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Exploring the influence of the substituent at position 4 in a series of 3,4-dihydropyrimidin-2(1*H*)-one A_{2B} adenosine receptor antagonists

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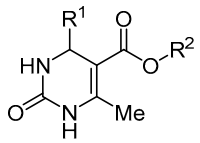

R^1 = H, Cyp, Cy, Ph, 2-XC_6H_4
R^2 = Et, *i*-Pr

In the context of a program to identify selective adenosine A_{2B} receptor antagonists, we have obtained a focused library of 4-substituted 3,4-dihydropyrimidin-2(1*H*)-ones and its affinity for the four human adenosine receptor subtypes was determined. The synthesis was accomplished by using an experimentally simple and efficient Biginelli approach. The biological evaluation of the library revealed that all the documented derivatives exhibit low or negligible affinity for the A_{2B} receptor, thus highlighting the critical importance of the substituent at position 4 of the 3,4-dihydropyrimidin-2(1*H*)-one chemotype.

Keywords: 3,4-dihydropyrimidin-2(1*H*)-ones, adenosine antagonists, A_{2B} adenosine receptor, A_{2B} antagonists, structure–activity relationship.

Adenosine, an endogenous nucleoside released from almost all mammalian cells,[1] is structurally and metabolically related to the bioactive nucleotides,[2] RNA and the coenzymes A, NAD and FAD.[2] Adenosine is also a key signalling molecule, that mediates a plethora of physiological effects through the stimulation of a family of G-protein-coupled receptors (GPCRs) named adenosine receptors (ARs: A_1, A_{2A}, A_{2B}, and A_3).[3] Human ARs, exhibit high sequence homology[4] and differ in their affinity for adenosine,[5] the localization, and the G-protein-mediated downstream signalling pathways. Adenosine receptors have proved to participate in diverse physiological processes,[1,2,6] being considered attractive drug targets in several pathophysiological events.[6–9]

The A_{2B} adenosine receptor (A_{2B}AR) is ubiquitously expressed in the body[10–11] and remains the poorer characterized AR. Recent evidences confirm that the A_{2B}AR is implicated in major biological processes (e.g., vascular tone, cardiac myocyte contractility, glucose homeostasis, pulmonary inflammation, inflammatory response, and pain).[11] Whereas the interest in A_{2B}AR signalling is currently increasing significantly,[11] the compendium of ligands that selectively target the A_{2B}AR remains scarce.[12] The pursuit of A_{2B}AR antagonists has traditionally focused on the pharmacomodulation of naturally occurring xanthines (Fig. 1, compounds **1**–**5**).[7–9] In clear contrast, very few examples of potent, selective, and structurally simple non-xanthine A_{2B}AR antagonists have been described (Fig. 1, compounds **6**–**11**).[6–9,11,12] Accordingly, there is a demand for novel scaffolds representative of diverse topologies, physicochemical features, and binding modes. In this context, we recently reported the discovery of a novel

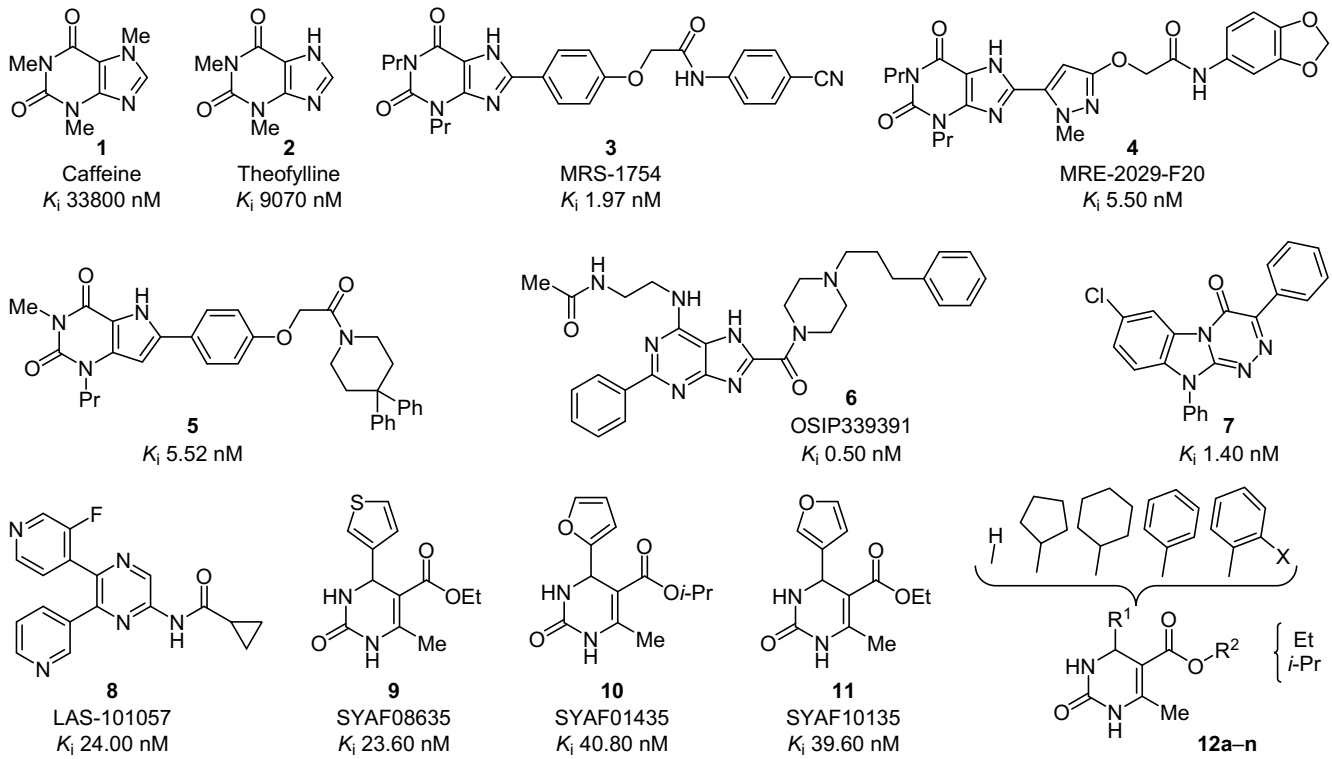

Figure 1. Representative A_{2B}AR antagonists (compounds **1–8**), model A_{2B}AR antagonists (compounds **9**–**11**) and herein targeted compounds **12a**–**n**.

family of A_{2B}AR antagonists based on the 3,4-dihydropyrimidin-2(1*H*)-one scaffold.[13] As part of this study we have demonstrated the excellent pharmacological profile of this novel chemotype, preliminarily exploring the SAR at positions 1, 2, 5, and 6. In the frame of this program, we herein document the synthesis of a collection of 3,4-dihydropyrimidin-2(1*H*)-ones **12** designed to evaluate the effect of substituting the heterocyclic ring at position 4 of the scaffold by different groups.

With the aim to investigate the effect produced by the replacement of the pentagonal heterocyclic moieties (e.g., 2- or 3-furan and 2- or 3-thiophene) at position 4 of previously identified hit compounds (Fig. 1, compounds **9**–**11**) we designed a small library of 3,4-dihydropyrimidin-2(1*H*)-ones (compounds **12**). The 3,4-dihydropyrimidin-2(1*H*)-ones **12a**–**b** (R^1 = H) would allow to evaluate the real contribution of the substituent at position 4 during the interaction with the A_{2B} receptor. In a similar fashion, the introduction of cyclopentyl (Cyp) and cyclohexyl (Cy) rings, phenyl and 2-substituted phenyl groups was undertaken to explore new substituents providing different topologies, physicochemical properties, and binding modes. In this study, we retained the most advantageous substituents at positions 2 (O), 3 (H), 6 (Me), and 5 (CO_2Et or CO_2*i*-Pr).

The required 4-substituted 3,4-dihydropyrimidin-2(1*H*)-ones **12a**–**n** were assembled by following environmentally friendly protocol[14] using the highly reliable Biginelli reaction,[15] thus demonstrating the potential of the multicomponent reactions as preparative tools in drug discovery.[16] The synthetic transformations are depicted in Scheme 1. Briefly, the reaction of urea (**15**), the corresponding carbaldehyde **13a**–**g**, the selected alkyl acetoacetate **14a**,**b**, and $ZnCl_2$ were heated at 70°C in THF for 12 h to afford the targeted 3,4-dihydropyrimidin-2(1*H*)-ones **12a**–**n** in satisfactory to excellent yields (Table 1).

The pharmacological profiles of compounds **12** obtained were studied *in vitro* at the four human adenosine receptor subtypes using radioligand-binding assays.[13,17] The affinities for the four human adenosine receptor subtypes (A_1, A_{2A}, A_{2B}, and A_3) of the 4-substituted 3,4-dihydropyrimidin-2(1*H*)-ones **12a**–**n** were determined *in vitro* with radioligand binding assays according to experimental protocols described by our group[13,17] and reported in Table 1. In the previous[13] and current study, all compounds were evaluated as racemic mixtures. Human adenosine receptors expressed in transfected CHO (A_1AR), HeLa (A_{2A}AR and A_3AR), and HEK-293 (A_{2B}AR) cells were employed. [^{3}H]-1,3-Dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) for A_1AR and A_{2B}AR, [^{3}H]ZM241385 for A_{2A}AR, and [^{3}H]NECA for A_3AR were employed as radioligands in binding assays. The biological data are expressed as K_i ± SEM in nM (n = 3) or percentage of inhibition of specific binding at 1 mM (n = 2, average) for those compounds that did not fully displace radioligand.

Examination of the binding data obtained for herein documented compounds (Table 1) revealed that all synthesized 4-substituted 3,4-dihydropyrimidin-2(1*H*)-ones were deprived of any significant affinity for the A_{2B}AR. The obtained data unequivocally confirms the relevant role exercised by pentagonal heterocyclic cores (i.e., 2- or 3-furan or 3-thiophene)[13,17] at position 4 during the interaction with the A_{2B}AR. Such a role was early detected when comparing

Scheme 1

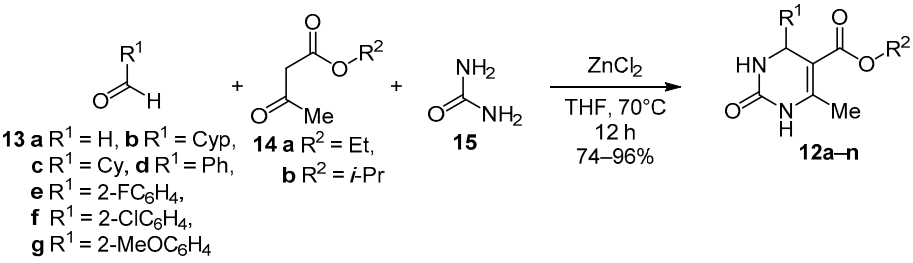

Table 1. Structure and binding data for 3,4-dihydropyridin-2(1*H*)-ones **12a**–**n** at the human adenosine receptors

Compound	R^1	R^2	Yield, %	K_i at 1 mM			
				hA_1**	hA_{2A}***	hA_{2B}*[4]	hA_3*[5]
12a[18]	H	Et	74	1%	3%	3%	1%
12b*	H	*i*-Pr	76	2%	5%	1%	2%
12c*	Cyp	Et	74	3%	1%	2%	18%
12d*	Cyp	*i*-Pr	76	1%	1%	3%	7%
12e[19]	Cy	Et	81	4%	10%	2%	11%
12f*	Cy	*i*-Pr	84	2%	8%	5%	9%
12g[20]	Ph	Et	96	1%	10%	2%	2%
12h[21]	Ph	*i*-Pr	93	2%	1%	2%	2%
12i[19]	2-FC_6H_4	Et	91	26%	10%	54%	42%
12j*	2-FC_6H_4	*i*-Pr	93	12%	3%	38%	18%
12k[22]	2-ClC_6H_4	Et	87	37%	6%	1321 nM	50%
12l[23]	2-ClC_6H_4	*i*-Pr	92	11%	13%	32%	19%
12m[24]	2-$MeOC_6H_4$	Et	90	31%	18%	4117 nM	40%
12n*	2-$MeOC_6H_4$	*i*-Pr	91	1%	4%	42%	12%
DPCPX*[6]	–	–	–	2.2 ± 0.2	157 ± 3	73.2 ± 2	1722 ± 11
ZM241385*[7]	–	–	–	683 ± 4.1	1.9 ± 0.1	65.7 ± 1	863 ± 4

* New compound.

** Displacement of specific [^{3}H]DPCPX binding in human CHO cells expressed as K_i ± SEM in nM (n = 3) or percentage displacement of specific binding at a concentration of 1 mM (n = 2).

*** Displacement of specific [^{3}H]-4-{2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl}phenol binding in human HeLa cells expressed as K_i ± SEM in nM (n = 3) or percentage displacement of specific binding at a concentration of 1 mM (n = 2).

*[4] Displacement of specific [^{3}H]DPCPX binding in human HEK-293 cells expressed as K_i ± SEM in nM (n = 3) or percentage displacement of specific binding at a concentration of 1 mM (n = 2).

*[5] Displacement of specific [^{3}H]NECA binding in human HeLa cells expressed as K_i ± SEM in nM (n = 3) or percentage displacement of specific binding at a concentration of 1 mM (n = 2).

*[6] DPCPX: 1,3-dipropyl-8-cyclopentylxanthine, potent and selective A_1 antagonist standard.

*[7] ZM241385: 4-{2-[7-amino-2-(2-furyl)[1,2,4]triazolo[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl}phenol, potent and selective A_{2A} antagonist standard.

the excellent data experienced (K_i 23–40 nM) for the reference compounds (Fig. 1, compounds **9**–**11**)[13,17] and the negligible affinity observed for compounds **12a**–**b** (R^1 = H).

Examination of the binding data of the 3,4-dihydropyrimidin-2(1*H*)-ones bearing cyclopentyl, cyclohexyl, and phenyl groups at position 4 (Table 1, compounds **12c**–**f** and **12g**–**h**) reinforced the idea that the pentagonal heterocyclic cores are the optimal substituents for this position. As observed, the only 3,4-dihydropyrimidin-2(1*H*)-ones exhibiting a modest affinity (micromolar) are those bearing a 2-substituted phenyl group at position 4 (i.e., compounds **12i**–**n**), with ethyl 4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**12k**) and ethyl 4-(2-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**12m**) being the most attractive ligands identified during this work. In any case, these ligands probed to be 57- and 179-fold less potent that model ligand **9**, respectively (Fig. 1). An additional observation emerging from the obtained data highlights that the synthesized ligands show a similar low affinity profile irrespectively of the alkoxy residue (EtO, *i*-PrO) present at the ester moiety, a fact that highlights the prominent influence of the residue at position 4 during the interaction of the 3,4-dihydropyrimidin-2(1*H*)-one scaffold with the A_{2B}AR. In addition, to reinforce the role of the pentagonal heterocyclic ring, the obtained data corroborate the binding modes proposed for model compounds **9**–**11** in previous papers.[13,17]

In summary, a focused library of 4-substituted 3,4-dihydropyrimidin-2(1*H*)-ones was synthesized and their affinity for the four human adenosine receptor subtypes (A_1, A_{2A}, A_{2B},

and A_3) determined *in vitro* with radioligand binding assays. The synthesis of the new derivatives was accomplished in satisfactory to excellent yields by using an experimentally simple and efficient Biginelli approach. The biological evaluation of the library revealed that all the documented derivatives exhibit low or negligible affinity at A_{2B}AR. The reported data highlight the critical importance of the substituent at position 4 of the 3,4-dihydropyrimidin-2(1*H*)-one scaffold during its interaction with the A_{2B}AR. Novel studies are currently in progress in our laboratories to explore the biological activity of novel 3,4-dihydropyrimidin-2(1*H*)-ones bearing heterocyclic scaffolds at position 4.

Experimental

^{1}H and ^{13}C NMR spectra were recorded on Bruker AM300 (300 MHz) spectrometer using TMS as internal standard. Due to long relaxation times of the quaternary carbon atoms of some compounds, particular signals of the quaternary carbon atoms were hardly observed. High-resolution mass spectra were obtained on an Autospec Micromass spectrometer (EI, 70 eV). Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. The reactions were monitored by thin-layer chromatography (TLC) on 2.5 mm Merck silica gel GF 254 strips, and the purified compounds each showed a single spot unless stated otherwise, UV light and/or iodine vapor were used to detect compounds. The Biginelli reactions were performed in coated Kimble vials on a PLS Organic Synthesizer with orbital stirring. Filtration and washing protocols for supported reagents were performed in a 12-channel vacuum manifold. Purification of isolated products was carried out by column chromatography (Kieselgel 0.040–0.063 mm, E. Merck) or medium pressure liquid chromatography on a CombiFlash Companion (Teledyne ISCO) with RediSep prepacked normal-phase silica gel (35–60 μm) columns followed by recrystallization.

Unless otherwise indicated, all starting materials, reagents, and solvents were purchased and used without further purification.

The Biginelli synthesis of 4-substituted 3,4-dihydropyrimidin-2(1*H*)-ones 12a–n (General method). A mixture of urea **15** (0.180 g, 3.0 mmol), the corresponding carbaldehyde **13** (2.0 mmol), alkyl acetoacetate **14** (2.0 mmol), and $ZnCl_2$ (0.027 g, 0.2 mmol) in THF (3 ml) was stirred at 70°C for 12 h. After completion of the reaction, as indicated by TLC, the reaction mixture was poured onto crushed ice and stirred for 5–10 min. The solid separated was filtered under vacuum, washed with ice-cold water (20 ml), dried in vacuum, purified by column chromatography (*n*-hexane–EtOAc, 4:1) on silica gel, and then recrystallized from EtOH.

(±)-Ethyl 6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12a).[18] Yield 273 mg (74%), white solid, mp 241–242°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.19 (3H, t, *J* = 7.0, $OCH_2C\underline{H}_3$); 2.15 (3H, s, CH_3); 3.86 (2H, s, CH_2); 4.03 (2H, q, *J* = 7.0, $OC\underline{H}_2CH_3$); 6.95 (1H, br. s, NH); 8.87 (1H, br. s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.2; 17.4; 43.2; 59.0; 94.5; 148.5; 152.8; 165.5.

(±)-Isopropyl 6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12b). Yield 301 mg (76%), white solid, mp 168–169°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.26 (3H, d, *J* = 1.8) and 1.29 (3H, d, *J* = 1.9, $OCH(C\underline{H}_3)_2$); 2.27 (3H, s, 6-CH_3); 3.84 (2H, s, CH_2); 4.97 (1H, dq, *J* = 12.7, *J* = 6.4, $OC\underline{H}(CH_3)_2$); 6.74 (1H, br. s, NH); 8.84 (1H, br. s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.2; 17.3; 44.2; 60.2; 97.1; 147.7; 151.9; 166.1. Found, *m*/*z*: 198.1007 $[M]^+$. $C_9H_{14}N_2O_3$. Calculated, *m*/*z*: 198.1004.

(±)-Ethyl 4-cyclopentyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12c). Yield 373 mg (74%), white solid, mp 142–143°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.28 (3H, t, *J* = 7.1, CH_3); 1.40–1.81 (8H, m, H Cyp); 2.00–2.20 (1H, m, H Cyp); 2.27 (3H, s, CH_3); 4.17 (2H, q, *J* = 7.0, $OC\underline{H}_2CH_3$); 4.31 (1H, dd, *J* = 5.5, *J* = 3.8, 6-CH); 5.86 (1H, br. s, NH); 7.92 (1H, br. s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 13.9; 16.4; 25.8; 25.9; 31.6; 31.7; 42.8; 54.7; 61.6; 96.9; 150.7; 154.9; 168.4. Found, *m*/*z*: 252.1476 $[M]^+$. $C_{13}H_{20}N_2O_3$. Calculated, *m*/*z*: 252.1474.

(±)-Isopropyl 4-cyclopentyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12d). Yield 405 mg (76%), white solid, mp 138–139°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.25 (3H, d, *J* = 1.8) and 1.27 (3H, d, *J* = 1.8, $OCH(C\underline{H}_3)_2$); 1.43–1.78 (8H, m, H Cyp); 2.01–2.18 (1H, m, H Cyp); 2.27 (3H, s, CH_3); 4.29 (1H, dd, *J* = 5.8, *J* = 3.7, 6-CH); 4.99–5.12 (1H, m, $OC\underline{H}(CH_3)_2$); 5.87 (1H, br. s, NH); 7.86 (1H, br. s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 16.5; 21.6; 25.8; 31.6; 31.7; 43.5; 54.6; 71.2; 98.4; 153.3; 154.7; 167.2. Found, *m*/*z*: 266.1633 $[M]^+$. $C_{14}H_{22}N_2O_3$. Calculated, *m*/*z*: 266.1630.

(±)-Ethyl 4-cyclohexyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12e).[19] Yield 431 mg (81%), white solid, mp 138–139°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.96–1.21 (5H, m, H Cy); 1.28 (3H, t, *J* = 7.1, $OCH_2C\underline{H}_3$); 1.42–1.56 (3H, m, H Cy); 1.58–1.81 (3H, m, H Cy); 2.28 (3H, s, CH_3); 4.05–4.28 (3H, m, $OC\underline{H}_2CH_3$, 6-CH); 5.67 (1H, br. s, NH); 7.83 (1H, br. s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 14.1; 16.6; 25.7; 25.8; 26.3; 29.6; 29.7; 40.8; 55.6; 60.7; 96.9; 151.6; 153.9; 169.5. Found, *m*/*z*: 266.1632 $[M]^+$. $C_{14}H_{22}N_2O_3$. Calculated, *m*/*z*: 266.1630.

(±)-Isopropyl 4-cyclohexyl-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12f). Yield 471 mg (84%), white solid, mp 134–135°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 0.95–1.21 (5H, m, H Cy); 1.24 (3H, d, *J* = 1.8) and 1.27 (3H, d, *J* = 1.8, $OCH(C\underline{H}_3)_2$); 1.42–1.56 (3H, m, H Cy); 1.58–1.81 (3H, m, H Cy); 2.28 (3H, s, CH_3); 4.18 (1H, t, *J* = 3.5, 6-CH); 5.05 (1H, dq, *J* = 12.7, *J* = 6.4, $OC\underline{H}(CH_3)_2$); 5.53 (1H, br. s, NH); 7.45 (1H, br. s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 16.5; 22.5; 22.6; 25.1; 25.2; 25.7; 29.6; 29.7; 41.8; 54.3; 71.1; 98.4; 152.4; 153.8; 166.3. Found, *m*/*z*: 280.1791 $[M]^+$. $C_{15}H_{24}N_2O_3$. Calculated, *m*/*z*: 280.1787.

(±)-Ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12g).[20] Yield 499 mg (96%), white solid, mp 209–211°C. ^{1}H NMR spectrum (DMSO-d_6),

δ, ppm (*J*, Hz): 1.05 (3H, t, *J* = 6.9, $OCH_2C\underline{H}_3$); 2.24 (3H, s, CH_3); 3.96 (2H, q, *J* = 6.9, $OC\underline{H}_2CH_3$); 5.46 (1H, s, 6-CH); 7.17–7.30 (5H, m, H Ar); 7.74 (1H, s, NH); 9.23 (1H, s, NH).

(±)-Isopropyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12h).[21] Yield 510 mg (93%), white solid, mp 198–200°C. ^{1}H NMR spectrum ($CDCl_3$), δ, ppm (*J*, Hz): 1.24 (3H, d, *J* = 1.9) and 1.26 (3H, d, *J* = 1.8, $OCH(C\underline{H}_3)_2$); 2.34 (3H, s, CH_3); 4.90–5.01 (1H, m, $OC\underline{H}(CH_3)_2$); 5.38 (1H, d, *J* = 2.7, CH); 5.65 (1H, s, NH); 7.19–7.37 (5H, m, H Ar); 7.88 (1H, s, NH).

(±)-Ethyl 4-(2-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12i).[19] Yield 506 mg (91%), white solid, mp 234–236°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 1.20 (3H, t, *J* = 7.1, $OCH_2C\underline{H}_3$); 2.31 (3H, s, CH_3); 4.07 (2H, q, *J* = 7.1, $OC\underline{H}_2CH_3$); 5.62 (1H, s, 6-CH); 7.28–7.48 (4H, m, H Ar); 7.87 (1H, s, NH); 9.43 (1H, s, NH).

(±)-Isopropyl 4-(2-fluorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12j).[22] Yield 543 mg (93%), white solid, mp 168–169°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 1.27 (3H, d, *J* = 1.9) and 1.29 (3H, d, *J* = 1.9, $OCH(C\underline{H}_3)_2$); 2.32 (3H, s, CH_3); 5.08–5.12 (1H, m, $OC\underline{H}(CH_3)_2$); 5.39 (1H, s, 6-CH); 7.21–7.36 (4H, m, H Ar); 7.80 (1H, s, NH); 9.38 (1H, s, NH).

(±)-Ethyl 4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12k).[23] Yield 513 mg (87%), white solid, mp 213–215°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 1.08 (3H, t, *J* = 7.5, $OCH_2C\underline{H}_3$); 2.32 (3H, s, CH_3); 3.91 (2H, q, *J* = 7.5, $OC\underline{H}_2CH_3$); 5.04 (1H, s, 6-CH); 7.16–7.23 (4H, m, H Ar); 7.72 (1H, s, NH); 9.30 (1H, s, NH).

(±)-Isopropyl 4-(2-chlorophenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12l). Yield 568 mg (92%), mp 168–169°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 1.21 (3H, d, *J* = 1.9) and 1.24 (3H, d, *J* = 1.9, $OCH(C\underline{H}_3)_2$); 2.31 (3H, s, CH_3); 5.11–5.15 (1H, m, $OC\underline{H}(CH_3)_2$); 5.60 (1H, d, *J* = 2.6, 6-CH); 7.18–7.29 (4H, m, H Ar); 7.70 (1H, s, NH); 9.34 (1H, s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 16.4; 21.7; 21.8; 50.8; 71.2; 97.8; 125.7; 129.7; 130.4; 131.7; 133.5; 138.7; 155.8; 157.6; 163.8. Found, *m*/*z*: 308.0931 $[M]^+$. $C_{15}H_{17}ClN_2O_3$. Calculated, *m*/*z*: 308.0928.

(±)-Ethyl 4-(2-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12m).[24] Yield 522 mg (90%), white solid, mp 260–262°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 1.08 (3H, t, *J* = 7.5, $OCH_2C\underline{H}_3$); 2.30 (3H, s, CH_3); 3.80 (3H, s, OCH_3); 3.93 (2H, q, *J* = 7.5, $OC\underline{H}_2CH_3$); 5.50 (1H, d, *J* = 3.0, 6-CH); 6.87–7.05 (3H, m, H Ar); 7.14–7.21 (2H, m, NH, H Ar); 9.10 (1H, br. s, NH).

(±)-Isopropyl 4-(2-methoxyphenyl)-6-methyl-2-oxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (12n). Yield 554 mg (91%), white solid, mp 239–241°C. ^{1}H NMR spectrum (DMSO-d_6), δ, ppm (*J*, Hz): 1.21 (3H, d, *J* = 2.0) and 1.24 (3H, d, *J* = 2.1, $OCH(C\underline{H}_3)_2$); 2.31 (3H, s, CH_3); 3.86 (3H, s, OCH_3); 5.04–5.09 (1H, m, $OC\underline{H}(CH_3)_2$); 5.46 (1H, d, *J* = 3.1, 6-CH); 6.85–7.08 (3H, m, H Ar); 7.11–7.23 (2H, m, NH, H Ar); 9.12 (1H, br. s, NH). ^{13}C NMR spectrum ($CDCl_3$), δ, ppm: 16.5; 22.1; 22.2; 47.2; 55.4; 70.4; 97.6; 110.8; 121.1; 127.2; 129.9; 130.9; 155.2; 156.4; 157.6; 163.8. Found, *m*/*z*: 304.1427 $[M]^+$. $C_{16}H_{20}N_2O_4$. Calculated, *m*/*z*: 304.1423.

Pharmacology. Radioligand binding competition assays were performed *in vitro* using A_{2A} and A_{2B} human receptors expressed in transfected HeLa (*h*A_{2A}) and HEK-293 (*h*A_{2B}) cells as described previously.[17] A brief description is given below. Adenosine A_1 receptor competition binding experiments were carried out in membranes from CHO–A_1 cells labelled with 2 nM [^{3}H]DPCPX. Nonspecific binding was determined in the presence of 10 μM R-PIA. The reaction mixture was incubated at 25°C for 60 min. Adenosine A_{2A} receptor competition binding experiments were carried out in membranes from HeLa–A_{2A} cells labelled with 3 nM [^{3}H]ZM241385. Nonspecific binding was determined in the presence of 50 μM NECA. The reaction mixture was incubated at 25°C for 30 min. Adenosine A_{2B} receptor competition binding experiments were carried out in membranes from HEK-293–A_{2B} cells (Euroscreen, Gosselies, Belgium) labelled with 35 nM [^{3}H]DPCPX. Nonspecific binding was determined in the presence of 400 μM NECA. The reaction mixture was incubated at 25°C for 30 min. Adenosine A_3 receptor competition binding experiments were carried out in membranes from HeLa–A_3 cells labelled with 30 nM [^{3}H]NECA. Nonspecific binding was determined in the presence of 100 μM R-PIA. The reaction mixture was incubated at 25°C for 180 min. cAMP assays were performed at adenosine receptors transfected using a cAMP enzyme immunoassay kit (Amersham Biosciences). HEK-293 cells were seeded (10000 cells/well) in 96-well culture plates and incubated at 37°C in an atmosphere with 5% CO_2 in Eagle's Medium Nutrient Mixture F-12 (EMEM F-12), containing 10% foetal calf serum (FCS) and 1% L-glutamine. Cells were washed with 3 × 200 μl assay medium (EMEM-F12 and 25 mM HEPES pH 7.4) and preincubated with assay medium containing 30 μM rolipram and test compounds at 37°C for 15 min. 10 μM NECA was incubated for 15 min at 37°C (total incubation time 30 min). Reaction was stopped with lysis buffer supplied in the kit and the enzyme immunoassay was carried out for detection of intracellular cAMP at 450 nm in an Ultra Evolution detector (Tecan). Data analysis: IC_{50} values were obtained by fitting the data with non-linear regression using Prism 2.1 software (GraphPad, San Diego, CA). For those compounds that showed either little affinity or poor solubility a percentage inhibition of specific binding is reported. Results are the mean of 3 experiments (n = 3) each performed in duplicate. The experimental details and dose-response curves obtained during the functional assays are described in the supporting information.

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