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1,3-Dipropyl-8-(1-phenylacetamide-1*H*-pyrazol-3-yl)-xanthine derivatives as highly potent and selective human A_{2B} adenosine receptor antagonists

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Abstract—A new series of 1,3-dipropyl-8-(1-phenylacetamide-1*H*-pyrazol-3-yl)-xanthine derivatives has been identified as potent A_{2B} adenosine receptor antagonists. The products have been evaluated for their binding affinities for the human A_{2B}, A_1, A_{2A}, and A_3 adenosine receptors. *N*-(4-chloro-phenyl)-2-[3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl] (**11c**) showed a high affinity for the human A_{2B} adenosine receptor K_i = 7 nM and good selectivity (A_1, A_{2A}, A_3/A_{2B} > 140). Synthesis and SAR of this novel class of compounds is presented herein.

1. Introduction

The biological activity of adenosine occurs through the activation of specific receptors located on cell membranes and belonging to the extensive family of G-protein coupled receptors.[1,2] Adenosine activates four subtypes of receptors (ARs) named as A_1, A_{2A}, A_{2B}, and A_3.[1,3] Adenosine receptors are transmembrane G-protein coupled with approximately 21–28 amino acids in each transmembrane region. The N-terminus of the receptor is extracellular and all receptors, with the exception of the A_{2A} adenosine receptor, have a palmitoylation site near the C-terminus.[4] While A_1 and A_3 receptors are coupled to G_i-protein, A_{2A} and A_{2B} adenosine receptors are linked to adenylate cyclase-stimulatory G_s-proteins.[1] Reverse transcription coupled to the polymerase chain reaction (RT-PCR) and in situ hybridization of rat tissue demonstrates low levels of A_{2B} receptor in all brain regions tested,[5] whereas, high levels can be found in the cecum, large intestine, and urinary bladder. Lower levels of the A_{2B} adenosine receptor can be detected in spinal cord, vas deferens, pituitary, in a variety of skin cells, and fibroblasts.[6] It has been reported the presence of A_{2B} receptors in HMC-1 cells (human mast cells). Several compounds have been studied in this cell line and evaluated for their potency to decrease NECA-stimulated cAMP production showing a high potency.[7] It has been also investigated the expression and functional coupling of A_{2B} receptors in peripheral blood cells by using a novel selective antagonist radioligand [^{3}H]MRE-2029F20.[8,9]

The A_{2B} adenosine receptor has been also implicated in several biological events. This receptor plays a role in mediating vasodilatation in some vessels, such as guinea pig and mouse aorta.[10,11] Inhibition of growth of rat aortic smooth muscle cells has been achieved through selective A_{2B} receptor activation and appears to be secondary to blockade of the mitogen-activated protein kinase (MAPK) pathway.[12,13] Recently it has been revealed the presence of A_{2B} adenosine receptors in human lung peripheral parenchyma to investigate the

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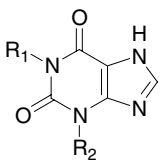
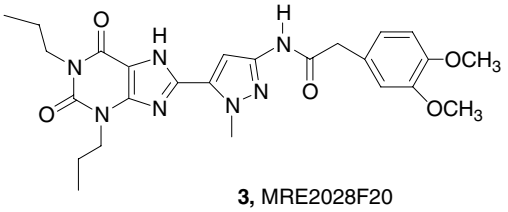
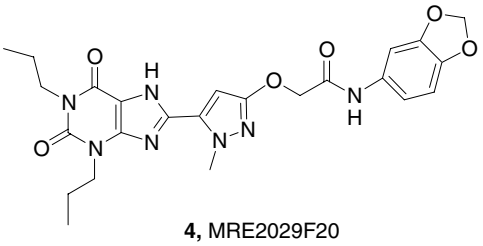


1, Theophylline: $R_1 = R_2 =$ Methyl
2, Enprophylline: $R_1 = H$, $R_2 =$ propyl



3, MRE2028F20
K_i (hA_{2B}) = 38 nM, K_i (hA_1, hA_{2A}, hA_3) > 1000 nM



4, MRE2029F20
K_i (hA_{2B}) = 5.5 nM, K_i (hA_1) = 200 nM, (hA_{2A}, hA_3) > 1000nM

Figure 1. Representative structures of xanthines as A_{2B} adenosine receptor antagonists.

expression, affinity, and density of adenosine receptors in peripheral lung parenchyma from age-matched smokers with chronic obstructive pulmonary disease (COPD) and smokers with normal lung function.[14] In addition a role of adenosine in asthma has been demonstrated on the basis of key experimental evidence such as the increase of adenosine concentration in hypoxia and cellular inflammation in bronchoalveolar fluids of asthmatics and in plasma (upon contact with allergens).[15,16] Moreover, adenosine (in the form of AMP) induces bronchoconstriction in asthmatics, but not in healthy individuals.[17,18] Finally, theophylline, a dimethylxanthine with a well-established role in the therapy of asthma, was shown to block selectively the AMP-induced bronchoconstriction.[16] The bronchodilating activity of theophylline **1** and its structural analog enprofylline **2** (Fig. 1) recently has been attributed to a selective, albeit small, antagonism at the A_{2B}AR.[15] These findings prompted several groups to design and test a large number of xanthine derivatives in the search for new, more potent, and A_{2B}-selective ligands.

We have recently discovered a number of 8-heterocycle-substituted xanthine derivatives as potent and selective A_{2B}AR antagonists.[19] These MRE compounds (Fig. 1) shared high affinity for the A_{2B} adenosine receptor. The dimethoxy compound MRE2028F20 (**3**) had a very high selectivity and affinity of 38 nM. The highest affinity was obtained with MRE2029F20 (**4**), $K_i = 5.5$ nM combined with strong selectivity (>36-fold) with respect to the other three adenosine receptor subtypes.

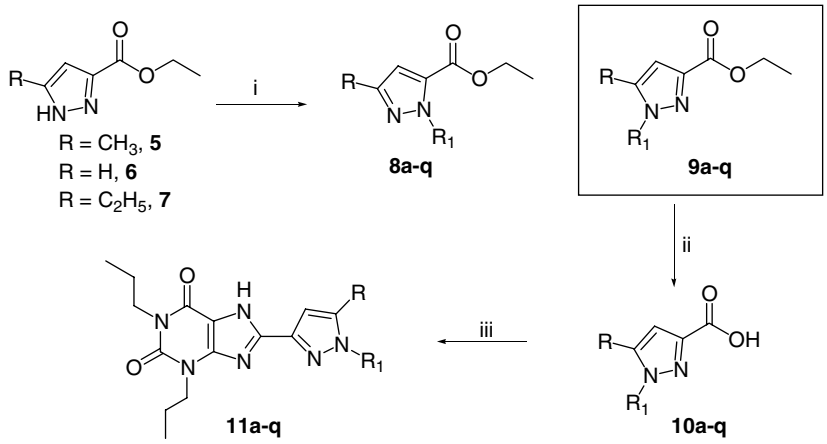
Herein we report some of the SAR we developed and the characterization of a new series of compounds (**11a–q**) obtained by replacement of the side chain from the 3-position of the pyrazole ring to the 1-position. Next to these derivatives, we have also prepared analogs **16a–c** to investigate the presence of a halogen atom at the 4-position of pyrazole ring.

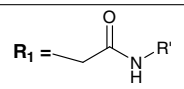
The affinity of the synthesized products was evaluated in human CHO cells by using specific radioligands of A_{2B}, A_1, A_{2A}, and A_3 receptors; all compounds were tested for the inhibition of NECA-induced cAMP accumulation mediated by the adenosine A_{2B} receptor.

2. Chemistry

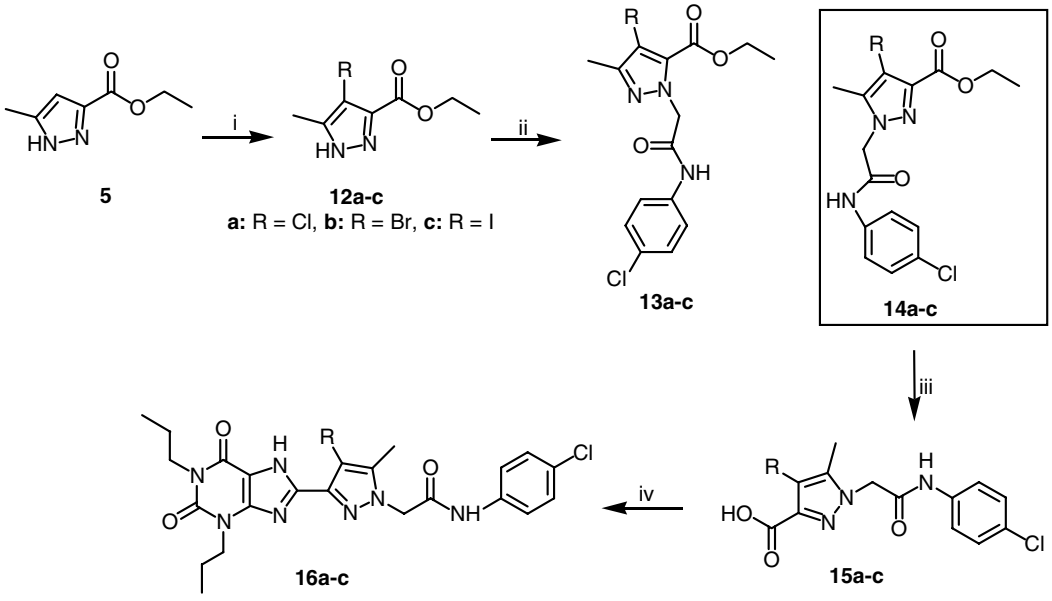
The synthetic routes to obtain the target compounds **11a–q** are outlined in Scheme 1. Appropriately substituted anilines are acylated with bromoacetyl bromide to provide the corresponding bromoacetanilides. *N*-alkylation of pyrazoles **5–7** in anhydrous ethanol with bromoacetanilides in the presence of sodium ethoxide gave an approximately 1:4 mixture of the N^1-3/N^1-5-(un)substituted pyrazoles (compounds **8a–q** and **9a–q**). The two isomers were separated by flash chromatography. Hydrolysis of pyrazole ethyl esters **9a–q** in 2N KOH yielded the corresponding carboxylic acids **10a–q**. The coupling reaction of derivatives **10a–q** with 1,3-dipropyl-5,6-diaminouracil was achieved by a two-step sequence involving reaction in methanol solution using 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDAC) as condensing agent, followed by ring closure in the presence of sodium hydroxide at 70 °C yielding the target xanthines **11a–q**. The low yields of this step are due to the partial hydrolysis of the amide group, which occurs during the cyclization in the presence of NaOH. The target compounds **16a–c** were synthesized by the same procedures except using the 4-halo-pyrazoles **12a–c**, which are prepared by halogenation of pyrazole ester **5** in the presence of the appropriate *N*-halo-succinamide (Scheme 2).

In Table 1 are reported the ^{13}C NMR signals of several alkyl pyrazoles examined. The comparison of the carbon chemical shifts of the two *N*-alkyl-pyrazole series (**8acd**, **14a**, **9acd**, **13a**) showed that the *C*-3 and *C*-5 signals of



Comp 11	R	R_1 =  R'
		R'
a	CH_3	phenyl
b	CH_3	4-F-phenyl
c	CH_3	4-Cl-phenyl
d	CH_3	4-Br-phenyl
e	CH_3	4-I-phenyl
f	CH_3	4-tolyl
g	CH_3	4-methoxy-phenyl
h	CH_3	4-*N*$(CH_3)_2$-phenyl
i	CH_3	4-*sec*-butyl-phenyl
j	CH_3	3-methoxy-phenyl
k	CH_3	3-Cl-phenyl
l	CH_3	3,4-dichloro-phenyl
m	CH_3	3,4-dimethoxy-phenyl
n	CH_3	3,4-dimethyl-phenyl
o	CH_3	1-naphtyl
p	CH_3CH_2	4-Cl-phenyl
q	H	4-Cl-phenyl

Scheme 1. Reagents and conditions: (i) appropriate bromoacetanilides, C_2H_5 ONa, C_2 H_5 OH, rt; (ii) KOH, dioxan temperature, (iii) a—1,3-dipropyl-5,6-diamino-uracil, EDAC, CH_3OH, room temperature; b—NaOH, 80 °C.



Scheme 2. Reagents and conditions: (i) N–Cl/Br/I-succinimide, DMF; (ii) 2-bromo-*N*-(4-chloro-phenyl)-acetamide, C_2H_5ONa,C_2H_5OH, room temperature; (iii) KOH, dioxane, rt; (iv) a—1,3-dipropyl-5,6-diamino-uracil, EDAC,CH_3OH, room temperature; b—NaOH, 80 °C.

Table 1. ^{13}C NMR parameters of several pyrazole isomers

Compound	C_3	C_5	C_4	N-CH_2
8a	146.55	133.13	110.91	54.95
9a	141.56	141.61	107.60	52.63
8d	149.60	135.80	112.30	54.86
9d	143.99	142.18	108.94	53.32
8c	149.40	135.18	111.70	54.96
9c	143.97	142.19	108.93	53.29
13a	144.86	137.44	112.80	55.70
14a	139.58	137.20	109.22	53.63

the 1-alkyl-3-methyl derivatives (**8acd**, **13a**), in accordance with Pugmire and Grant's principle,[20] resonate upfield and downfield, respectively, with respect to the corresponding signals in the spectrum of the 1-alkyl-5-methyl derivatives (**9acd**, **14a**). For the pyrazole derivatives **9acd**, **14a** the ^{13}C NMR resonance of *C*-5 is downfield of the corresponding signals in the spectrum of the N^1-3-methyl-pyrazole isomer. These data coincide with our previous studies.[21]

3. Results and conclusions

As depicted in Table 2 a number of selected analogs were prepared, showing good binding affinities for the A_{2B} receptor in the radioligand assay, as well as good selectivity versus A_1, A_{2A}, and A_3 ARs. Unsubstituted phenylacetamide chain on the *N*-position of pyrazole ring (compound **11a**) was found to show high potency and selectivity. The derivatives **11b–o** were prepared to study the effect of substituting the 3- and/or 4-position of the phenyl ring with electron-donating (EDG) and electron-withdrawing groups (EWG). The substitution at the *para*-position of the phenyl ring of **11a** with electron-withdrawing (EWG) groups, such as halogen atoms (**11b–e**), resulted in an increase of selectivity while retaining affinity at the A_{2B} receptor. The 4-chlorophenyl derivative **11c** was found to be the most potent (K_iA_{2B} = 7 nM) and selective compound within the series (A_1, A_{2A}, $A_3/A_{2B} > 140$). The substitution at the *para*-position of the phenyl ring of **11a** with electron-donating groups (EDG), such as CH_3 (**11f**, K_i = 56) and OCH_3 (**11g**, K_i = 12), also showed good affinity at the A_{2B}, retaining selectivity at AR subtypes (Table 2). Bulky lipophilic groups, such as the 4-*sec*-butyl (**11i**) and dimethylamino (**11h**) groups, led to a loss of affinity. Shifting of substituents from the *para* to the *meta* position of the phenyl ring resulted in compounds with lower binding affinity at the A_{2B} receptor compared to the corresponding *para*-chloro (**11c**) and *para*-methoxy (**11g**) analogs, (**11j**: *m*-methoxy K_i = 65) and (**11k**: *m*-chloro, K_i = 28). The *m*-methoxy derivative also showed a slight loss of selectivity versus the A_1 adenosine (hA_1 K_i = 480 nM). Disubstituted phenyl analogs which contain a 3,4-substituent (3,4-dichloro **11l**, dimethoxy **11m**, and dimethyl **11n** derivatives) were found to have lower A_{2B} AR affinity than mono-substituted analogs. In particular the dichloro and dimethoxy derivatives showed a 5- to 6-fold decrease of affinity compared to that of compounds **11c** and **11g** (Table 2). The replacement of the phenyl group on the *N*-position of acetamido-chain with a naphthyl ring resulted in compound **11o** which displayed a loss of affinity at ARs. Hence the analogs **11p** and **11q** were investigated to determine the effect of substitution at the 5-position of pyrazole ring in comparison to the reference compound **11c**. Introduction of an

Table 2. Adenosine receptor affinities and functional data of A_{2B} adenosine derivatives **11a–q**, **16a–c**

Compound	K_i (nM)				IC_{50} (nM)
	hA_1[a]	**hA_{2A}**[b]	**hA_3**[c]	**hA_{2B}**[d]	**hA_{2B}**[e]
11a	350 (275–482)	>1000	>1000	15 (10–21)	58 (45–74)
11b	500 (420–595)	>1000	>1000	14 (10–20)	68 (51–91)
11c	>1000	>1000	>1000	7.0 (5.0–9.0)	43 (31–60)
11d	>1000	>1000	>1000	35 (27–45)	156 (123–198)
11e	>1000	>1000	>1000	28 (18–43)	115 (84–159)
11f	>1000	>1000	>1000	56 (45–70)	212 (162–277)
11g	>1000	>1000	>1000	12 (8–18)	61 (44–84)
11h	>1000	>1000	>1000	>1000	>1000
11i	>1000	>1000	>1000	>1000	>1000
11j	480 (444–518)	>1000	>1000	65 (56–75)	266 (216–327)
11k	675 (569–800)	>1000	>1000	28 (19–41)	106 (81–139)
11l	>1000	>1000	>1000	40 (25–66)	188 (166–213)
11m	>1000	>1000	>1000	60 (44–82)	222 (203–242)
11n	>1000	>1000	>1000	48 (35–65)	200 (182–219)
11o	>1000	>1000	>1000	>1000	>1000
11p	>1000	>1000	>1000	>1000	>1000
11q	140 (123–159)	>1000	>1000	20 (15–26)	80 (63–100)
16a	>1000	>1000	>1000	222 (181–273)	>1000
16b	>1000	>1000	>1000	250 (205–306)	>1000
16c	>1000	>1000	>1000	>1000	>1000

[a] Displacement of specific [^{3}H]DPCPX binding at human A_1 receptors expressed in CHO cells (n = 3–6).
[b] Displacement of specific [^{3}H]ZM241385 binding at human A_{2A} receptors expressed in CHO cells (n = 3–6).
[c] Displacement of specific [^{3}H]MRE3008-F20 binding at human A_3 receptors expressed in CHO cells (n = 3–6).
[d] Displacement of specific [^{3}H] MRE2029F20 binding at human A_{2B} receptors expressed in HEK293 cells (n = 3–6).
[e] cAMP assay in CHO cells expressing human A_{2B} adenosine receptors IC_{50} (nM). Data are expressed as geometric means with 95% confidence limits.

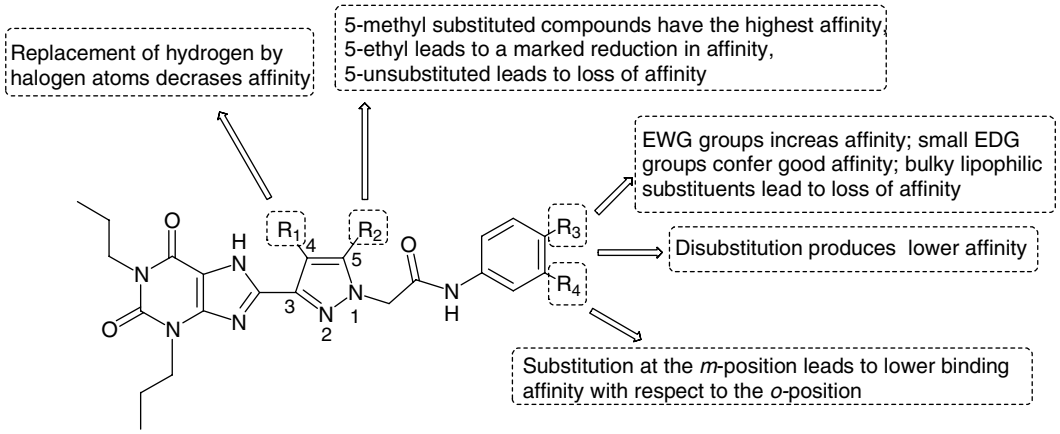


Figure 2. Illustration of structure–activity relationship of synthesized compounds.

ethyl moiety at the 5-position (**11p**) led to a loss of affinity and the derivative with a hydrogen atom at the same position (**11q**) displayed a 3-fold decrease of affinity and selectivity (hA_{2B} $K_i = 20$, hA_1 $K_i = 140$ nM) compared to that of compound **11c**. We also explored the effect of 4-substituted pyrazoles on A_{2B} AR affinity with the 4-halo-derivatives **16a**–**c**. This resulted in a loss of affinity for compound **16c** (4-I-derivative) and a decrease of affinity (30-fold) for analogs **16a** and **16b** relative to that of the unsubstituted derivative **11c**. The structure–activity relationship (SAR) of synthesized compounds is illustrated in Figure 2.

Figure 3A reports the competition curves of specific [^{3}H]-MRE 2029F20 binding to human A_{2B} receptors of new, potent, and selective A_{2B} adenosine antagonists showing affinity values in the range 7–40 nM. The log dose-response curves of cAMP accumulation for selected antagonists to human A_{2B} receptors representing the capability of the new compounds to block the stimulatory effect of 100 nM NECA on cAMP levels are shown in Figure 3B. All adenosine antagonists are able to inhibit cAMP accumulation, displaying an order of potency strictly similar to that observed in binding affinities. The new A_{2B} adenosine antagonists **11c** and **11g** are potent in binding ($K_i = 7$ and 12 nM, respectively) and in functional assays ($IC_{50} = 43$ and 61 nM, respectively). The comparison of K_i and IC_{50} values (Fig. 3C) indicates that an high correlation exists between data obtained from binding and functional assays ($r = 0.99$, $P < 0.01$).

4. Conclusion

Synthesis of a new class of 8-(pyrazol-3-yl) xanthine derivatives led to the identification of several A_{2B} AR antagonists with high affinity and very good selectivity for the adenosine receptor subtypes. 8-[5-Methyl-(4-chlorophenylacetamido-1*H*-pyrazol)-3-yl]-1,3-dipropylxanthine (**11c**) had the highest affinity and selectivity in this series that will be useful in understanding the physiological role of the A_{2B} AR, allowing for the targeted synthesis of potent and selective antagonist of the A_{2B} AR.

5. Experimental

5.1. General information

Reagent grade solvents were dried according to standard techniques. Sodium sulfate was used as a drying agent for water containing organic phases. Reactions were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{245} Merck plates). Chromatographic spots were visualized by UV light. Purification of crude compounds and separation of reaction mixtures were carried out by column chromatography on silica gel 60 (230–400 mesh from Merck) using appropriate eluents. Melting points (uncorrected) were determined in a Buchi-Tottoli melting point apparatus. Chemical shifts (*d*) are reported in parts per million (ppm) relative to the solvent central peak. ^{1}H NMR spectra were recorded at 200 MHz on a Bruker AC 200 spectrometer. Electrospray Ionization Mass Spectrometry (ESI/MS) was performed with an Agilent 1100 Series LC/MSD model in positive scan mode. The molecular weights from the MS spectra (not reported) were in full agreement with the proposed chemical structures of target compounds. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica University of Ferrara. All final products had satisfactory C, H, N analyses (within ±0.40 of theoretical values). Unambiguous assignment for all proton and carbon resonances of the two isomeric N^1-3/N^1-5-substituted or unsubstituted pyrazoles (compounds **8a**–**q** and **9a**–**q**) was achieved *via* ^{13}C NMR chemical shifts and *via* a strong NOE between *N*-CH_2 of the side chain and CH_3 on the 5 position of the pyrazole ring.

1*H*-Pyrazole-3-carboxylic acid ethyl ester **6** was commercially available. The 5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester **5** and 5-ethyl-1*H*-pyrazole-3-carboxylic acid ethyl ester **7** were prepared by cyclization of 2,4-dioxo-pentanoic- or -hexanoic acid ethyl esters with hydrazine salts in ethanol at reflux.

5.2. General synthesis of 2-bromo-*N*-phenylacetamide

A solution of aniline (0.016 mol) in 40 mL of dichloromethane was cooled on ice; 1.0 equiv of bromoacetyl

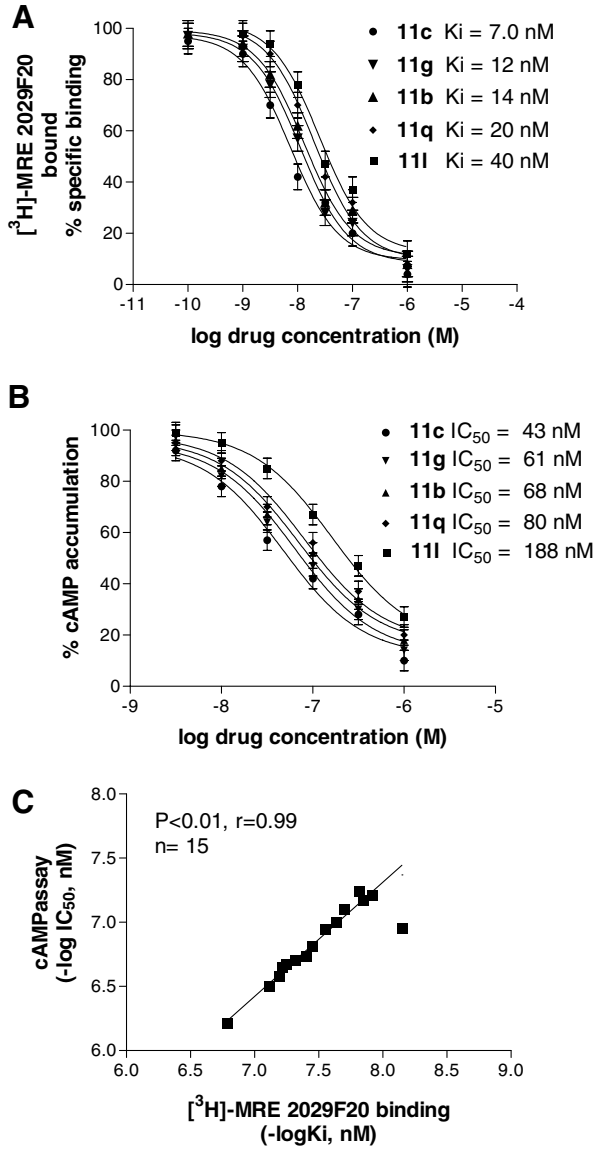


Figure 3. Affinity and potency of selected new A_{2B} adenosine antagonists: (A) Competition curves of specific [^{3}H]-MRE 2029F20 binding to human A_{2B} receptors; (B) Inhibition curves of cAMP accumulation to human A_{2B} receptors representing the capability of the antagonists to block the stimulatory effect of 100 nM NECA on cAMP levels. Curves are the mean of three independent experiments; (C) Comparison between affinity values (K_i) of [^{3}H]-MRE 2029F20 binding to human A_{2B} receptors and potency values (IC_{50}) obtained in cAMP assays to human A_{2B} receptors.

bromide in 3 mL of dichloromethane was added dropwise, followed by 0.019 mol of triethylamine. The solution turned dark and a precipitate formed, as the reaction mixture was warmed to ambient temperature over 3 h. The mixture was concentrated and taken up in ethyl acetate and extracted with water; the organic phase was dried over Na_2SO_4. After filtration and concentration, the solid was purified by crystallization (ethyl acetate), yield: 80–95%. All anilines are commercially available. Analytical data of bromo-phenyl-acetamides are reported in Table 3.

5.3. General procedure for preparation of 5-(alkyl)-1-(substituted)phenylcarbamoylmethyl-1*H*-pyrazole-3-carboxylic acid ethyl esters 8a–q and 9a–q

To a magnetically stirred mixture of pyrazole **5**–**7** (0.02 mol) and sodium ethoxide (0.22 mol) in anhydrous ethanol (20 mL) was added the appropriate 2-bromo-*N*-phenyl-acetamide (0.02 mol). The resulting suspension was stirred at room temperature for 3 h. After concentration in vacuo, EtOAc was added, and the resulting solution was washed twice with water and dried over Na_2SO_4. After filtration and concentration, the residue that contained the alkylated pyrazoles **8** and **9a**–**q** as a separable mixture of N^1-3- and N^1-5-substituted isomers (ratio approximately 1:4) was further purified by flash chromatography (ethyl acetate/hexane).

5.3.1. 5-Methyl-1-phenylcarbamoylmethyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9a). Yield: 65%; mp: 139–140 °C; ^{1}H NMR DMSO-d_6 δ 1.26–1.29 (t, 3H); 2.28 (s, 3H); 4.23–4.25 (q, 2H); 5.06 (s, 2H); 6.56 (s, 1H); 7.07 (m, 1H); 7.32 (m, 2H); 7.57 (m, 2H); 10.41 (s, 1H).

5.3.2. 1-[(4-Fluoro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9b). Yield: 62%; mp: 149–153 °C; ^{1}H NMR DMSO-d_6 δ 1.40–1.42 (t, 3H); 2.57 (s, 3H); 4.41–4.43 (q, 2H); 4.95 (s, 2H); 6.67 (s, 1H); 6.98 (m, 2H); 7.39 (m, 2H); 8.09 (s, 1H).

5.3.3. 1-[(4-Chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9c). Yield: 62%; mp: 216–218 °C; ^{1}H NMR DMSO-d_6 δ 1.37–1.39 (t, 3H); 2.38 (s, 3H); 4.38 (q, 2H); 5.07 (s, 2H); 6.65 (s, 1H); 7.18 (d, 2H); 7.33 (d, 2H); 8.78 (s, 1H).

5.3.4. 1-[(4-Bromo-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9d). Mp: Yield: 62%; 195–198 °C; ^{1}H NMR DMSO-d_6 δ1.37 (t, 3H); 2.38 (s, 3H); 4.39 (q, 2H); 5.06 (s, 2H); 6.65 (s, 1H); 7.27 (m, 2H); 7.32 (m, 2H); 8.77 (s, 1H).

5.3.5. 1-[(4-Iodo-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9e). Yield: 60%; mp: 209–210 °C; ^{1}H NMR DMSO-d_6 δ1.26–1.28 (m, 3H); 2.27 (s, 3H); 4.23 (q, 2H); 5.06 (s, 2H); 6.56 (s, 1H); 7.41 (m, 2H); 7.66 (m, 2H); 10.52 (s, 1H).

5.3.6. 5-Methyl-1-(*p*-tolylcarbamoyl-methyl)-1*H*-pyrazole-3-carboxylic acid ethyl ester (9f). Yield: 65%; mp: 244–246 °C; ^{1}H NMR DMSO-d_6 δ 1.24–1.28 (m, 3H); 2.25 (s, 3H); 2.28 (s, 3H); 4.20–4.24 (q, 2H); 5.04 (s, 2H); 6.55 (s, 1H); 7.12 (d, 2H); 7.46 (d, 2H); 10.32 (s, 1H).

5.3.7. 1-[(4-Methoxy-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9g). Yield: 65%; mp: 239–241 °C; ^{1}H NMR DMSO-d_6 δ 1.44–1.47 (t, 3H); 2.47 (s, 3H); 3.69 (s, 3H); 4.45 (q, 2H); 4.91 (s, 2H); 6.49 (s, 1H); 6.77 (m, 2H); 7.52 (m, 2H); 9.83 (s, 1H).

5.3.8. 1-[(4-Dimethylamino-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9h). Yield: 55%; mp: 168–171 °C; ^{1}H NMR DMSO-d_6 δ

Table 3. Analytical data of bromophenylacetamides

Compound	Mp (°C)	^{1}H NMR: $CDCl_3$ (δ)
2-Bromo-*N*-phenyl-acetamide	131–135	4.01 (s, 2H); 7.18 (m, 1H); 7.36 (m, 2H); 7.54 (2H); 8.09 (s, 1H)
2-Bromo-*N*-(4-fluoro-phenyl)-acetamide	137–141	4.01 (s, 2H); 7.05 (m, 2H); 7.49 (m, 2H); 8.14 (s, 1H)
2-Bromo-*N*-(4-chloro-phenyl)-acetamide	153–155	4.02 (s, 2H); 7.31 (m, 2H); 7.49 (m, 2H); 8.09 (s, 1H)
2-Bromo-*N*-(4-bromo-phenyl)-acetamide	163–165	4.05 (s, 2H); 7.36 (m, 2H); 7.54 (2H); 8.09 (s, 1H)
2-Bromo-*N*-(4-iodo-phenyl)-acetamide	183–185	4.01 (s, 2H); 7.32 (m, 2H); 7.67 (m, 2H); 8.09 (s, 1H)
2-Bromo-*N*-*p*-tolyl-acetamide	155–159	2.24 (s, 3H); 4.06 (s, 2H); 7.25 (d, 2H); 7.29 (d, 2H); 8.09 (s, 1H)
2-Bromo-*N*-(4-methoxy-phenyl)-acetamide	125–127	3.75 (s, 3H); 4.04 (s, 2H); 6.85 (d, 2H); 7.58 (d, 2H); 8.05 (s, 1H)
2-Bromo-*N*-(4-dimethylamino-phenyl)-acetamide	212–214	2.85 (s, 6H); 4.03 (s, 2H); 6.71 (d, 2H); 7.47 (d, 2H); 8.04 (s, 1H)
2-Bromo-*N*-(4-*sec*-butyl-phenyl)-acetamide	90–93	0.81 (t, 3H); 1.19 (d, 3H); 1.54 (m, 2H); 2.54 (m, 1H); 4.95 (s, 2H); 7.11 (d, 2H); 7.35 (d, 2H); 8.01 (s, 1H)
2-Bromo-*N*-(3-methoxy-phenyl)-acetamide	78–81	3.69 (s, 3H) 4.02 (s, 2H); 7.14–7.66 (m, 4H); 8.09 (s, 1H)
2-Bromo-*N*-(3-chloro-phenyl)-acetamide	66–68	4.22 (s, 2H); 7.20–7.61 (m, 4H); 8.02 (s, 1H)
2-Bromo-*N*-(3,4-dichloro-phenyl)-acetamide	98–99	4.24 (s, 2H); 7.210 (d, 1H); 7.26 (d, 1H); 7.32 (s, 1H); 8.01 (s, 1H)
2-Bromo-*N*-(3,4-dimethoxy-phenyl)-acetamide	153–155	3.38 (d, 6H); 4.01 (s, 2H); 6.82 (d, 1H); 7.92 (d, 1H); 7.26 (1H); 8.09 (s, 1H)
2-Bromo-*N*-(3,4-dimethyl-phenyl)-acetamide	118–120	2.23 (s, 3H); 2.25 (s, 3H); 4.01 (s, 2H); 7.10 (d, 1H); 7.24 (d, 1H); 7.29 (s, 1H); 8.09 (s, 1H)
2-Bromo-*N*-naphthalen-1-yl-acetamide	153–154	4.22 (s, 2H); 7.31–8.10 (m, 7H); 8.09 (s, 1H)

1.22–1.24 (t, 3H); 2.80 (s, 6H); 3.29 (s, 3H); 4.19 (q, 2H); 4.96 (s, 2H); 6.51 (s, 1H); 6.65–6.67 (d, 2H); 7.31–7.34 (d, 2H); 10.06 (s, 1H).

5.3.9. 1-[(4-*sec*-Butyl-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9i). Yield: 58%; mp: 116–118 °C; ^{1}H NMR DMSO-d_6 δ 0.78 (t, 3H); 1.18 (d, 3H); 1.39–1.42–1.45 (t, 3H); 1.54 (m, 2H); 2.36 (s, 3H); 2.54 (m, 1H); 4.42 (q, 2H); 4.95 (s, 2H); 6.66 (s, 1H); 7.10–7.14 (d, 2H); 7.31–7.35 (d, 2H); 10.55 (s, 1H).

5.3.10. 1-[(3-Methoxy-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9j). Yield: 65%; mp: 133–135 °C; ^{1}H NMR DMSO-d_6 δ 1.26–1.29 (t, 3H); 2.28 (s, 3H); 3.71 (s, 3H); 4.23–4.31 (q, 2H); 5.06 (s, 2H); 6.56 (s, 1H); 6.65 (m, 1H); 7.07–7.30 (m, 3H); 0.43 (s, 1H).

5.3.11. 1-[(3-Chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9k). Yield: 60%; mp: 184–186 °C; ^{1}H NMR DMSO-d_6 δ 1.24–1.28 (m, 3H); 2.28 (s, 3H); 4.21–4.26 (q, 2H); 5.08 (s, 2H); 6.56 (s, 1H); 7.13–7.16 (m, 1H); 7.34–7.45 (m, 2H); 7.78 (d, 1H), 10.65 (s, 1H).

5.3.12. 1-[(3,4-Dichloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9l). Yield: 58%; mp: 207–209 °C; ^{1}H NMR DMSO-d_6 δ 1.24–1.28 (m, 3H); 2.27 (s, 3H); 4.20–4.26 (q, 2H); 5.09 (s, 2H); 6.56 (s, 1H); 7.46 (d, 1H); 7.58 (d, 1H); 7.96 (d, 1H); 10.7 5 (s, 1H).

5.3.13. 1-[(3,4-Dimethoxy-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9m). Yield: 65%; mp: 133–135 °C; ^{1}H NMR DMSO-d_6 δ 1.39–1.42 (t, 3H); 2.38 (s, 3H); 3.83 (s, 6H), 4.41 (q, 2H); 4.96 (s, 2H); 6.67 (s, 1H); 6.78 (m, 2H); 7.14 (s, 1H); 7.98 (s, 1H).

5.3.14. 1-[(3,4-Dimethyl-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9n). Yield: 65%; mp: 104–109 °C; ^{1}H NMR DMSO-d_6 δ 1.40–1.42 (t, 3H); 2.19 (s, 6H); 2.37 (s, 3H); 4.40 (q, 2H); 4.94 (s, 2H); 6.66 (s, 1H); 7.04–7.18 (m, 3H); 7.90 (s, 1H).

5.3.15. 5-Methyl-1-(naphthalen-1-ylcarbamoylmethyl)-1*H*-pyrazole-3-carboxylic acid ethyl ester (9o). Yield: 65%; mp: 170–174 °C; ^{1}H NMR DMSO-d_6 δ 1.24–1.31 (t, 3H); 2.32 (s, 3H); 4.20–4.30 (q, 2H); 5.28 (s, 2H); 6.58 (s, 1H); 7.46–8.18 (m, 7H); 10.56 (s, 1H).

5.3.16. 1-[(4-Chloro-phenylcarbamoyl)-methyl]-5-ethyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (9p). Yield: 60%; mp: 203–208 °C; ^{1}H NMR DMSO-d_6 δ 1.16–1.25 (m, 6H); 2.54–2.58 (q, 2H); 4.20–4.25 (q, 2H); 5.18 (s, 2H); 6.75 (s, 1H); 7.35–7.38 (d, 2H); 7.60 (m, 2H); 10.56 (s, 1H).

5.3.17. 1-[(4-Chloro-phenylcarbamoyl)-methyl]-1*H*-pyrazole-3-carboxylic acid ethyl ester (9q). Yield: 65%; mp: 209–211 °C; ^{1}H NMR DMSO-d_6 δ 1.24–1.31 (t, 3H); 4.24–4.27 (q, 2H); 5.14 (s, 2H); 6.76 (d, 1H); 7.36–7.41 (d, 2H); 7.59–7.63 (d, 2H); 7.89 (d, 1H); 10.56 (s, 1H).

5.4. General procedure for preparation of 5-(alkyl)-1-(substituted)phenylcarbamoylmethyl-*1H*-pyrazole-3-carboxylic acids 10a–q

To a mixture of pyrazole esters **9a**–**q** (3.0 mmol) in dioxane (50 mL) was added a 2 N KOH solution (5 mL) and the resulting mixture was stirred at room temperature for 3 h. After concentration in vacuo, the residue was cooled and acidified with a 10% HCl and the resulting precipitate was collected by filtration (quantitative yield).

5.4.1. 5-Methyl-1-phenylcarbamoylmethyl-1*H*-pyrazole-3-carboxylic acid (10a). Mp: 273–275 °C; ^{1}H NMR DMSO-d_6 δ 2.28 (s, 3H); 5.06 (s, 2H); 6.56 (s, 1H); 7.07 (m, 1H); 7.32 (m, 2H); 7.57 (m, 2H); 10.41 (s, 1H); 12.50 (s,1H).

5.4.2. 1-[(4-Fluoro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10b). Mp: 236–241 °C; ^{1}H NMR DMSO-d_6 δ 2.58 (s, 3H); 4.90 (s, 2H); 6.63

(s, 1H); 6.95 (m, 2H); 7.43 (m, 2H); 8.11 (s, 1H); 12.04 (s, 1H).

5.4.3. 1-[(4-Chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10c). Mp: 254–257 °C; ^{1}H NMR DMSO-d_6 δ 2.40 (s, 3H); 5.12 (s, 2H); 6.71 (s, 1H); 7.38 (d, 2H); 7.63 (d, 2H); 10.61 (1H); 12.98 (s, 1H).

5.4.4. 1-[(4-Bromo-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10d). Mp: 288–291 °C; ^{1}H NMR DMSO-d_6 δ 2.36 (s, 3H); 5.03 (s, 2H); 6.50 (s, 1H); 7.41 (d, 2H); 7.66 (d, 2H); 10.51 (1H); 12.55 (s, 1H).

5.4.5. 1-[(4-Iodo-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10e). Mp: 238–244 °C; ^{1}H NMR DMSO-d_6 δ 2.25 (s, 3H); 5.04 (s, 2H); 6.58 (s, 1H); 7.45 (d, 2H); 7.69 (d, 2H); 10.50 (s, 1H); 12.35 (s, 1H).

5.4.6. 5-Methyl-1-(*p*-tolylcarbamoyl-methyl)-1*H*-pyrazole-3-carboxylic acid (10f). Mp: 253–255 °C; ^{1}H NMR DMSO-d_6 δ 2.24 (s, 3H); 2.27 (s, 3H); 5.021 (s, 2H); 6.49 (s, 1H); 7.12 (d, 2H); 7.16 (d, 2H); 10.32 (s, 1H); 12.54 (s, 1H).

5.4.7. 1-[(4-Methoxy-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10g). Mp: 230–234 °C; ^{1}H NMR DMSO-d_6 δ 2.49 (s, 3H); 3.68 (s, 3H); 4.89 (s, 2H); 6.46 (s, 1H); 6.75 (d, 2H); 7.49 (d, 2H); 9.80 (s, 1H); 12.03 (s, 1H).

5.4.8. 1-[(4-Dimethylamino-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10h). Mp: 189–201 °C; ^{1}H NMR DMSO-d_6 δ 2.82 (s, 6H);3.30 (s, 3H); 4.98 (s, 2H); 6.51 (s, 1H); 6.67 (d, 2H); 7.36 (d, 2H); 10.10 (s, 1H); 12.01 (s, 1H).

5.4.9. 1-[(4-*sec*-Butyl-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10i). Mp: 160–162 °C; ^{1}H NMR DMSO-d_6 δ 0.76 (t, 3H); 1.16 (d, 3H); 1.51 (m, 2H); 2.52 (m, 4H); 5.09 (s, 2H); 6.76 (s, 1H); 7.09 (d, 2H); 7.38 (d, 2H); 10.09 (s, 1H); 12.06 (s, 1H).

5.4.10. 1-[(3-Methoxy-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10j). Mp: 226–230 °C; ^{1}H NMR DMSO-d_6 δ 2.29 (s, 3H); 3.74 (s, 3H); 5.08 (s, 2H); 6.53 (s, 1H); 6.66 (m, 1H); 7.06–7.32 (m, 3H); 10.46 (s, 1H); 12.04 (s, 1H).

5.4.11. 1-[(3-Chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10k). Mp: >300 °C; ^{1}H NMR DMSO-d_6 δ 2.37 (s, 3H); 5.07 (s, 2H); 6.51 (s, 1H); 7.12–7.48 (m, 3H); 7.79 (s, 1H); 10.76 (s, 1H); 12.06 (s, 1H).

5.4.12. 1-[(3,4-Dichloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10l). Mp: 219–220 °C; ^{1}H NMR DMSO-d_6 δ 2.27 (s, 3H); 5.07 (s, 2H); 6.51 (s, 1H); 7.50–7.97 (m, 3H); 10.77 (s, 1H); 12.04 (s, 1H).

5.4.13. 1-[(3,4-Dimethoxy-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (10m). Mp: 204–207 °C; ^{1}H NMR DMSO-d_6 δ 2.39 (s, 3H); 3.85 (s, 6H), 4.99 (s, 2H); 6.76 (s, 1H); 6.88 (m, 2H); 7.13 (s, 1H); 7.99 (s, 1H); 12.02 (s, 1H).

5.4.14. 1-[(3,4-Dimethyl-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3 carboxylic acid ethyl ester (10n). Mp: 232–236 °C; ^{1}H NMR DMSO-d_6 δ 2.21 (s, 6H); 2.40 (s, 3H); 4.99 (s, 2H); 6.68 (s, 1H); 7.05–7.25 (m, 3H); 7.94 (s, 1H); 12.20 (s, 1H).

5.4.15. 5-Methyl-1-(naphthalen-1-ylcarbamoylmethyl)-1*H*-pyrazole-3-carboxylic acid (10o). Mp: 227–229 °C; ^{1}H NMR DMSO-d_6 δ 2.32 (s, 3H); 5.28 (s, 2H); 6.58 (s, 1H); 7.46–8.18 (m, 7H); 10.56 (s, 1H); 12.09 (s, 1H).

5.4.16. 1-[(4-Chloro-phenylcarbamoyl)-methyl]-5-ethyl-1*H*-pyrazole-3-carboxylic acid (10p). Mp: 189–195 °C; ^{1}H NMR DMSO-d_6 δ 1.22 (t, 3H); 2.60 (q, 2H); 4.98 (s, 2H); 6.46 (s, 1H), 7.40 (d, 2H); 7.56 (d, 2H); 10.43 (s, 1H); 12.45 (1H).

5.4.17. 1-[(4-Chloro-phenylcarbamoyl)-methyl]-1*H*-pyrazole-3-carboxylic acid (10q). Mp: 239–242 °C; ^{1}H NMR DMSO-d_6 δ 5.12 (s, 2H); 6.79 (s, 1H); 7.40 (d, 2H); 7.63 (d, 2H); 7.88 (m, 1H); 10.54 (s, 1H); 12.31 (s, 1H).

5.5. General procedure for preparation of 2-[3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-(substituted)-pyrazol-1-yl]-*N*-(substituted)phenyl-acetamides (11a–q)

To a solution of 1,3-dipropyl-5,6-diaminouracil (2.20 mmol), in methanol (10 mL), was added an equimolar amount of the appropriate carboxylic acid (**10a–q**) and EDAC (2.21 mmol). The reaction mixture was stirred at room temperature for 4–5 h with being monitored by TLC. Methanol was concentrated and the amide intermediate derivative was precipitated by the addition of water. After filtration, the solid was dissolved in methanol (10 mL) and NaOH (2.5 N, 15 mL) and warmed at 50–60 °C for 1 h. The methanol was distilled off, and the residue was taken up in H_2O and acidified with HCl to pH 4–5. The precipitate was filtered off, washed with water, and purified by flash chromatography by elution with different mixtures of ethyl acetate-petroleum ether. Compounds **11a–q** were also purified by crystallization from methanol or dioxane.

5.5.1. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-phenyl-acetamide (11a). Yield: 45%; mp: 255–257 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.56 (q, 2H); 1.70 (q, 2H); 2.32 (s, 3H); 3.84 (m, 2H); 3.98 (m, 2H); 5.08 (s, 2H); 6.74 (s, 1H); 7.08 (m, 1H); 7.33 (m, 2H); 7.59 (m, 2H); 10.41 (s, 1H); 13.85 (s, 1H).

5.5.2. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-fluoro-phenyl)-acetamide (11b). Yield: 38%; mp: 205–207 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.56 (q, 2H); 1.70 (q, 2H); 2.31 (s, 3H); 3.84 (t, 2H); 3.97 (t, 2H); 5.07 (s, 2H);

6.74 (s, 1H); 7.17 (d, 2H); 7.61 (d, 2H); 10.48 (s, 1H); 13.80 (s, 1H).

5.5.3. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-chloro-phenyl)-acetamide (11c). Yield: 40%; mp: 261–262 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.56 (q, 2H); 1.70 (q, 2H); 2.31 (s, 3H); 3.82 (m, 2H); 3.95 (m, 2H); 5.07 (s, 2H); 6.73 (s, 1H); 7.37 (d, 2H); 7.60 (d, 2H); 10.55 (s, 1H); 13.68 (s, 1H).

5.5.4. 2-[3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-bromo-phenyl)-acetamide (11d). Yield: 42%; mp: 270–271 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.56 (q, 2H); 1.70 (q, 2H); 2.31 (s, 3H); 3.85 (m, 2H); 3.98 (m, 2H); 5.08 (s, 2H); 6.75 (s, 1H); 7.51 (d, 2H); 7.57 (d, 2H); 10.56 (s, 1H); 13.69 (s, 1H).

5.5.5. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-iodo-phenyl)-acetamide (11e). Yield: 42%; mp: 287 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.56 (q, 2H); 1.71 (q, 2H); 2.30 (s, 3H); 3.84 (t, 2H); 3.97 (t, 2H); 5.07 (s, 2H); 6.74 (s, 1H); 7.42 (d, 2H); 7.66 (d, 2H); 10.52 (s, 1H); 13.69 (s, 1H).

5.5.6. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-*p*-tolyl-acetamide (11f). Yield: 32%; mp: >300 °C; ^{1}H NMR DMSO-d_6 δ 0.88 (m, 6H); 1.57 (q, 2H); 1.72 (q, 2H); 2.25 (s, 3H); 2.31 (s, 3H); 3.84 (t, 2H); 3.99 (t, 2H); 5.05 (s, 2H); 6.73 (s, 1H); 7.20 (d, 2H); 7.46 (d, 2H); 10.32 (s, 1H); 13.85 (br s, 1H).

5.5.7. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-methoxy-phenyl)-acetamide (11g). Yield: 39%; mp: 295–296 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.56 (q, 2H); 1.70 (q, 2H); 2.31 (s, 3H); 3.71 (s, 3H); 3.84 (t, 2H); 3.97 (t, 3H) 5.03 (s, 2H); 6.72 (s, 1H); 7.90 (d, 2H); 7.49 (d, 2H); 10.28 (s, 1H); 13.80 (s, 1H).

5.5.8. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-dimethyl-amino-phenyl)-acetamide (11h). Yield: 28%; mp > 300 °C; ^{1}H NMR DMSO-d_6 δ 0.88 (m, 6H); 1.56 (q, 2H); 1.70 (q, 2H); 2.31 (s, 3H); 2.84 (s, 6H); 3.85 (t, 2H); 3.94 (t, 2H); 5.01 (s, 2H); 6.67 (s, 1H); 6.72 (d, 2H); 7.42 (d, 2H); 10.11 (s, 1H); 13.70 (s, 1H).

5.5.9. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-*sec*-butyl-phenyl)-acetamide (11i). Yield: 25%; mp: 269 °C; ^{1}H NMR DMSO-d_6 δ 0.74 (t, 3H);0.88 (m, 6H); 1.15 (q, 3H); 1.48–1.55 (m, 7H); 2.31 (s, 3H); 3.85 (t, 2H); 3.97 (t, 2H); 5.06 (s, 2H); 6.74 (s, 1H); 7.14 (d, 2H); 7.49 (d, 2H);10.33 (s, 1H); 13.75 (s, 1H).

5.5.10. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(3-methoxyl-phenyl)-acetamide (11j). Yield: 29%; mp: 276–277 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.58 (q, 2H); 1.68 (q, 2H); 2.31 (s, 3H); 3.71 (s, 3H); 3.84 (t, 3H); 3.97 (t, 2H); 5.06 (s, 2H); 6.63–6.68 (m, 1H); 6.72 (s, 1H); 7.08–7.31 (m, 3H); 10.48 (s, 1H); 13.80 (s, 1H).

5.5.11. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(3-chloro-phenyl)-acetamide (11k). Yield: 28%; mp: 304–305 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.56 (q, 2H); 1.70 (q, 2H); 2.31 (s, 3H); 3.84 (t, 2H); 3.95 (t, 2H); 5.09 (s, 2H); 6.73 (s, 1H); 7.15–7.80 (m, 4H); 10.48 (s, 1H); 13.80 (s, 1H).

5.5.12. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(3,4-dichloro-phenyl)-acetamide (11l). Yield: 30%; mp: 298 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.57 (q, 2H); 1.73 (q, 2H); 2.31 (s, 3H); 3.86 (t, 2H); 3.95 (t, 2H); 5.01 (s, 2H); 6.75 (s, 1H); 7.47–7.59 (m, 2H); 7.97 (d, 1H); 10.73 (s, 1H); 13.75 (s, 1H).

5.5.13. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(3,4-dimethoxy-phenyl)-acetamide (11m). Yield: 35%; mp: 289–290 °C; ^{1}H NMR DMSO-d_6 δ 0.88 (m, 6H); 1.57 (q, 2H); 1.70 (q, 2H); 2.32 (s, 3H); 3.71 (s, 6H); 3.84 (t, 2H); 3.88 (t, 2H); 5.03 (s, 2H); 6.73 (s, 1H); 6.90 (d, 1H); 7.05 (d, 1H); 7.32 (s, 1H); 10.29 (s, 1H); 13.88 (s, 1H).

5.5.14. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(3,4-dimethyl-phenyl)-acetamide (11n). Yield: 43%; mp: 260–263 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.56 (q, 2H); 1.70 (q, 2H); 2.17 (m, 6H); 2.31 (s, 3H); 3.84 (t, 2H); 3.97 (t, 2H); 5.03 (s, 2H); 6.70 (s, 1H); 7.06 (d, 1H); 7.29 (d, 1H); 7.37 (s, 1H) 10.24 (s, 1H); 13.80 (s, 1H).

5.5.15. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-naphthalen-1-yl-acetamide (11o). Yield: 35%; mp >300 °C; ^{1}H NMR DMSO-d_6 δ 0.89 (m, 6H); 1.59 (q, 2H); 1.72 (q, 2H); 2.36 (s, 3H); 3.85 (t, 2H); 3.97 (t, 2H); 5.30 (s, 2H); 6.76 (s, 1H); 7.46–7.81 (m, 5H); 7.93–78.19 (m, 2H); 10.36 (s, 1H); 13.72 (s, 1H).

5.5.16. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-ethyl-pyrazol-1-yl]-*N*-(4-chlorophenyl) acetamide (11p). Yield: 37%; mp: 256–257 °C; ^{1}H NMR DMSO-d_6 δ 0.88 (m, 6H); 1.25 (m, 3H); 1.55 (q, 2H); 1.70 (q, 2H); 2.66 (q, 2H); 3.84 (t, 2H); 3.99 (t, 2H); 5.08 (s, 2H); 6.77 (s, 1H); 7.40 (d, 2H); 7.63 (d, 2H); 10.56 (s, 1H); 13.67 (s, 1H).

5.5.17. 2-[3-(2,6-Dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-pyrazol-1-yl]-*N*-(4-chlorophenyl)acetamide (11q). Yield: 33%; mp: 283–285 °C; ^{1}H NMR DMSO-d_6 δ 0.87 (m, 6H); 1.52 (q, 2H); 1.66 (q, 2H); 3.85 (t, 2H); 3.98 (t, 2H); 5.14 (s, 2H); 6.94 (d, 1H); 7.37 (d, 2H); 7.60 (d, 2H); 7.89 (d, 1H); 10.57 (s, 1H); 13.69 (s, 1H).

5.6. Synthesis of 4-halogeno-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl esters 12a–c

To a magnetically stirred solution of pyrazole ester **5** (3 mmol) in 10 mL anhydrous DMF at 0 °C was added

N-halogensuccinimide (4 mmol). The solution was stirred at room temperature for 5 h. The solvent was evaporated to a half of the original volume, water was added and the reaction mixture was allowed to cool over ice. The precipitate was collected by filtration and purified by crystallization to afford the desired compounds **12a–c** as a white solid.

5.6.1. 4-Chloro-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (12a). Yield: 85%, mp: 106–107 °C; ^{1}H NMR DMSO-d_6 δ 1.25–1.32 (t, 3H); 2.21 (s, 3H); 4.22–4.32 (q, 2H); 13.59 (s, 1H).

5.6.2. 4-Bromo-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (12b). Yield: 88%, mp: 150–152 °C; ^{1}H NMR DMSO-d_6 δ 1.25–1.33 (t, 3H); 2.24 (s, 3H); 4.22–4.31 (q, 2H); 13.61 (s, 1H).

5.6.3. 4-Iodo-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (12c). Yield: 80%, mp: 113–114 °C; ^{1}H NMR DMSO-d_6 δ 1.25–1.35 (t, 3H); 2.24 (s, 3H); 4.20–4.32 (q, 2H); 13.62 (s, 1H).

5.7. General procedure for preparation of 4-halogeno-1-[(4-chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl esters 14a–c

Same procedure as **9a–q**, *except used* **12a–c**.

5.7.1. 4-Chloro-1-[(4-chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (14a). Mp: 208–210 °C; ^{1}H NMR DMSO-d_6 δ 1.26–1.29 (t, 3H); 2.25 (s, 3H); 4.26–4.28 (q, 2H); 5.14 (s, 2H); 7.39 (d, 2H); 7.60 (d, 2H); 10.60 (s, 1H).

5.7.2. 4-Bromo-1-[(4-chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (14b). Mp: 221–223 °C; ^{1}H NMR DMSO-d_6 δ 1.25–1.28 (t, 3H); 2.27 (s, 3H); 4.26–4.28 (q, 2H); 5.16 (s, 2H); 7.39 (d, 2H); 7.60 (d, 2H); 10.60 (s, 1H).

5.7.3. 4-Iodo-1-[(4-chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid ethyl ester (14c). Mp: 234–235 °C; ^{1}H NMR DMSO-d_6 δ 1.26–1.30 (t, 3H); 2.30 (s, 3H); 4.26–4.28 (q, 2H); 5.19 (s, 2H); 7.39 (d, 2H); 7.60 (d, 2H); 10.59 (s, 1H).

5.8. General procedure for preparation of 4-halo-1-[(4-chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acids 15a–c

Same procedure as **10a–q**, *except used* **14a–c**.

5.8.1. 4-Chloro-1-[(4-chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (15a). Mp: 244–245 °C; ^{1}H NMR DMSO-d_6 δ 2.24 (s, 3H); 5.11 (s, 2H); 7.39 (d, 2H); 7.60 (d, 2H); 10.60 (s, 1H); 12.98 (s, 1H).

5.8.2. 4-Bromo-1-[(4-chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (15b). Mp: 247–248 °C; ^{1}H NMR DMSO-d_6 δ 2.26 (s, 3H); 5.14 (s, 2H); 7.41 (d, 2H); 7.61 (d, 2H); 10.60 (s, 1H); 12.96 (s, 1H).

5.8.3. 4-Iodo-1-[(4-chloro-phenylcarbamoyl)-methyl]-5-methyl-1*H*-pyrazole-3-carboxylic acid (15c). Mp: 248 °C; ^{1}H NMR DMSO-d_6 δ 2.30 (s, 3H); 5.17 (s, 2H); 7.37 (d, 2H); 7.61 (d, 2H); 10.58 (s, 1H); 12.96 (s, 1H).

5.9. General procedure for preparation of 2-[4-halo-3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)- 5-methyl-pyrazol-1-yl]-*N*-(4-chlorophenyl)-acetamides 16a–c

Same procedure as **11a–q**, *except used* **15a–c**.

5.9.1. 2-[4-Chloro-3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-chlorophenyl)-acetamide (16a). Yield: 58%; mp: 297–298 °C; ^{1}H NMR DMSO-d_6 δ 0.88 (m, 6H); 1.62 (q, 2H); 1.70 (q, 2H); 2.31 (s, 3H); 3.85 (t, 2H); 4.00 (t, 2H); 5.15 (s, 2H); 7.39 (d, 2H), 7.61 (d, 2H); 10.59 (s, 1H); 13.89 (s, 1H).

5.9.2. 2-[4-Bromo-3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-chlorophenyl)-acetamide (16b). Yield: 58%; mp: 298–299 °C; ^{1}H NMR DMSO-d_6 δ 0.88 (m, 6H); 1.57 (q, 2H); 1.72 (q, 2H); 2.321 (s, 3H); 3.91 (t, 2H); 4.02 (t, 2H); 5.19 (s, 2H); 7.39 (d, 2H), 7.61 (d, 2H); 10.68 (s, 1H); 13.79 (s, 1H).

5.9.3. 2-[4-Iodo-3-(2,6-dioxo-1,3-dipropyl-2,3,6,7-tetrahydro-1*H*-purin-8-yl)-5-methyl-pyrazol-1-yl]-*N*-(4-chlorophenyl)-acetamide (16c). Yield: 55%; mp: 296–297 °C; ^{1}H NMR DMSO-d_6 δ 0.90 (m, 6H); 1.59 (q, 2H); 1.74 (q, 2H); 2.35 (s, 3H); 3.87 (t, 2H); 4.03 (t, 2H); 5.19 (s, 2H); 7.40 (d, 2H), 7.59 (d, 2H); 10.58 (s, 1H); 13.87 (s, 1H).

5.10. Biology experiments

5.10.1. Membrane preparation. The human A_1, A_{2A}, A_{2B}, and A_3 receptors were transfected in CHO or HEK cells, were grown adherently and maintained in Dulbecco's modified Eagle's medium with nutrient mixture F12 (DMEM/F12) without nucleosides, containing 10% fetal calf serum, penicillin (100 U/ml), streptomycin (100 μg/ml), L-glutamine (2 mM), and Geneticin (G418, 0.2 mg/ml) at 37 °C in 5% CO_2/95% air.[9,22] For membrane preparation the culture medium was removed and the cells were washed with PBS and scraped off T75 flasks in ice-cold hypotonic buffer (5 mM Tris–HCl, 2 mM EDTA, pH 7.4). The cell suspension was homogenized with Polytron and the homogenate was spun for 10 min at 1000*g*. The supernatant was then centrifuged for 30 min at 100,000*g*. The membrane pellet was suspended in 50 mM Tris–HCl buffer, pH 7.4, for A_1 adenosine receptors; in 50 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.4, for A_{2A} adenosine receptors; in 50 mM Tris–HCl, 10 mM $MgCl_2$, 1 mM EDTA, pH 7.4, for A_{2B} and A_3 adenosine receptors.

5.10.2. Human cloned A_1, A_{2A}, A_{2B}, and A_3 adenosine receptor binding assay. The synthesized compounds have been tested to evaluate their affinity to human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors. Displacement experiments of [^{3}H]-DPCPX to CHO cells transfected with the human recombinant A_1 adenosine receptors were performed for 120 min at 25 °C incubating diluted membranes (50 μg of protein/assay) and at least 6–8 different concentrations of examined antagonists.[23] Non specific binding was determined in the presence of 10 μM of DPCPX and this was always ⩽ 10% of the total binding. Binding of [^{3}H]-ZM 241385 to CHO cells transfected with the human recombinant A_{2A} adenosine receptors was performed using a suspension of membranes (50 μg of protein/assay) and at least 6–8 different concentrations of studied antagonists for an incubation time of 60 min at 4 °C. Non specific binding was determined in the presence of 1 μM ZM 241385 and was about 20% of total binding.[24] Competition binding experiments of [^{3}H]-MRE 2029F20 to CHO cells transfected with the human recombinant A_{2B} adenosine receptors were carried out incubating for 120 min at 4 °C diluted membranes (50 μg of protein/assay) and at least 6–8 different concentrations of examined compounds.[9] Non-specific binding was defined as binding in the presence of 1 μM MRE 2029F20 and was about 25% of total binding. Competition binding experiments of [^{3}H]-MRE 3008F20 to CHO cells transfected with the human recombinant A_3 adenosine receptors were carried out incubating for 120 min at 4 °C diluted membranes (50 μg of protein/assay) and at least 6–8 different concentrations of examined ligands.[22] Non-specific binding was defined as binding in the presence of 1 μM MRE 3008F20 and was about 25% of total binding. Bound and free radioactivity were separated by filtering the assay mixture through Whatman GF/B glass fiber filters using a Micro-Mate 196 cell harvester (Packard Instrument Co). The filter bound radioactivity was counted on a Top Count (efficiency 58%) with Micro-Scint 20.

5.10.3. Measurement of cyclic AMP levels in CHO cells transfected with human A_{2B} adenosine receptors. CHO cells transfected with human A_{2B} adenosine receptors were washed with phosphate-buffered saline and centrifuged for 10 min at 200*g*. The pellet containing the CHO cells (1×10^6 cells/assay) was suspended in 0.5 ml of incubation mixture (mM): NaCl 15, KCl 0.27, NaH_2PO_4 0.037, $MgSO_4$ 0.1, $CaCl_2$ 0.1, Hepes 0.01, $MgCl_2$ 1, glucose 0.5, pH 7.4, at 37 °C, 2 IU/ml adenosine deaminase, and 4-(3-butoxy-4-methoxybenzyl)-2-imidazolidinone (Ro 20-1724) as phosphodiesterase inhibitor, and preincubated for 10 min in a shaking bath at 37 °C. The potency of antagonists studied was determined by antagonism of NECA (100 nM)-induced stimulation of cyclic AMP levels. The reaction was terminated by the addition of cold 6% trichloroacetic acid (TCA). The TCA suspension was centrifuged at 2000*g* for 10 min at 4 °C and the supernatant was extracted four times with water saturated diethyl ether. The final aqueous solution was tested for cyclic AMP levels by a competition protein binding assay. Samples of cyclic AMP standard (0–10 pmole) were added to each test tube containing the incubation buffer (trizma base 0.1 M, aminophylline 8.0 mM, 2 mercaptoethanol 6.0 mM, pH 7.4) and [^{3}H] cyclic AMP in a total volume of 0.5 ml. The binding protein previously prepared from beef adrenals was added to the samples previously incubated at 4 °C for 150 min, and after the addition of charcoal centrifuged at 2000*g* for 10 min. The clear supernatant was counted in a Packard scintillation counter TR2500 with an efficiency of 57%.

5.10.4. Data analysis. The protein concentration was determined according to a Bio-Rad method with bovine albumin as a standard reference.[25] Inhibitory binding constant, K_i, values were calculated from those of IC_{50} according to Cheng & Prusoff equation $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where [C*] is the concentration of the radioligand and K_D^* its dissociation constant.[26] A weighted non linear least-squares curve fitting program LIGAND was also used for computer analysis of inhibition experiments.[27] IC_{50} values obtained in cyclic AMP assay were calculated by non linear regression analysis using the equation for a sigmoid concentration-response curve (GraphPad Prism, San Diego, CA, USA). Data are expressed as geometric means with 95% confidence limits in parentheses.

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Supplementary data

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