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Letter

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Discovery of 3,4-Dihydropyrimidin-2(1*H*)-ones As a Novel Class of Potent and Selective A_{2B} Adenosine Receptor Antagonists

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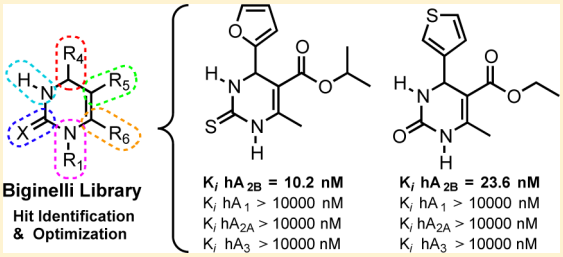
Supporting Information

ABSTRACT: We describe the discovery and optimization of 3,4-dihydropyrimidin-2(1*H*)-ones as a novel family of (nonxanthine) A_{2B} receptor antagonists that exhibit an unusually high selectivity profile. The Biginelli-based hit optimization process enabled a thoughtful exploration of the structure−activity and structure−selectivity relationships for this chemotype, enabling the identification of ligands that combine structural simplicity with excellent hA_{2B} AdoR affinity and remarkable selectivity profiles.



KEYWORDS: *Adenosine antagonists, A_{2B} receptor antagonists, 3,4-dihydropyrimidin-2(1H)-ones, Biginelli reaction*

The endogenous neuromodulator adenosine is a signaling nucleoside that is widely distributed in diverse tissues in both the peripheral and central nervous system.[1] Adenosine is involved in different biochemical processes, such as energy transfer and signal transduction, but it is also a key building block for biologically relevant molecules.[1] Once in the extracellular space, adenosine modifies cell function by triggering specific cell membrane G protein-coupled receptors (adenosine receptors AdoRs, namely, A_1, A_{2A}, A_{2B}, and A_3) that modulate diverse effector systems.[1] The improved understanding of the physiology, pharmacology, and the structural and molecular biology of adenosine and its receptors has provided solid evidence that supports the ability of adenosine to regulate diverse physiopathological events.[2] These findings, along with the identification of potent and selective ligands,[3,4] embrace the emergence of conceptually novel therapeutic strategies to address significant unmet medical needs.[5] The A_{2B} AdoRs have generally been defined as low affinity receptors that remain silent under physiological conditions but are rapidly activated during chronic highly oxidative stress conditions such as hyperglycemia or mast cell activation.[1,2] The A_{2B} AdoR subtype has been recognized as regulating a wide range of physiopathological events,[6] being particularly involved in modulating cardiovascular functions and the genesis of inflammation processes. The A_{2B} AdoR antagonists are promising drug candidates[7] toward clinical use for the treatment of diabetes[8] and diabetes retinopathy,[9] as well as asthma and chronic obstructive pulmonary disease (COPD).[10] From a structural point of view, A_{2B} AdoR antagonists are planar heterocyclic compounds (Figure 1)[3−5,7] that can be classified into two families: xanthines (**I**, **IV**, and deaza-analogues **II**)[7,11] and nonxanthine derivatives **III**, **V**−**VIII** (e.g., purines, 2-aminopyrimidines, and 2-aminopyrazines).[12−15] The structural elaboration of these prototypes has provided potent and selective ligands, some of which have entered into clinical trials or preclinical development.[7]

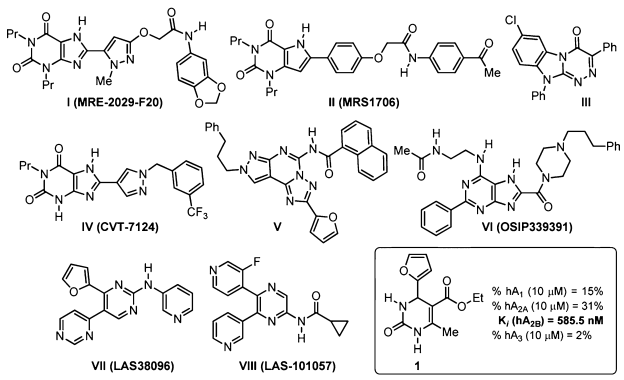


Figure 1. Representative A_{2B} AdoR antagonists and hit compound **1**.

Within the framework of an HTS-based program aimed at accelerating the discovery of ligands that target adenosine receptors, employing the ComBioMed screening library (our multicomponent-assembled exploratory collection), we identified 3,4-dihydropyrimidin-2(1*H*)-one **1** (Figure 1); which exhibited a submicromolar affinity (K_i = 585.5 nM) toward

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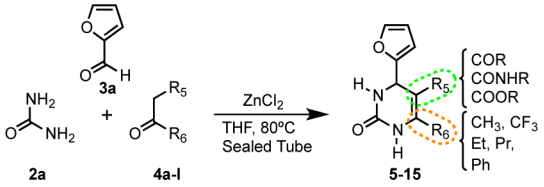
Table 1. Synthesis, Affinity Data at the *h*AdoRs of the Early Hit (1), and Preliminary SAR at Positions 5 and 6 of the Exploratory Library[18]



			K_i (nM) ± SEM or % at 10 μM[a]			
compd	R_5	R_6	hA_1[b]	hA_{2A}[c]	hA_{2B}[d]	hA_3[e]
1[19]	CO_2Et	Me	15%	31%	585.5 ± 89	2%
5[20]	COMe	Me	30%	21%	38%	14%
6*	$CONH_2$	Me	2%	1%	2%	2%
7*	CONHPh	Me	24%	7%	31%	5%
8[21]	CO_2Me	Me	34%	25%	2159 ± 232	13%
9*	CO_2-*n*Pr	Me	43%	55%	206.3 ± 16	2%
10*	CO_2-*i*Pr	Me	18%	23%	40.8 ± 2.3	4%
11*	CO_2-*t*Bu	Me	4%	7%	44%	7%
12*	CO_2Et	CF_3	15%	32%	2%	2%
13*	CO_2Et	Et	29%	41%	714.6 ± 81	3%
14*	CO_2Et	*n*Pr	50%	602.4 ± 76	256.1 ± 42	19%
15*	CO_2Et	Ph	3%	17%	28%	2%

[a] $n \geq 3$ unless otherwise noted. [b] Displacement of specific [^{3}H]DPCPX binding in membranes obtained from hA_1 AR stably expressed in CHO cells. [c] Displacement of specific [^{3}H]ZM241385 binding in membranes obtained from hA_{2A} AR stably expressed in HeLa cells. [d] Displacement of specific [^{3}H]DPCPX binding in membranes obtained from hA_{2B} AR stably expressed in HEK-293 cells. [e] Displacement of specific [^{3}H]NECA binding in membranes obtained from hA_3 AR stably expressed in HeLa cells.

the A_{2B} AdoR. The attractive affinity/selectivity profile observed for ligand **1**, along with the well-documented drug-likeness and synthetic feasibility of the 3,4-dihydropyrimidin-2(1*H*)-one scaffold,[16] led us to start an optimization study based on this chemotype. Herein, we describe the discovery and optimization of a novel class of (nonxanthine and nonadenine) potent and structurally simple A_{2B} AdoR antagonists that exhibit unusually elevated subtype selectivity. It should be pointed out that hit compound **1** (Figure 1) was the only derivative to exhibit noticeable activity among a subset of 30 3,4-dihydropyrimidin-2(1*H*)-ones screened (incorporating diverse residues at position 4 of the heterocycle). As a consequence, we further focused on derivatives containing furan or thiophene heterocycles at position 4.

An exploratory library of 3,4-dihydropyrimidin-2(1*H*)-ones (Table 1) was prepared for a preliminarily assessment of the effect of substitution at positions 5 and 6 on the affinity/selectivity profile. The required structures (**5**−**15**) were obtained by following an environmentally friendly protocol[17] involving the reliable Biginelli reaction with urea (**2a**) and furfural (**3a**) but varying the ketone component (**4a**−**l**). Novel compounds are indicated in the Tables with an asterisk, the original articles describing already known compounds were incorporated in the references section and Supporting Information. The pharmacological profiles of the compounds obtained (**5**−**15**) were studied in vitro at the four human adenosine receptor subtypes using radioligand-binding assays.[18] All compounds were tested as racemic mixtures.

The prospective screening of congeners of **1** (Table 1) revealed preliminary facets of the SAR in this series, highlighting the role exerted by substituents at positions 5 and 6. It was observed (Table 1) that replacement of the ester group by ketone (**5**) or amide (**6**−**7**) functions had a deleterious impact on the activity. Within the ester series (compounds **8**−**11**), substitution of the alkoxy residue in hit compound (**1**) by methyl (**8**), propyl (**9**), isopropyl (**10**), or *tert*-butyl (**11**) groups produced derivatives that elicited attenuated (methyl and *tert*-butyl) or improved (propyl and isopropyl) affinity. It is worth noting the high affinity (K_i = 40.8 nM) and excellent selectivity exhibited by the isopropyl ester **10** (14-fold increase in potency with respect to **1**), while its skeletal isomer (**9**) showed moderate affinity (K_i = 206.3 nM).

Preliminary exploration of the substituent at position 6 (Table 1, compounds **1** and **12**−**15**) emphasized the effectiveness of a methyl residue. Thus, the introduction of a trifluoromethyl (**12**) group or phenyl ring (**15**) drastically reduces the activity, while superior homologues (**13**−**14**) provide moderately active ligands, albeit with a rather subtype promiscuous profile.

Structural modification at positions 3 and 4 of the heterocyclic core in the parent compound (**1**) [e.g., aromatization (**16**) or acetylation (**17**), Scheme 1] afforded derivatives with (6−7-fold) attenuated affinity; this finding suggests a probable role for the NH group and the stereogenic center during interaction with A_{2B} AdoRs.

The pharmacological data obtained for compounds **1** and **5**−**17** guided the design of a focused library exploring positions 1,

Scheme 1. Synthesis of Derivatives 16 and 17

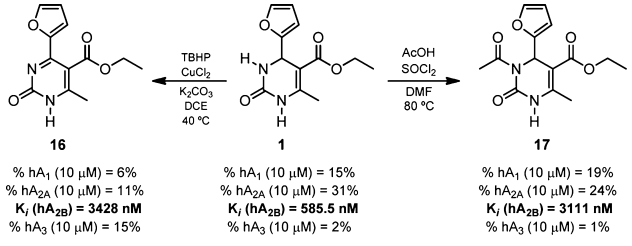


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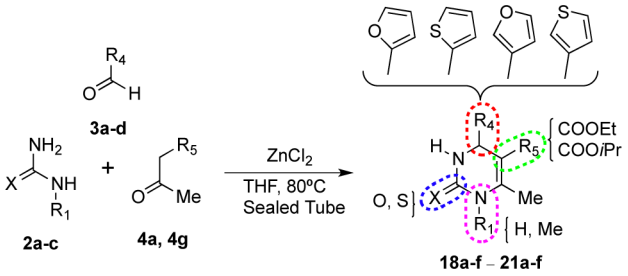
Table 2. Synthesis and Affinity Data at the *h*AdoRs of the 3,4-Dihydropyrimidin-2(1*H*)-ones 18−21[18]



					K_i (nM) or % at 10 μM[a]			
compd	R_1	X	R_4	R_5	hA_1[b]	hA_{2A}[c]	hA_{2B}[d]	hA_3[e]
18a[19] (1)	H	O	2-furyl	CO_2Et	15%	31%	585.5 ± 89	2%
18b* (10, SYAF014)	H	O	2-furyl	CO_2-*i*Pr	18%	23%	40.8 ± 2.3	4%
18c[22]	H	S	2-furyl	CO_2Et	1%	32%	608.4 ± 31	5%
18d[23] (SYAF030)	H	S	2-furyl	CO_2-*i*Pr	15%	18%	10.2 ± 0.5	4%
18e[24]	Me	O	2-furyl	CO_2Et	17%	5%	6544 ± 124	25%
18f*	Me	O	2-furyl	CO_2-*i*Pr	28%	31%	1151 ± 38	18%
19a[19]	H	O	2-thienyl	CO_2Et	3%	33%	39%	2%
19b*	H	O	2-thienyl	CO_2-*i*Pr	37%	19%	44%	14%
19c[19]	H	S	2-thienyl	CO_2Et	2%	23%	6247 ± 101	4%
19d[25]	H	S	2-thienyl	CO_2-*i*Pr	23%	15%	1572 ± 93	20%
19e[26]	Me	O	2-thienyl	CO_2Et	2%	3%	11%	6%
19f*	Me	O	2-thienyl	CO_2-*i*Pr	22%	24%	28%	18%
20a[27] (SYAF101)	H	O	3-furyl	CO_2Et	21%	24%	39.6 ± 1.1	1%
20b*	H	O	3-furyl	CO_2-*i*Pr	25%	26%	1486 ± 62	3%
20c*	H	S	3-furyl	CO_2Et	18%	25%	43.1 ± 1.6	15%
20d*	H	S	3-furyl	CO_2-*i*Pr	18%	33%	194.2 ± 12	23%
20e*	Me	O	3-furyl	CO_2Et	17%	24%	4259 ± 110	1%
20f*	Me	O	3-furyl	CO_2-*i*Pr	23%	2%	35%	1%
21a[28] (SYAF080)	H	O	3-thienyl	CO_2Et	16%	34%	23.6 ± 1.0	11%
21b* (SYAF020)	H	O	3-thienyl	CO_2-*i*Pr	26%	25%	56.6 ± 1.3	5%
21c[29]	H	S	3-thienyl	CO_2Et	23%	28%	1103 ± 94	2%
21d*	H	S	3-thienyl	CO_2-*i*Pr	16%	20%	415.7 ± 7.3	3%
21e*	Me	O	3-thienyl	CO_2Et	30%	24%	2968 ± 150	3%
21f*	Me	O	3-thienyl	CO_2-*i*Pr	33%	20%	1811 ± 112	14%

[a] $n \geq 3$ unless otherwise noted. [b] Displacement of specific [^{3}H]DPCPX binding in membranes obtained from hA_1 AR stably expressed in CHO cells. [c] Displacement of specific [^{3}H]ZM241385 binding in membranes obtained from hA_{2A} AR stably expressed in HeLa cells. [d] Displacement of specific [^{3}H]DPCPX binding in membranes obtained from hA_{2B} AR stably expressed in HEK-293 cells. [e] Displacement of specific [^{3}H]NECA binding in membranes obtained from hA_3 AR stably expressed in HeLa cells.

2, 4, and 5 of the heterocycle (Table 2). The novel 3,4-dihydropyrimidin-2(1*H*)-one collection (compounds **18**−**21**) was prepared (Table 2) by utilizing three urea derivatives (**2a**−**c**), ethyl or isopropyl acetoacetate (**4a** and **4g**) and a subset of pentagonal heterocyclic carbaldehydes (**3a**−**d**). This approach enabled us to install at position 4 of the pyrimidine scaffold the furan or thiophene backbones attached through positions 2 or 3. Examination of the binding data (Table 2) confirms the identification of novel ligands that elicit potent A_{2B} affinity but also excellent subtype selectivity (compounds **18b**, **18d**, **20a**, **20c**, **21a**, and **21b**).

A primary observation emerging from the pharmacological evaluation of the four 3,4-dihydropyrimidine subsets (Table 2, **18**−**21**) illustrates the different biological profiles produced by the variation in R_4. As observed (Table 2), derivatives bearing (2- or 3-) furyl and 3-thienyl residues (**18** and **20**−**21**) elicited superior affinity at the A_{2B} AdoRs, while its 2-thienyl congeners (**19**) does not exhibit significant activity. As anticipated from preliminary SAR studies (Table 1), the group at position 5 (R_5), more precisely the alkoxy residue of the ester moiety, exerts a remarkable effect on ligand affinity (Table 2, compare ligands **a**−**b**, **c**−**d**, and **e**−**f** in each subset). A detailed inspection of the biological data (Table 2) illustrates how the effect of the ester moiety (R_5) is modulated by the neighborhood of the pentagonal heterocyclic framework (vide infra), more specifically by the position on which the furan or thiophene core is attached (2- or 3-) to the 3,4-dihydropyrimidin-2(1*H*)-one scaffold. Thus, isopropyl esters provide optimal affinity at A_{2B} AdoR when a 2-furyl group is present at position 4 of the azinone (e.g., compounds **18b** and **18d**, K_i = 40.8 and 10.2 nM, respectively). Conversely, for derivatives that incorporate an ethoxycarbonyl group at position 5 a better affinity profile was found for those derivatives that bear 3-furyl or 3-thienyl counterparts (e.g., compounds **20a** and **21a**, K_i = 39.6 and 23.6 nM, respectively). It should be noted that derivative **21b**, bearing COO-*i*Pr and 3-thienyl groups and exhibiting a K_i = 56.6 nM, constitutes an exception to the aforementioned trend.

The pharmacological data obtained for the 3,4-dihydropyrimidin-2(1*H*)-ones and their 2-thia-analogues (Table 2,

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compounds **a**–**d** in each subset) reveals the effect of the C=O/C=S substitution. As previously observed for positions 4 and 5, the impact of bioisosteric replacement at position 2 seems to be dependent on the alkoxy residue of the ester function (R_5) and the substituent at R_4. Thus, for compounds that incorporate an isopropyloxycarbonyl group, the 2-thioderivatives proved to be 4–7-fold more potent than the 2-oxo-analogues (Table 2, compare **18b** and **18d**, **19b** and **19d**, and **20b** and **20d**). A similar comparison for the 3,4-dihydropyrimidines bearing an ethyl ester group shows that C=O/C=S substitution has negligible impact on A_{2B} AdoR affinity (Table 2, compare **18a** and **18c**, **19a** and **19c**, and **20a** and **20c**). It should be noted that the 3-thienyl subset (**21**) constitutes an exception to this trend since in this series the introduction of a thiocarbonyl group produces derivatives with attenuated affinity regardless of the alkoxy residue at the ester function (Table 1, compare **21a**/**21c** and **21b**/**21d**). An additional feature emerging from the pharmacological data illustrates that methylation at position 1 generates ligands with remarkably attenuated affinity (Table 2, compounds **e**–**f** in each subset). It is noteworthy that, in contrast with the effect discussed for substituents at positions 2, 4, and 5 (which proved to be highly coordinated), the detrimental effect of methylation is observed for all compounds evaluated regardless of the substitution patterns on the 3,4-dihydropyrimidine scaffold.

All the compounds with high A_{2B} affinity (i.e., $K_i \leq 100$ nM) were docked with GOLD[30] into a homology-based model of the A_{2B} AdoR,[31] considering the two stereoisomers in all cases (see Supporting Information for details). The consensus binding mode obtained for each stereoisomer (Supplementary Figure S1) is illustrated in detail for compound **21a** in Figure 2

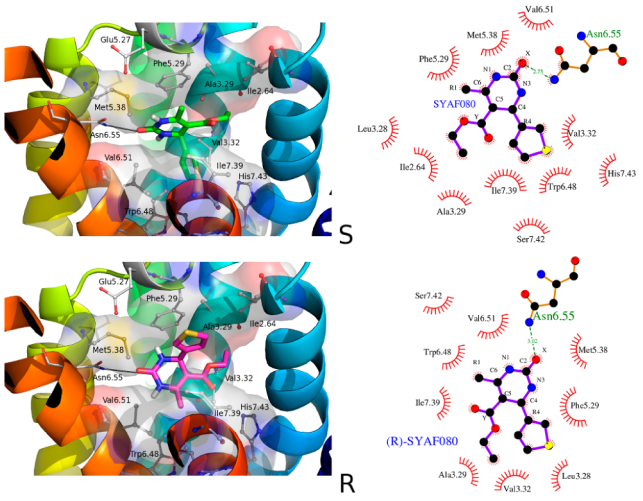

Figure 2. Proposed binding mode of the *S* (top) or *R* (bottom) stereoisomers of compound **21a** at the $A2_B$ AdoR. The Conolly surface is shown in gray for the residues in the binding site and a black line depicts H-bond with Asn254$^{6.55}$.

and provides valuable structural insights to rationalize their potency and selectivity profiles. Both stereoisomers show a hydrogen bond of the heteroatom at position 2 (oxygen in **21a**) with the highly conserved Asn254$^{6.55}$. In the case of the *S*-enantiomer, position N1 could make additional, water-mediated hydrogen bonds with residues Asn254$^{6.55}$ and Glu169$^{5.27}$ (see Supplementary Figure S2), an interaction that might explain the diminished activity observed by methylation of this position. In this enantiomer, the methyl at position 6 points toward the extracellular region, while in the *R* enantiomer it is the heterocycle at position 4 that sits at the equivalent subsite. It can also be appreciated the optimal shape complementarity of residue Val250$^{6.51}$ with the surface of the dihydropyrimidine core, more precisely around the region where the substituent at either position 4 (*S*-stereoisomer) or 6 (*R*) is located. Interestingly, this residue is one of the few differences in the transmembrane region between A_{2B} and the closely related A_{2A} AdoR,[18] where the bigger Leu$^{6.51}$ was found to be the key for the binding of antagonists.[32] Consequently, this interaction with Val250$^{6.51}$ might provide a rationale for the highly selective profile of the present series toward the A_{2B} AdoR. The substituent at position 5 sits on a narrow region between TM2 and TM3, in a way that the ester function would displace the water molecules that eventually fill this region according to the last crystal structure of the A_{2A} AdoR,[33] while the alkyl chain (Et or *i*Pr) shows vdW interactions with residues Ile67$^{2.64}$, Ala82$^{3.29}$, and Val85$^{3.32}$.[34]

Among the collection of compounds herein described, derivatives **18d** and **21a** warrant particular attention since they combine structural simplicity with excellent affinity/selectivity profiles (with K_i = 10.2 and 23.6 nM, respectively). In addition to its excellent potency and selectivity profile, a remarkable feature of the ligands reported here concerns their structural novelty. A comparative analysis of the novel ligands optimized during this work with established selective A_{2B} receptor antagonists (Figure 1) confirms the discovery of a singular chemotype that provides distinctive structural features, particularly its simple nonplanar structure and potential for rapid diversification.

The competition curves of specific [^{3}H]DPCPX binding (B_{max} = 1941 fmol/mg protein, K_D = 25.8 nM) to human A_{2B} receptors obtained for compounds **18d** and **21a** are shown in the Supporting Information (Figure A). As part of the pharmacological characterization, **18d** and **21a** were tested in c-AMP assays in order to evaluate their capability to inhibit NECA-stimulated (100 nM) c-AMP production. The log dose–response curves of c-AMP accumulation for selected antagonists to human A_{2B} receptors are shown in the Supporting Information. These experiments demonstrated that 3,4-dihydropyrimidin-2(1*H*)-ones **18d** and **21a** inhibit c-AMP accumulation, unequivocally validating the antagonistic behavior of these compounds at A_{2B} AdoRs. A comparison of K_i and K_B values obtained for the novel A_{2B} AdoR antagonists studied (**18d** and **21a**) reveals the high correlation between data obtained from binding (K_i = 10.2 and 23.6 nM, respectively) and functional assays (K_B = 4.7 and 25.2 nM, respectively). With the aim of completing the preliminary characterization of this novel class of selective A_{2B} antagonists, the in vitro cytochrome P450 (CYP450) inhibition profiles of **18d** and **21a** were studied (see Supporting Information). They both showed no relevant inhibition of human CYP450 cytochromes at concentrations 100-fold superior to the K_i values.

In summary, we have discovered a novel class of (non-xanthine) A_{2B} receptor antagonists derived from the 3,4-dihydropyrimidin-2(1*H*)-one chemotype. The robustness and exploratory potential of the Biginelli reaction accelerated the hit optimization process, enabling the rapid identification of ligands that combine structural simplicity with excellent hA_{2B} AdoR affinity and remarkable selectivity profiles (**18d**, **20a**, and **21a**). The antagonistic behavior of two representative

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derivatives was unequivocally validated through functional cAMP experiments.

■ ASSOCIATED CONTENT

Supporting Information

Detailed experimental protocols and characterization data for compounds described. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS

AdoRs, adenosine receptors; GPCRs, G protein-coupled receptors; hA_{2B} AdoR, human A_{2B} adenosine receptors; COPD, chronic and obstructive pulmonary disease

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