphate (cAMP) production and inhibition in live cells. We extended our investigation to study the effect of compounds (**15**–**26**) on $A_{2A}R$ mediated inhibition of cAMP accumulation.[28] Functional antagonism study points that all the compounds (**15**–**26**) significantly decreased the cAMP concentration compared to agonist NECA (0.75 pmol/ml) and showed better inhibition of cAMP than SCH58261 (Table 1).

In summary, all the urea derivatives (**15**–**21**) showed binding affinity with $A_{2A}R$ in nanomolar range. However, the compound **16** displayed K_i value of 0.0038 nM with (737-fold) binding selectivity versus A_1R and compound **20** exhibited reasonably good $A_{2A}R$ binding affinity (K_i value 0.017) and (>100-fold) selectivity over A_1R. The inhibition of cAMP accumulation with the compound **16** and **20** was 0.14 pmol and 0.076 pmol, respectively, better than

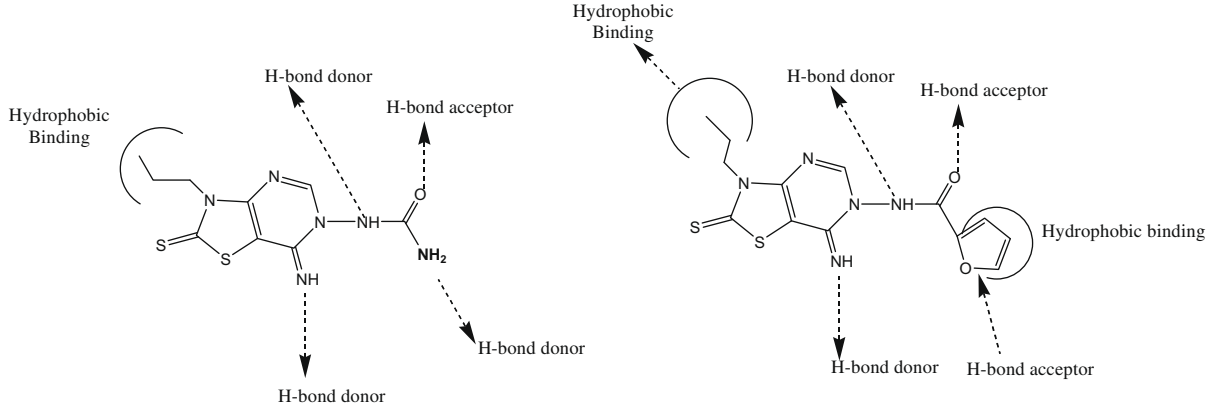
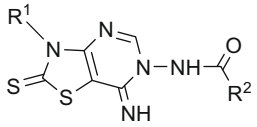
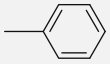
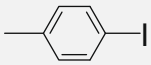
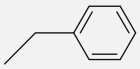
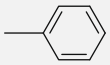


Figure 2. Potential binding site of thiazolopyrimidine urea derivative **16** and thiazolopyrimidine furonamide derivative **23**.

Table 1
Radioligand binding and cAMP functional assay of thiazolopyrimidine compounds (**15**–**26**)



Compounds	R^1	R^2	hA_{2A} binding $K_i \pm SD^a$ (nM)	hA_1 binding $K_i \pm SD^b$ (nM)	hA_1/hA_{2A} Ratio	cAMP (pmol/ml)
15	$-CH_2CH_3$	$-NH_2$	0.09 ± 0.01	0.00016 ± 0.007	0.002	0.085
16	$-CH_2CH_2CH_3$	$-NH_2$	0.0038 ± 0.001	2.8 ± 0.8	737	0.14
17	$-CH_2CH_2CH_2CH_3$	$-NH_2$	0.089 ± 0.01	1.04 ± 0.84	11	0.083
18	$-CHCHCH_3$	$-NH_2$	0.092 ± 0.01	0.47 ± 0.1	5	0.08
19		$-NH_2$	0.063 ± 0.008	1.5 ± 1.10	24	0.078
20		–NH2	0.017 ± 0.01	2.5 ± 1.1	147	0.076
21		$-NH_2$	0.023 ± 0.014	0.14 ± 0.086	6	0.067
22	$-CH_2CH_3$		1.2 ± 1.10	0.016 ± 0.01	0.01	0.12
23	$-CH_2CH_2CH_3$		0.024 ± 0.01	0.087 ± 0.05	3	0.092
24	$-CH_2CH_2CH_2CH_3$		0.029 ± 0.01	0.0053 ± 0.001	0.2	0.06
25	$CHCHCH_3$		0.17 ± 0.1	0.59 ± 0.11	3	0.084
26			0.33 ± 0.75	0.0085 ± 0.001	0.02	0.048
SCH58261			1.23	594.1	483	0.25
NECA			14	20	0.7[32]	0.75
Untreated HEK293 cells (stably expressing $A_{2A}R$)						0.4

[a] Standard deviation (SD) of binding affinity (K_i in nM) of compound **15-26** for human $A_{2A}R$.
[b] Standard deviation (SD) of binding affinity (K_i in nM) of compound **15-26** for human A_1R.

SCH58261 (0.25 pmol). The results demonstrated that the compound **16** was most potent $A_{2A}R$ antagonist among all compounds (**15**–**26**) and thiazolopyrimidine with urea extension represent a novel pharmacophore template for further development of selective and potent $A_{2A}R$ antagonist.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.11.133.

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28. In the functional assay, cAMP concentrations were determined using direct cAMP EIA kit (Assay designs, USA) in treated HEK293 cells (stably transfected with cDNA encoding human $A_{2A}R$). Cells (1×10^6) were treated with 100 nM of compounds (**15**–**26**), 100 nM SCH58261 and 100 nM of NECA for 24 h, 0.1 N HCl was added to release endogenous cAMP, centrifuged at 1000*g* for 5 min at 4 °C. cAMP concentrations were measured (P. Tijssen, 'Practice & theory of enzyme immunoassays', (1985) Amsterdam, Elsevier) in the treated cells, and the results were compared with untreated cells (representing basal level of cAMP in the cells).
29. A mixture of isothiocyanate (1 equiv), malononitrile (1 equiv), and sulfur powder (1 equiv), in DMF (50 ml) was stirred in ice bath. After 15 min, triethylamine (5 ml) was added, and the reaction was continued for 4 h. The reaction mixture was poured in water and the resulting precipitate was dissolved in absolute ethanol to give pure crystalline 4-amino-3-substituted -2-thioxo-2,3-dihydro-thiazole-5-carbonitrile derivatives (**1**–**7**). The carbonitrile compounds **1**–**7** (1 equiv) were refluxed with triethylorthoformate (1 equiv), and *p*-toluene sulfonic acid (catalytic amount) in toluene (20 ml) for 6 h. Toluene was evaporated under reduced pressure and crude product was dissolved in ethyl acetate, washed with water and dried with Na_2SO_4. Solution was concentrated under reduced pressure to give pure crystalline imino-ether derivatives (**8**–**14**). A Mixture of imino-ether derivatives **8**–**14** (1 equiv), semicarbazide hydrochloride(1 equiv), and TEA (1–2 ml) in ethanol (10 ml) was stirred at rt for 12 h. The precipitate was filtered, washed with absolute ethanol and water to give pure compounds (**15**–**21**). imino-ether derivatives **8**–**11**(1 equiv), were stirred with furoic acid hydrazide (1 equiv), and TEA (1–2 ml) in ethanol (10 ml) at rt for 12 h. The precipitate was filtered, washed with ethanol and water to give the compounds (**22**–**26**). Spectroscopic data for **16**. *7-Imino-3-propyl-2-thioxo-3,7-dihydro-2H-thiazolo[4,5-d]pyrimidin-6-yl)-urea* Yield: 86% (100% purity by HPLC), mp: 220 °C, IR (KBr): 3249, 3163 (NH_2), 2957, 2782 (alkyl), 1673 (C═O) cm^{-1}, ^1H NMR (DMSO-d_6): δ 0.89 (t, 3H, J = 7.2 Hz, CH_3), 9.70 (s, 1H, NH), 1.67–1.79 (m, 2H, CH_2), 4.28 (t, 2H, J = 7.2 Hz, CH_2), 6.35 (s, 2H, NH_2), 8.40 (s, 1H, N═CH), 9.70 (s, 1H, NH), ^{13}C NMR (DMSO-d_6): δ 10.9, 20.2, 46.3, 98.1, 154.1, 155.8, 158.5, 159.0, 190.3, LC–MS: m/z 284 (M^+), 285 (M+1).
30. *Adenosine $A_{2A}R$ and A_1R binding assays:* [^{3}H]ZM241385 and [^{3}H]DPCPX binding assays for adenosine $A_{2A}R$ and A_1Rs, respectively, were performed in HEK293 cells (procured from National Center for Cell Sciences, Pune, India). Briefly, 10 μg HEK293 cell membranes isolated from stably transfected HEK293 cells with human $A_{2A}R$ and A_1R cDNA were incubated with different concentrations (1 pM to 1 μM) of compounds and 1 nM [^{3}H]ZM241385 in 200 μl incubation buffer containing 50 mM Tris–Cl, 1 mM EDTA, pH 7.4 and 2.5 U/ml adenosine deaminase. Adenosine A_1R assays were performed on 10 μg of HEK293 cell membranes expressing human adenosine A_1Rs and 1 nM [^{3}H]DPCPX in 200 μl incubation buffer. Reactions were carried out for 60 min at 26 °C and were terminated by rapid filtration over 96-well plates equipped with GF/B filters (Millipore, USA). Filters were washed three times with 300 μl of cold washing buffer containing 50 mM Tris–Cl, 10 mM $MgCl_2$, pH 7.4, air dried, and radioactivity retained on filters were counted in 1450 LSC & Luminescence counter (Wallac Microbeta Trilux, Perkin–Elmer, USA). The K_d values for radioligands [^{3}H]ZM241385 and [^{3}H]DPCPX were 0.99 and 1.79 nM, respectively, obtained by saturation binding assays. Nonspecific binding for adenosine $A_{2A}R$ and A_1R were determined in the presence of 50 μM NECA and 50 μM CPA, respectively. Assays were performed in duplicates and compounds were tested twice. Data were fitted in one site competition-binding model for IC_{50} determination using the program GraphPad Prism 4.0 (GraphPad Software, Inc., San Diego, CA) and K_i values were calculated using Cheng and Prusoff formula.
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