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N^6-[(Hetero)aryl/(cyclo)alkyl-carbamoyl-methoxy-phenyl]-(2-chloro)-5′-*N*-ethylcarboxamido-adenosines: The first example of adenosine-related structures with potent agonist activity at the human A_{2B} adenosine receptor

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Abstract—A new series of N^6-[(hetero)aryl/(cyclo)alkyl-carbamoyl-methoxy-phenyl]-(2-chloro)-5′-*N*-ethylcarboxamido-adenosines (**24**–**43**) has been synthesised and tested in binding assays at hA_1, hA_{2A} and hA_3 adenosine receptors, and in a functional assay at the hA_{2B} subtype. The examined compounds displayed high potency in activating A_{2B} receptors with good selectivity versus A_{2A} subtypes. The introduction of an unsubstituted 4-[(phenylcarbamoyl)-methoxy]-phenyl chain at the N^6 position of 5′-*N*-ethyl-carboxamido-adenosine led us to the recognition of compound **24** as a full agonist displaying the highest efficacy of the series (EC_{50} hA_{2B} = 7.3 nM). These compounds represent the first report about adenosine-related structures capable of activating hA_{2B} subtype in the low nanomolar range.

1. Introduction

Adenosine is a ubiquitous nucleoside involved in important biochemical processes, such as energy transfer (ATP and ADP), as well as in signal transduction as cyclic adenosine monophosphate (cAMP), and as a building block for some biologically significant molecules such as NAD (nicotinamide-adenine-dinucleotide), FAD (flavin-adenine-dinucleotide), SAM (*S*-adenosyl-L-methionine), DNA and RNA. Four adenosine receptors, classified as A_1, A_{2A}, A_{2B} and A_3, have been cloned from many mammalian and some non-mammalian species.[1,2] The adenosine receptors have extensive tissue distribution and can be co-expressed in the same cell type. All the adenosine receptor subclasses belong to the family of cell membrane G protein-coupled receptors. Different signal transduction mechanisms have been identified for each subtype. In particular, A_{2B} receptors are associated with stimulation of adenylate cyclase and activation of phospholipase C through the coupling to Gs and Gq/11 proteins, respectively. A_{2B} receptors have been found on practically every cell in most species and their sequences are highly similar across species.[3] A role of A_{2B} adenosine receptors in regulation of vascular tone, neurosecretion and neurotransmission, intestinal tone and smooth muscle growth has been suggested.[3] RT-PCR studies revealed their highest expression in caecum, large intestine and urinary bladder, but lower levels in brain, spinal cord, lung and vas deferens.[4] Recent evidence of a probable involvement of A_{2B} adenosine receptors in tumur growth and development has been reported.[5] The expression of A_{2B} receptors is regulated in T cell activation events, thus indicating that A_{2B} receptors are involved in the regulation of signalling processes occurring in human lymphocytes by adenosine.[6] There is growing evidence that adenosine plays a role in asthma and chronic obstructive pulmonary disease (COPD).[7] It has been recently demonstrated that adenosine stimulates production of IL-4 and IL-13 in

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mast cells. This effect seems to be mediated via A_{2B} adenosine receptors.[8]

The treatment of asthma with selective A_{2B} adenosine receptor antagonists is a promising therapeutic aim.[9] The use of A_{2B} adenosine receptor agonists for the treatment of atherosclerosis, septic shock[10] and impotence[3] has been investigated. However, the lack of selective pharmacological tools, in particular, the lack of selective A_{2B} receptor agonists, has certainly determined the general scarceness of information about the physiological functions of this potential therapeutic target.

Up to now, several non-selective adenosine analogues with poor affinity have been identified and employed for the characterization of the A_{2B} adenosine receptor, which has been classified as a low-affinity adenosine receptor.[10,11] A leading relevance has been ascribed for years to some nucleoside ligands such as NECA (5′-*N*-ethylcarboxamido-adenosine) and (*S*)-PHPNECA((*S*)-2-phenylhydroxypropynylNECA)[10] which show the capability to activate the A_{2B} receptor with EC_{50} values in the micromolar range based on the measurement of receptor-stimulated adenylate cyclase activity.[10,12] An innovative series of 2-amino-pyridine-3,5-dicarbonitrile derivatives has been recently reported as the first example of high-potency non-adenosine full and partial agonists of the human A_{2B} receptor.[13,14]

For the design of new nucleoside A_{2B} adenosine receptor agonists, we considered the possibility to exploit some information deriving from the structural analysis of known A_{2B} adenosine receptor antagonists. In this field, several compounds with high affinity and selectivity have been identified among structures based upon a xanthine core,[15] bearing different substituents at the 1-, 3- and 8-positions. Kim et al.[16] reported that a (substituted)phenylcarbamoyl-methoxy-phenyl chain at the 8-position of a series of 1,3-dipropyl-xanthines selectively addresses the antagonists' affinity to the adenosine A_{2B} receptor. Further evidence of the important role of the substituent at the 8-position, as the selective structural element for the design of potent A_{2B} antagonists, has been recently provided by our group.[17]

The strategy here reported to obtain new A_{2B} selective NECA derivatives has been furthermore suggested by SAR studies which would indicate the N^6 a useful position for A_{2B} binding site recognition. A docking study related to a small collection of known A_{2B} agonists highlighted the interaction of the exocyclic amino group at the 6-position of NECA with a residue of asparagine 254 belonging to the VI transmembrane receptor helix.[18] We have recently confirmed the importance of the N^6-position for A_{2B} adenosine receptor recognition, by the synthesis of a series of 1-deoxy-1-[6-[((hetero)aryl-carbonyl)-hydrazino]-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide and 1-deoxy-1-[2-chloro-6-[((hetero)aryl-carbonyl)-hydrazino]-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide derivatives which have been found to be the first examples of both potent and selective A_{2B} adenosine receptor agonists.[19]

In light of this, and thanks to the identification of a versatile synthetic route which permitted us to functionalize the N^6-position of the known non-selective adenosine agonists NECA[20,21] and 2-Cl-NECA (2-chloro-5′-*N*-ethylcarboxamido-adenosine),[22] we designed a series of adenosine analogues bearing a (substituted) phenylcarbamoyl-methoxy-phenyl chain at the N^6-NECA position. Our attempt was to evaluate the possibility of identifying a molecular hybrid obtained by the molecular combination of the nucleoside core responsible for receptor activation with the structural element able to grant A_{2B} selectivity to the cited series of xanthine derivatives.[16]

We have then synthesised a new series of N^6-[(substituted)phenyl/cycloalkyl/benzyl/heteroaryl-carbamoyl-methoxy-phenyl]-5′-*N*-ethylcarboxamido-adenosine and 2-chloro-adenosine derivatives (**24**–**43**; Fig. 1, Table 1). The N^6 mono-substitution demonstrated to be well

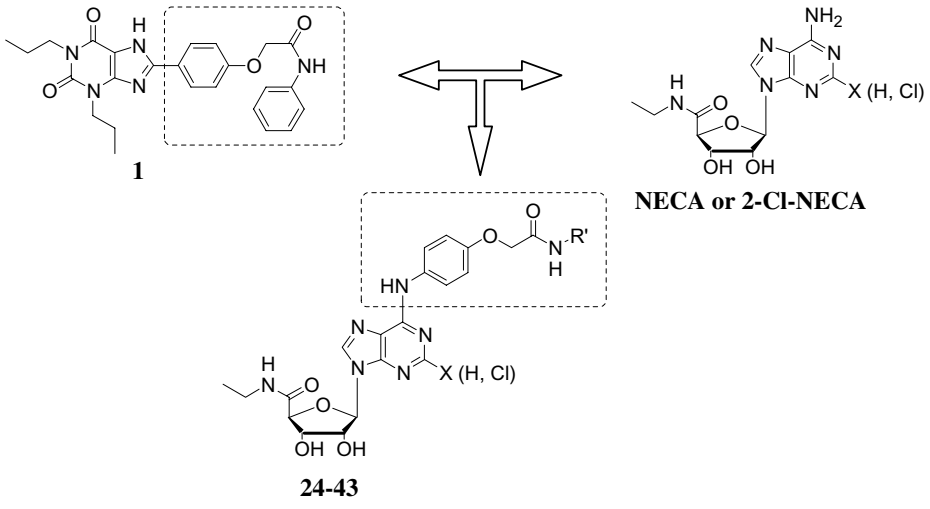
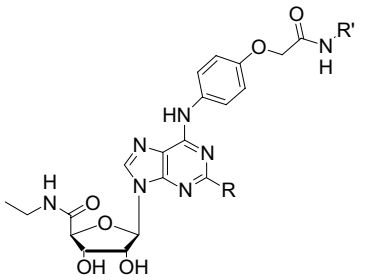


Figure 1. Design of new N^6-substituted-NECA derivatives as A_{2B} adenosine receptor agonists based on a hybridization approach between known selective A_{2B} AR antagonists and non-selective ARs agonist.

Table 1. Structures and physicochemical parameters of the synthesised N^6-substituted-NECA derivatives



Compound	R	R′	Mp (°C)	M_W	Formula
24	H	Ph	145–146	533.54	$C_{26}H_{27}N_7O_6$
25	H	4-F–Ph	274–275	551.19	$C_{26}H_{26}FN_7O_6$
26	H	4-Cl–Ph	279–280	567.98	$C_{26}H_{26}ClN_7O_6$
27	H	4-Br–Ph	272	612.43	$C_{26}H_{26}BrN_7O_6$
28	H	4-I–Ph	284–285	659.43	$C_{26}H_{26}IN_7O_6$
29	H	2-CF_3–Ph	222	601.53	$C_{27}H_{26}F_3N_7O_6$
30	H	4-OCH_3–Ph	249–250	563.56	$C_{27}H_{29}N_7O_7$
31	H	3,4-$(OCH_3)_2$–Ph	240	593.59	$C_{28}H_{31}N_7O_8$
32	H	3,5-$(OCH_3)_2$–Ph	219–220	593.59	$C_{28}H_{31}N_7O_8$
33	H	3,4-OCH_2O–Ph	270–272	577.55	$C_{27}H_{27}N_7O_8$
34	H	4-*tert*-Butyl–Ph	155–156	589.26	$C_{30}H_{35}N_7O_6$
35	H	4-*N*-Morpholino–Ph	274	618.26	$C_{30}H_{34}N_8O_7$
36	H	4-Pyridyl	145	534.20	$C_{25}H_{26}N_8O_6$
37	H	Benzyl	129–130	547.56	$C_{27}H_{29}N_7O_6$
38	H	4-OCH_3–benzyl	115–116	577.23	$C_{28}H_{31}N_7O_7$
39	H	Cyclohexyl	211–212	539.58	$C_{26}H_{33}N_7O_6$
40	Cl	Ph	261–262	567.98	$C_{26}H_{26}ClN_7O_6$
41	Cl	4-OCH_3–Ph	264–265	598.01	$C_{27}H_{28}ClN_7O_7$
42	Cl	Benzyl	235–236	582.01	$C_{27}H_{28}ClN_7O_6$
43	Cl	Cyclohexyl	275–276	574.03	$C_{26}H_{32}ClN_7O_6$

tolerated by the A_{2B} receptor in a series of adenosine and 5′-*N*-ethyl-carboxamidoadenosine derivatives previously reported.[23] The literature in the field of A_{2B} agonists also indicated that a second possible site of modification was the 2-position of the purine nucleus. Compounds such as (*S*)-PHPNECA and 2-alkynyl-5′-*N*-alkylcarboxamidoadenosines[10,12] have been synthesised and identified as quite potent agonists at the A_{2B} receptors. We chose to introduce in some of the N^6-substituted-NECA derivatives here reported, a chlorine atom at the 2-position of the purine moiety.

All of the synthesised compounds were evaluated in radioligand binding assays to define their affinities for human A_1, A_{2A} and A_3 adenosine receptors (Table 2). Efficacy of the compounds at the hA_{2B} adenosine receptors was assessed via functional tests, based on the estimation of cyclic AMP levels in stably transfected CHO cells. In light of our biological evaluation, we can report the identification of the first series of adenosine analogues endowed with potent agonist activity for the human A_{2B} adenosine receptor with EC_{50} values ranging from 7.3 to 360 nM.

2. Chemistry

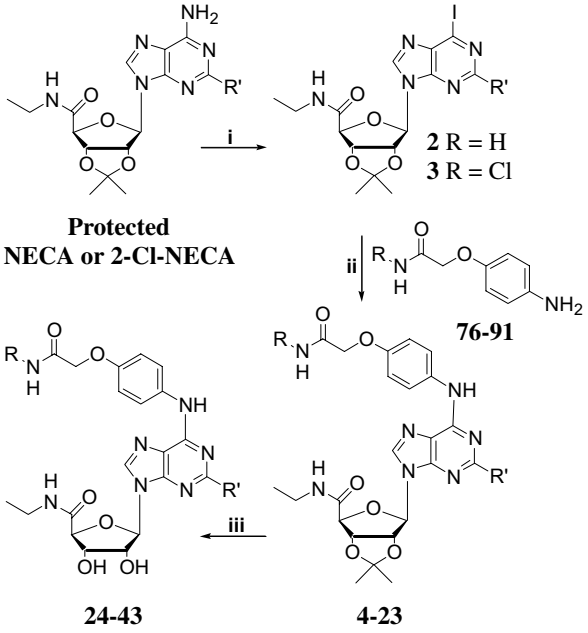
Scheme 1 depicts the synthetic strategy adopted to prepare the target compounds **24**–**43**. 2′,3′-*O*-isopropylidene-5′-*N*-ethylcarboxamidoadenosine (2′,3′-*O*-isopropylidene-NECA)[24] and 2′,3′-*O*-isopropylidene-2-chloro-5′-*N*-ethylcarboxamidoadenosine (2′,3′-*O*-isopropylidene-2-chloro-NECA)[22] were quite effectively converted into the corresponding 6-iodo derivatives (**2** and **3**) by treatment with diiodomethane and isopentylnitrite, as reported by Nair (yield 60%).[19,25] Intermediates **2** and **3** proved to be useful key substrates for the subsequent substitution reactions with the appropriate 2-(4-aminophenoxy)-N-substituted-acetamide **76**–**91**, which were performed in a steel bomb at 100 °C for 7–8 h (yield 20–50%) to provide intermediates **4**–**23**. Despite the drastic reaction condition, in no case did we observe the by-products arising from substitution of the 2-chloro atom. Protected N^6-substituted nucleoside derivatives **4**–**23** were stirred for about 3 h at room temperature in a 1:1 mixture of water and trifluoroacetic acid to give unprotected final compounds **24**–**43** in nearly quantitative yield.

2-(4-Amino-phenoxy)-N-substituted-acetamides **76**–**91** were synthesised according to the procedure described in Scheme 2. Chloroacetyl chloride was reacted with (substituted)phenyl/heteroaryl/(substituted)benzyl/cycloalkyl-amines to give the corresponding 2-chloro-N-substituted-acetamides **44**–**59**.[26] Subsequent alkylation of 4-nitro-phenol with intermediates **44**–**59** furnished the 2-(4-nitro-phenoxy)-N-substituted-acetamides **60**–**75** which were efficiently converted into the desired 2-(4-amino-phenoxy)-N-substituted-acetamides **76**–**91** by reduction with $NaBH_4$ and 10% Pd/C.

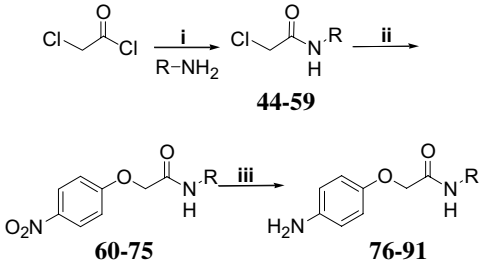
Table 2. Binding affinities and functional parameters of the synthesised N^6-substituted-NECA derivatives at the human A_1, A_{2A}, A_{2B} and A_3 adenosine receptors expressed in CHO cells

Compound	[^{3}H]CHA binding[a] hA_1 CHO cells K_i (nM)	[^{3}H]CGS21680 binding[b] hA_{2A} CHO cells K_i (nM)	cAMP assay[c] hA_{2B} CHO cells EC_{50} (nM)	[^{125}I]ABMECA binding[d] hA_3 CHO cells K_i (nM)
NECA	18.2 ± 2.1	12.4 ± 2.7	155 ± 12	35.7 ± 3.3
S-PHPNECA	2.1[10]	2.0[10]	220[10]	0.75[10]
24	8.5 ± 0.8	>1000 (45%)	7.3 ± 0.6	38.4 ± 3.7
25	2.3 ± 0.2	>1000 (48%)	15.2 ± 2.1	72.3 ± 7.4
26	3.1 ± 0.3	>1000 (35%)	12.3 ± 1.4	34.2 ± 3.7
27	3.5 ± 0.4	>1000 (26%)	10.5 ± 1.2	36.4 ± 3.7
28	5.2 ± 0.5	>1000 (28%)	30.2 ± 2.8	85.2 ± 8.3
29	31.6 ± 3.3	>1000 (1%)	107 ± 10	115 ± 12
30	4.7 ± 0.4	>1000 (42%)	32.4 ± 3.3	25.3 ± 2.6
31	7.7 ± 0.5	>1000 (30%)	45.4 ± 5.3	53.2 ± 5.4
32	15.3 ± 2.2	>1000 (13%)	53.6 ± 5.4	78.6 ± 7.5
33	8.4 ± 0.9	>1000 (5%)	35.5 ± 2.7	81.4 ± 8.3
34	18.6 ± 2.1	>1000 (1%)	16.4 ± 2.1	40.2 ± 3.9
35	77.5 ± 4.6	>1000 (1%)	300 ± 35	320 ± 35
36	11.2 ± 1.3	>1000 (37%)	32.3 ± 2.4	42.3 ± 4.7
37	20.4 ± 2.1	>1000 (49%)	150 ± 17	82.7 ± 8.9
38	28.3 ± 3.7	>1000 (1%)	240 ± 23	65.4 ± 6.8
39	16.2 ± 2.4	>1000 (40%)	315 ± 34	72.5 ± 7.5
40	30.5 ± 3.3	>1000 (36%)	42.6 ± 4.2	107 ± 10
41	50.4 ± 3.5	>1000 (27%)	360 ± 32	101 ± 9
42	22.6 ± 2.4	>1000 (49%)	175 ± 14	75.7 ± 7.4
43	30.8 ± 3.6	>1000 (46%)	302 ± 16	95.8 ± 9.6

[a] Displacement of specific [^{3}H]CHA binding at human A_1 receptors expressed in CHO cells (n = 3–6).
[b] Displacement of specific [^{3}H]CGS21680 binding at human A_{2A} receptors expressed in CHO cells (n = 3–6).
[c] cAMP assay in CHO cells expressing human A_{2B} adenosine receptors IC_{50} (nM).
[d] Displacement of specific [^{125}I]ABMECA binding at human A_3 receptors expressed in CHO cells (n = 3–6). Data are expressed as geometric means with 95% confidence limits. In parentheses is indicated the percentage of displacement of the examined compounds.



Scheme 1. Reagents and conditions: (i) CH_2I_2, isopentylnitrite, 80 °C, 1 h; (ii) TEA, EtOH, steel bomb, 100–110 °C, 12 h; (iii) TFA/H_2O 1:1, rt, 3 h.



Scheme 2. Reagents and conditions: (i) K_2CO_3, CH_2Cl_2, reflux, 4 h; (ii) 4-NO_2-phenol, K_2CO_3, acetone, reflux, 12 h; (iii) $NaBH_4$, 10% Pd/C, CH_3OH, H_2O, rt, 3 h.

3. Results and discussion

Competition binding experiments[27] were performed to evaluate the affinity of the synthesised compounds **9b**–**26b** to hA_1, hA_{2A} and hA_3 receptors expressed in CHO cells using as radioligands [^{3}H]-CHA, [^{3}H]-CGS 21680 and [^{125}I]-AB-MECA, respectively. The compounds were also evaluated in a functional assay,[17] measuring their capacity to modulate cAMP levels in CHO cells expressing hA_{2B} receptors.

Structures, chemical properties and biological data of the synthesised compounds are listed in Tables 1 and 2. Different kinds of substitutions have been considered at the nitrogen of the acetamide chain at the N^6-position of NECA: unsubstituted phenyl (**24**), phenyl bearing weak electron-withdrawing functions at the ortho (**29**) and para position (**25**–**28**), strong electron-donating substituents at several positions (**30**–**33**), hindered lipophilic (**34**) and hydrophilic moieties (**35**). The effect of the replacement of the phenyl nucleus with a pyridine (**36**), a benzyl moiety (**37**), a 4-OCH_3-benzyl (**38**) and a

cycloalkyl group (**39**) has also been investigated. A chlorine atom at the 2-position of the purine nucleus has also been introduced (compounds **40**–**43**).

In the absence of binding data on the adenosine A_{2B} receptor, caused by the substantial lack of a useful radiolabelled agonist, we deduced a selectivity profile in view of the ratio of K_i (A_1, A_{2A} and A_3) to EC_{50} (A_{2B}).

All the synthesised compounds showed the capacity to bind to the adenosine A_1 receptor and to activate the adenosine A_{2B} receptor in the low nanomolar range, displaying at the same time scarce affinity ($K_i > 1\mu M$) towards A_{2A} adenosine subtypes and a relevant capability to bind A_3 receptors. Considering the binding and functional profile of NECA (see Table 2) and (*S*)-PHPNECA (K_i $hA_1 = 2.1$ nM; K_i $hA_{2A} = 2.0$ nM; EC_{50} $hA_{2B} = 220$ nM; K_i $hA_3 = 0.75$ nM)[10] which are among the most potent adenosine-like A_{2B} adenosine receptor agonists reported thus far, the molecules described here represent a remarkable advance in this field, albeit the selectivity profile must be undoubtedly improved.

Substitution at the para-position of the phenyl ring with a halogen atom led to a substantial loss of activity in comparison with the unsubstituted phenyl derivative **24** (EC_{50} $hA_{2B} = 7.3$ nM). Among the 4-halogenated-phenyl derivatives, the 4-iodo **28** (EC_{50} $hA_{2B} = 30.2$ nM) showed to be less potent than the 4-fluoro **25** (EC_{50} $hA_{2B} = 15.2$ nM), the 4-chloro **26** (EC_{50} $hA_{2B} = 12.3$ nM) and the 4-bromo **27** (EC_{50} $hA_{2B} = 10.5$ nM) analogues in activating the A_{2B} adenosine receptor. The same behaviour has been observed by introducing functions with reverse electronic effects, such as the 4-methoxy group, which led us to the identification of compound **30** (EC_{50} $hA_{2B} = 32.4$ nM). The bis-methoxy derivatives **31** (EC_{50} $hA_{2B} = 45.4$ nM) and **32** (EC_{50} $hA_{2B} = 53.6$ nM) were slightly less potent than the 4-methoxy counterpart **30**. Derivative**33** displayed the same potency of the 4-methoxy-phenyl (**30**). The presence of the trifluoromethyl function at the 2-position of the phenyl ring was quite detrimental for the A_{2B} receptor activation potency (**29**, EC_{50} $hA_{2B} = 107$ nM). Increasing the steric hindrance around the para-position by the introduction of the branched and lipophilic *tert*-butyl chain in compound **34** permitted us to obtain a very potent agonist for the adenosine A_{2B} receptor with an EC_{50} value comparable to that of the unsubstituted-phenyl derivative **24**. Replacement of the *tert*-butyl substituent with the sterically demanding but more polar 4-morpholino group (**35**, EC_{50} $hA_{2B} = 300$ nM) leads to poor activity, suggesting a possible lipophilic interaction of the terminal side of the N^6-phenylcarbamoyl residue with the A_{2B} receptor binding site.

The replacement of the phenyl with the 4-pyridyl moiety resulted in a 4-fold decrease in the potency (**36**, EC_{50} $hA_{2B} = 32.3$ nM). Further modifications of the phenyl ring, such as the insertion of a methylene group between the acetamide nitrogen and the phenyl ring (benzyl **37**, EC_{50} $hA_{2B} = 150$ nM, and 4-OCH_3-benzyl derivatives **38**, EC_{50} $hA_{2B} = 240$ nM), or the complete saturation of the 6-carbon cycle (cyclohexyl derivative **39**, EC_{50} $hA_{2B} = 315$ nM), led to a severe reduction in the capability to activate A_{2B} receptors. The presence of a chlorine atom at the 2-position had little or a slightly detrimental effect in terms of A_{2B} receptor activation, as emerged from the comparison of the biological data of the 2-chloro derivatives **40**–**43** with the corresponding 2-unsubstituted **24**, **30**, **37** and **39** compounds.

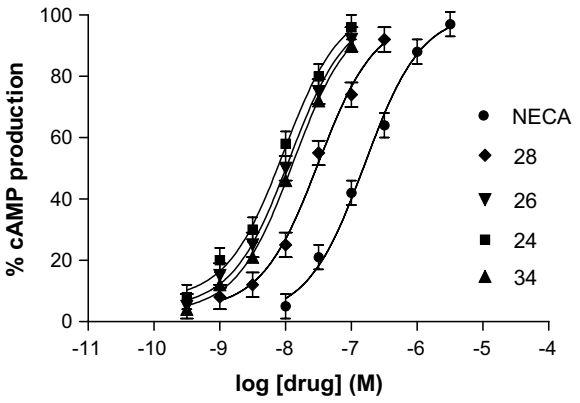


Figure 2. Stimulation curves representing the capability of the examined adenosine agonists to stimulate cAMP production in $CHOhA_{2B}$ cells.

Unfortunately, despite being very potent ligands for A_{2B} adenosine receptor, the reported series of N^6-[substituted-carbamoyl-methoxy-phenyl]-(2-chloro)-5′-*N*-ethylcarboxamido-adenosines exhibited very low, or no, selectivity versus the A_1 and A_3 adenosine receptor subtypes. Most of the examined molecules, in fact, preferentially bound to the A_1 receptor, with K_i binding values ranging from 2.3 to 77 nM. This experimental observation can be explained in light of the literature indicating that A_1 adenosine receptor selectivity is enhanced by monosubstitution of the exocyclic amino group at the 6-position of adenosine with bulky cycloalkyl or arylalkyl substituents.[28] A lower, but significant, affinity for the A_3 receptor was observed. The most selective compounds versus A_3 adenosine subtype were the unsubstituted phenyl derivative (**24**) and the 4-halo-phenyl derivatives (**25**–**28**).

Figure 2 reports the dose–response curves of NECA and compounds **24**, **26**, **28** and **34** in $hA_{2B}CHO$ cells showing that the designed molecules behave as full A_{2B} agonists.

4. Conclusions

In the present study, we described the affinity at the human adenosine receptors A_1, A_{2A} and A_3 and the efficacy at the human A_{2B} receptor of a new series of N^6-[(hetero)aryl/(cyclo)alkyl-carbamoyl-methoxy-phenyl]-(2-chloro)-5′-*N*-ethylcarboxamido-adenosines. These molecules can be considered the first example of adenosine-related structures able to exert very high potency (EC_{50} values from functional assay in the low nanomolar range) in activating the A_{2B} adenosine subtype. The

lack of agonists endowed with satisfactory levels of A_{2B} potency and selectivity has hampered the pharmacological characterization of this potential therapeutic target. Even though non-selective versus the adenosine A_1 and A_3 adenosine receptors, the synthesised molecules may represent a promising device for the development of useful ligands to be employed for pharmacological investigation of the physiological functions mediated by adenosine through the A_{2B} receptors.

5. Experimental

5.1. Chemistry

Reaction progress and product mixtures were monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) and visualized with aqueous potassium permanganate or a methanolic solution of H_2SO_4. ^{1}H NMR were determined in $CDCl_3$ or DMSO-d_6 solutions with a Varian VXR 200 spectrometer or a Varian Mercury Plus 400 spectrometer; peak positions are given in parts per million (δ) downfield from tetramethylsilane as internal standard and J values are given in Hertz. Light petroleum refers to the fractions boiling at 40–60 °C. Melting points were determined on a Buchi-Tottoli instrument and are uncorrected. Chromatography was performed on Merck 230–400 mesh silica gel. Organic solutions were dried over anhydrous sodium sulfate. Elemental analyses were performed by the microanalytical laboratory of Dipartimento di Chimica, University of Ferrara, and were within ±0.4% of the theoretical values for C, H and N.

5.2. General procedure for the preparation of 1-deoxy-1-[(2-chloro)-6-{4-[(substituted-phenyl/heteroayl/benzyl/cycloalkylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide derivatives 4–23

A mixture of the 6-iodo derivatives **2** or **3**[19] (0.17 mmol), TEA (0.21 mmol) and the appropriate 2-(4-amino-phenoxy)-N-substituted-acetamide **76–91** (0.21 mmol) in absolute EtOH (3 mL) was heated in a steel bomb at 100–110 °C overnight. The solvent was evaporated and the residue partitioned between H_2O (10 mL) and EtOAc (3 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4, filtered and the solvent evaporated under vacuum to give a residue which was purified by flash chromatography.

5.2.1. 1-Deoxy-1-[6-{4-[(phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (4). The product was purified by column chromatography on silica gel eluting with light petroleum/EtOAc 1:9: yield 34%; pale yellow solid; mp 215 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 0.60 (t, 3H, J = 7.2), 1.35 (s, 3H), 1.54 (s, 3H), 2.81 (m, 2H), 4.56 (s, 1H), 4.68 (s, 2H), 5.44 (s, 2H), 6.41 (s, 1H), 6.96–7.11 (m, 3H), 7.28–7.36 (m, 2H), 7.55 (br t, 1H), 7.63–7.81 (m, 4H), 8.29 (s, 1H), 8.41 (s, 1H), 9.85 (s, 1H), 10.09 (s, 1H).

5.2.2. 1-Deoxy-1-[6-{4-[(4-fluoro-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (5). The product was purified by column chromatography on silica gel eluting with EtOAc: yield 43%; pale yellow solid; mp 120–121 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.60 (t, 3H, J = 7.2), 1.35 (s, 3H), 1.54 (s, 3H), 2.78 (m, 2H), 4.56 (s, 1H), 4.67 (s, 2H), 5.44 (m, 2H), 6.40 (s, 1H), 6.96–7.22 (m, 4H), 7.55 (br t, 1H), 7.64–7.82 (m, 4H), 8.29 (s, 1H), 8.42 (s, 1H), 10.00 (s, 1H).

5.2.3. 1-Deoxy-1-[6-{4-[(4-chloro-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (6). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 1:1 to EtOAc: yield 33%; pale yellow solid; mp 122–123 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.90 (t, 3H, J = 7.2), 1.39 (s, 3H), 1.64 (s, 3H), 3.13 (m, 2H), 4.63 (s, 2H), 4.73 (s, 1H), 5.39 (m, 2H), 6.07 (d, 1H, J = 2.3), 7.01 (m, 3H), 7.34 (d, 2H, J = 8), 7.48 (d, 2H, J = 8), 7.69 (s, 1H), 7.74 (d, 2H, J = 8), 7.91 (s, 1H), 8.29 (br s, 1H), 8.45 (s, 1H).

5.2.4. 1-Deoxy-1-[6-{4-[(4-bromo-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (7). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 1:1 to light petroleum/EtOAc 3:7: yield 35%; pale yellow solid; mp 113–114 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.91 (t, 3H, J = 7.3), 1.40 (s, 3H), 1.64 (s, 3H), 3.13 (m, 2H), 4.62 (s, 2H), 4.73 (s, 1H), 5.40 (m, 2H), 6.07 (d, 1H, J = 2.3), 7.01 (br m, 3H), 7.50 (m, 4H), 7.74 (m, 3H), 7.91 (s, 1H), 8.29 (br s, 1H), 8.45 (s, 1H).

5.2.5. 1-Deoxy-1-[6-{4-[(4-iodo-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (8). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 1:1 to EtOAc: yield 44%; pale yellow solid; mp 130–131 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.90 (t, 3H, J = 7.2), 1.39 (s, 3H), 1.64 (s, 3H), 3.13 (m, 2H), 4.61 (s, 2H), 4.73 (s, 1H), 5.39 (br s, 2H), 6.07 (d, 1H, J = 2.3), 7.03 (m, 3H), 7.40 (d, 2H, J = 8), 7.64–7.66 (m, 5H), 7.91 (s, 1H), 8.27 (br s, 1H), 8.45 (s, 1H).

5.2.6. 1-Deoxy-1-[6-{4-[(2-trifluoromethyl-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2, 3-*O*-(1-methylethylidene)- β-D-ribofuranuronamide (9). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 1:1 to EtOAc: yield 35%; white solid; mp 110–112 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.91 (t, 3H, J = 7.2), 1.40 (s, 3H), 1.64 (s, 3H), 3.13 (m, 2H), 4.66 (s, 2H), 4.73 (s, 1H), 5.40 (m, 2H), 6.07 (d, 1H, J = 2.3), 7.01 (m, 3H), 7.56–7.76 (m, 5H), 7.91 (s, 1H), 8.41 (d, 2H, J = 8), 8.45 (s, 1H), 8.97 (br s, 1H).

5.2.7. 1-Deoxy-1-[6-{4-[(4-methoxy-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (10). The

product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 1:1 to light petroleum/EtOAc 3:7: yield 40%; pale yellow solid; mp 139–140 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.91 (t, 3H, J = 7.2), 1.39 (s, 3H), 1.64 (s, 3H), 3.13 (m, 2H), 3.81 (s, 3H), 4.62 (s, 2H), 4.73 (s, 1H), 5.40 (m, 2H), 6.07 (d, 1H, J = 2.3), 6.89 (d, 2H, J = 8), 7.03 (m, 3H), 7.49 (d, 2H, J = 8), 7.71 (m, 3H), 7.90 (s, 1H), 8.20 (br s, 1H), 8.45 (s, 1H).

5.2.8. 1-Deoxy-1-[6-{4-[(3,4-dimethoxy-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (11). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 2:3 to light petroleum/EtOAc 1:4: yield 30%; pale yellow solid; mp 117 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.91 (t, 3H, J = 7.2), 1.40 (s, 3H), 1.64 (s, 3H), 3.13 (m, 2H), 3.88 (s, 3H), 3.92 (s, 3H), 4.62 (s, 2H), 4.73 (s, 1H), 5.40 (m, 2H), 6.07 (d, 1H, J = 2.3), 6.82–7.07 (m, 4H), 7.36 (d, 2H, 2.3), 7.73 (m, 3H), 7.91 (s, 1H), 8.20 (br s, 1H), 8.45 (s, 1H).

5.2.9. 1-Deoxy-1-[6-{4-[(3,5-dimethoxy-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (12). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 1:1 to EtOAc: yield 25%; white solid; mp 121–122 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.91 (t, 3H, J = 7.2), 1.40 (s, 3H), 1.64 (s, 3H), 3.13 (m, 2H), 3.80 (s, 6H), 4.61 (s, 2H), 4.73 (s, 1H), 5.40 (m, 2H), 6.07 (d, 1H, J = 2.3), 6.28 (m, 1H), 6.83 (m, 2H), 7.01–7.06 (m, 3H), 7.71 (s, 2H), 7.75 (s, 1H), 7.91 (s, 1H), 8.23 (br s, 1H), 8.45 (s, 1H).

5.2.10. 1-Deoxy-1-[6-{4-[(Benzo[1,3]dioxol-5-ylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)- β-D-ribofuranuronamide (13). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 1:1 to EtOAc: yield 48%; white solid; mp 145 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.90 (t, 3H, J = 7.2), 1.39 (s, 3H), 1.64 (s, 3H), 3.13 (m, 2H), 4.61 (s, 2H), 4.73 (s, 1H), 5.39 (m, 2H), 5.97 (s, 2H), 6.07 (d, 1H, J = 2.3), 6.75–6.91 (m, 2H), 7.30 (m, 3H), 7.31 (d, 1H, J = 2), 7.70 (s, 2H), 7.75 (s, 1H), 7.91 (s, 1H), 8.19 (br s, 1H), 8.45 (s, 1H).

5.2.11. 1-Deoxy-1-[6-{4-[(4-*tert*-butyl-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (14). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 1:4 to EtOAc: yield 32%; white solid; mp 130 °C; ^{1}H NMR (200 MHz, DMSO): δ (ppm) 0.61 (t, 3H, J = 7.4), 1.26 (s, 9H), 1.35 (s, 3H), 1.54 (s, 3H), 2.80 (m, 2H), 4.56 (s, 1H), 4.66 (s, 2H), 5.44 (s, 2H), 6.41 (s, 1H), 6.98 (d, 2H, J = 9.4), 7.33 (d, 2H, J = 8.6), 7.55 (d, 2H, J = 8.6), 7.78 (d, 2H, J = 9.0), 8.29 (s, 1H), 8.41 (s, 1H), 9.84 (s, 1H), 10.01 (s, 1H).

5.2.12. 1-Deoxy-1-[6-{4-[(4-morpholin-4-yl-phenylcarbamoyl)-methoxy]- phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (15). The product was purified by column chromatography on silica gel eluting with light petroleum/EtOAc 2:8: yield 25%; pale yellow solid; mp 190 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.90 (t, 3H, J = 7.2), 1.39 (s, 3H), 1.63 (s, 3H), 3.08–3.15 (m, 6H), 3.83–3.88 (m, 4H), 4.61 (s, 2H), 4.72 (m, 1H), 5.38 (m, 2H), 6.07 (d, 1H, J = 2.4), 6.88–6.92 (d, 2H, J = 9.0), 7.00–7.05 (d, 2H, J = 9), 7.46–7.50 (d, 2H, J = 8.8), 7.65–7.74 (m, 3H), 7.90 (s, 1H), 8.16 (br s, 1H), 8.44 (s, 1H).

5.2.13. 1-Deoxy-1-[6-{4-[(pyridin-4-yl-carbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (16). The product was purified by column chromatography on silica gel eluting with EtOAc: yield 35%; pale yellow solid; mp 150 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 0.91 (t, 3H, J = 7.2), 1.39 (s, 3H), 1.65 (s, 3H), 3.18 (m, 2H), 4.60 (s, 2H), 4.75 (s, 1H), 5.38 (m, 2H), 6.09 (d, 1H, J = 2.3), 7.04 (m, 3H), 7.20 (d, 2H, J = 9.0), 7.40 (d, 2H, J = 9.0), 7.55 (d, 2H, J = 9.0), 7.69 (s, 1H), 7.94 (s, 1H), 8.26 (br s, 1H), 8.45 (s, 1H).

5.2.14. 1-Deoxy-1-[6-{4-[(benzylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (17). The product was purified by column chromatography on silica gel eluting with EtOAc: yield 37%; pale yellow solid; mp 118 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 0.60 (t, 3H, J = 7.2), 1.35 (s, 3H), 1.54 (s, 3H), 2.82 (m, 2H), 4.35 (d, 2H, J = 6.2), 4.55 (m, 3H), 5.44 (s, 2H), 6.41 (s, 1H), 6.95 (m, 2H), 7.21–7.35 (m, 5H), 7.54 (br t, 1H), 7.78 (m, 2H), 8.29 (s, 1H), 8.41 (s, 1H), 8.65 (br t, 1H), 9.84 (br t, 1H).

5.2.15. 1-Deoxy-1-[6-{4-[(4-methoxy-benzylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (18). The product was purified by column chromatography on silica gel eluting with EtOAc: yield 38%; pale yellow solid; mp 117 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 0.61 (t, 3H, J = 7.2), 1.35 (s, 3H), 1.54 (s, 3H), 2.78 (m, 2H), 3.71 (s, 3H), 4.27 (br s, 2H), 4.51 (s, 2H), 4.56 (s, 1H), 5.44 (s, 2H), 6.41 (s, 1H), 6.83–6.97 (m, 4H), 7.18 (d, 2H, J = 8.4), 7.55 (br t, 1H), 7.75–7.81 (m, 2H), 8.30 (s, 1H), 8.41 (s, 1H), 8.57 (br t, 1H), 9.80 (br s, 1H).

5.2.16. 1-Deoxy-1-[6-{4-[(cyclohexylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (19). The product was purified by column chromatography on silica gel eluting with light petroleum/EtOAc 2:8: yield 45%; pale yellow solid; mp 132–135 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 0.61 (t, 3H, J = 7.2), 1.20–1.30 (br m, 6H), 1.35 (s, 3H), 1.54 (s, 3H), 1.68-1.73 (br m, 4H), 2.78 (m, 2H), 3.60 (br s, 1H), 4.42 (s, 2H), 4.56 (s, 1H), 5.44 (s, 2H), 6.41 (s, 1H), 6.93 (d, 2H, J = 9.4), 7.55 (br t, 1H), 7.77 (d, 2H, J = 9.2), 7.86 (d, 1H, J = 8.00), 8.29 (s, 1H), 8.41 (s, 1H), 9.82 (s, 1H).

5.2.17. 1-Deoxy-1-[2-chloro-6-{4-[(phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (20). The product was purified by column chromatography on silica gel eluting with a gradient from light petroleum/EtOAc 7:3 to EtOAc: yield 47%; white solid; mp 148–150 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 0.66 (t, 3H, J = 7.2), 1.35 (s, 3H), 1.54 (s, 3H), 2.84 (m, 2H), 4.56 (s, 1H), 4.70 (s, 2H), 5.39 (s, 2H), 6.35 (s, 1H), 6.99–7.11 (m, 3H), 7.28–7.36 (m, 2H), 7.55–7.71 (m, 5H), 8.39 (s, 1H), 10.09 (s, 1H), 10.30 (br s, 1H).

5.2.18. 1-Deoxy-1-[2-chloro-6-{4-[(4-methoxy-phenylcarbamoyl)-methoxy]- phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (21). The product was purified by column chromatography on silica gel eluting with EtOAc: yield 44%; pale yellow solid; mp 83–84 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 0.67 (t, 3H, J = 7.2), 1.35 (s, 3H), 1.55 (s, 3H), 2.85 (m, 2H), 3.70 (s, 3H), 4.52 (s, 1H), 4.73 (s, 2H), 5.41 (m, 2H), 6.09 (d, 1H, J = 2.3), 6.75–6.98 (m, 4H), 7.33–7.45 (m, 4H), 7.70 (s, 1H), 7.91 (br t, 1H), 8.38 (br s, 1H), 10.02 (s, 1H).

5.2.19. 1-Deoxy-1-[2-chloro-6-{4-[(benzylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (22). The product was purified by column chromatography on silica gel eluting with light petroleum/EtOAc 1:4: yield 43%; pale yellow solid; yield 53%; pale yellow solid; mp 121–122 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 0.66 (t, 3H, J = 7.2), 1.35 (s, 3H), 1.54 (s, 3H), 2.84 (m, 2H), 4.35 (d, 2H, J = 6.0), 4.55 (s, 3H), 5.40 (s, 2H), 6.36 (s, 1H), 6.98 (d, 2H, J = 8.00), 7.26–7.35 (m, 5H), 7.57–7.70 (m, 3H), 8.40 (s, 1H), 8.66 (br t, 1H), 10.27 (s, 1H).

5.2.20. 1-Deoxy-1-[2-chloro-6-{4-[(cyclohexylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (23). The product was purified by column chromatography on silica gel eluting with light petroleum/EtOAc 1:4: yield 43%; pale yellow solid; mp 120–121 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 0.66 (t, 3H, J = 7.2), 1.13–1.25 (br m, 6H), 1.35 (s, 3H), 1.54 (s, 3H), 1.68 (br m, 4H), 2.85 (m, 2H), 3.60 (br s, 1H), 4.44 (s, 2H), 4.57 (s, 1H), 5.39 (s, 2H), 6.35 (s, 1H), 6.95 (d, 2H, J = 9.0), 7.60 (br t, 1H), 7.67 (d, 2H, J = 9.2), 7.90 (d, 1H), 8.39 (s, 1H), 10.25 (s, 1H).

5.3. General procedure for the preparation of 1-deoxy-1-[(2-chloro)-6-{4-[(substituted-phenyl/heteroayl/benzyl/cycloalkylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide derivatives 24–43

The appropriate isopropylidene derivative **4**–**23** (0.6 mmol) was dissolved in a mixture of trifluoroacetic acid and water 1:1 (4 mL) and the resulting solution was stirred at room temperature for 3 h. The solvents were evaporated at reduced pressure to give a residue which was suspended with water (15 mL) and extracted with EtOAc (3 × 15 mL). The organic layer was dried over Na_2SO_4, filtered and the solvent evaporated under vacuum. Purification by flash chromatography afforded the desired unprotected compounds **24**–**43**.

5.3.1. 1-Deoxy-1-[6-{4-[(phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (24). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 9:1: yield 63%; white solid; mp 145–146 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.09 (t, 3H, J = 7.2), 3.20 (m, 2H), 4.16 (m, 1H), 4.33 (s, 1H), 4.64 (m, 3H) 5.63 (d, 1H, J = 6.4), 5.79 (d, 1H, J = 4.4), 6.03 (d, 1H, J = 7.2), 6.98–7.13 (m, 3H), 7.29–7.37 (m, 2H), 7.66 (d, 2H, J = 8.00), 7.80 (d, 2H, J = 8.00), 8.39 (s, 1H), 8.58 (s, 1H), 8.79 (br t, 1H), 9.96 (s, 1H), 10.11 (s, 1H). Anal. ($C_{26}H_{27}N_7O_6$) C, H, N.

5.3.2. 1-Deoxy-1-[6-{4-[(4-fluoro-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (25). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 9:1: yield 85%; white solid; mp 274–275 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.08 (t, 3H, J = 7.0), 3.23 (m, 2H), 4.16 (m, 1H), 4.33 (m, 1H), 4.63 (m, 3H), 5.63 (d, 1H, J = 6.4), 5.80 (d, 1H, J = 4.00), 6.03 (d, 1H, J = 7.2), 7.00 (d, 2H, J = 9), 7.13–7.23 (m, 2H), 7.65–7.84 (m, 4H), 8.39 (s, 1H), 8.58 (s, 1H), 8.78 (br t, 1H), 10.00 (s, 1H). Anal. ($C_{26}H_{26}FN_7O_6$) C, H, N.

5.3.3. 1-Deoxy-1-[6-{4-[(4-chloro-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (26). The product was purified by column chromatography on silica gel eluting with a mixture of CH_2Cl_2/CH_3OH 9.5:0.5: yield 80%; white solid; mp 279–280°C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.17 (t, 3H, J = 7.2), 3.35 (m, 2H), 4.25 (m, 1H), 4.44 (s, 1H), 4.64 (m, 3H), 5.44 (m, 1H), 5.71 (m, 1H), 6.04 (d, 1H, J = 7.4), 7.10 (d, 2H, J = 8), 7.28 (d, 2H, J = 8), 7.70 (d, 2H, J = 8), 7.83 (d, 2H, J = 8), 8.32 (s, 1H), 8.36 (s, 1H), 8.99 (br t, 1H), 9.72 (s, 1H), 10.01 (s, 1H). Anal. ($C_{26}H_{26}ClN_7O_6$) C, H, N.

5.3.4. 1-Deoxy-1-[6-{4-[(4-bromo-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (27). The product was purified by column chromatography on silica gel eluting with a mixture of CH_2Cl_2/CH_3OH 9.5:0.5: yield 45%; white solid; mp 272 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.08 (t, 3H, J = 7.2), 3.21 (m, 2H), 4.17 (br m, 1H), 4.32 (m, 1H), 4.63 (br m, 1H), 4.69 (s, 2H), 5.60 (d, 1H, J = 6.4), 5.77 (d, 1H, J = 4.3), 6.02 (d, 1H, J = 7.4), 7.00 (d, 2H, J = 8), 7.50 (d, 2H, J = 8), 7.65 (d, 2H, J = 8), 7.80 (d, 2H, J = 8), 8.38 (s, 1H), 8.57 (s, 1H), 8.78 (br t, 1H), 9.95 (s, 1H), 10.25 (s, 1H). Anal. ($C_{26}H_{26}BrN_7O_6$) C, H, N.

5.3.5. 1-Deoxy-1-[6-{4-[(4-iodo-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (28). The product was purified by column chromatography on silica gel eluting with a mixture of CH_2Cl_2/CH_3OH 9.5:0.5: yield 70%; white solid; mp 284-285°C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.22 (t, 3H, J = 7.2), 3.35 (m, 2H), 4.31 (m, 1H), 4.51 (s, 1H), 4.62 (s, 2H), 4.74 (br m, 1H), 5.60 (br s, 1H), 5.70 (br s, 1H), 6.01 (d, 1H, J = 7.4), 7.02 (d, 2H, J = 8), 7.49–7.63 (m, 4H), 7.83 (d, 2H, J = 8), 8.15 (s,

1H), 8.38 (s, 1H), 9.08 (br t, 1H), 9.41 (s, 1H), 9.68 (s, 1H). Anal. ($C_{26}H_{26}IN_7O_6$) C, H, N.

5.3.6. 1-Deoxy-1-[6-{4-[(2-trifluoromethyl-phenylcarbamoyl)-methoxy]- phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (29). The product was purified by column chromatography on silica gel eluting with a mixture of CH_2Cl_2/CH_3OH 9.5:0.5: yield 55%; white solid; mp 222°C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.20 (t, 3H, J = 7.2), 3.38 (m, 2H), 4.26 (m, 1H), 4.46 (s, 1H), 4.70 (br s, 3H), 5.40 (m, 1H), 5.68 (m, 1H), 6.05 (d, 1H, J = 7.4), 7.02 (d, 2H, J = 8), 7.40 (m, 1H), 7.61–7.71 (m, 2H), 7.89 (d, 2H, J = 8), 8.10 (d, 1H, J = 8), 8.28 (s, 1H), 8.37 (s, 1H), 9.01 (br t, 1H), 9.25 (s, 1H), 9.70 (s, 1H). Anal. ($C_{27}H_{26}F_3N_7O_6$) C, H, N.

5.3.7. 1-Deoxy-1-[6-{4-[(4-methoxy-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (30). The product was purified by column chromatography on silica gel eluting with a mixture of CH_2Cl_2/CH_3OH 9.5:0.5: yield 65%; white solid; mp 249–250 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.11 (t, 3H, J = 7.2), 3.21 (m, 2H), 3.72 (s, 3H), 4.17 (br t, 1H), 4.34 (m, 1H), 4.58-4.67 (m, 3H), 5.49 (d, 1H, J = 6.4), 5.71 (d, 1H, J = 4.3), 6.01 (d, 1H, J = 7.4), 6.84 (d, 2H, J = 8), 6.96 (d, 2H, J = 8), 7.53 (d, 2H, J = 8), 7.80 (d, 2H, J = 8), 8.33 (s, 1H), 8.44 (s, 1H), 8.84 (br t, 1H), 9.82 (br s, 2H). Anal. ($C_{27}H_{29}N_7O_7$) C, H, N.

5.3.8. 1-Deoxy-1-[6-{4-[(3,4-dimethoxy-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-β-D-ribofuranuronamide (31). The product was purified by column chromatography on silica gel eluting with a mixture of CH_2Cl_2/CH_3OH 9.5:0.5: yield 60%; white solid; mp 240 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.08 (t, 3H, J = 7.2), 3.21 (m, 2H), 3.71 (s, 3H), 3.72 (s, 3H), 4.17 (br m, 1H), 4.32 (m, 1H), 4.65 (br s, 3H), 5.60 (d, 1H, J = 6.4), 5.78 (d, 1H, J = 4.3), 6.02 (d, 1H, J = 7.4), 6.87–7.02 (m, 3H), 7.18 (m, 1H), 7.34 (m, 1H), 7.80 (d, 2H, J = 8), 8.39 (s, 1H), 8.57 (s, 1H), 8.78 (br t, 1H), 9.95 (s, 1H), 10.25 (s, 1H). Anal. ($C_{28}H_{31}N_7O_8$) C, H, N.

5.3.9. 1-Deoxy-1-[6-{4-[(3,5-dimethoxy-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (32). The product was purified by column chromatography on silica gel eluting with a mixture of CH_2Cl_2/CH_3OH 9.5:0.5: yield 50%; white solid; mp 219–220°C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.20 (t, 3H, J = 7.2), 3.35 (m, 2H), 3.76 (s, 6H), 4.28 (m, 1H), 4.48 (s, 1H), 4.61 (s, 2H), 4.73 (m, 1H), 5.33 (m, 1H), 5.65 (m, 1H), 6.03 (d, 1H, J = 7.4), 6.20 (m,1H), 6.93–7.02 (m, 4H), 7.82 (d, 2H, J = 8), 8.21 (s, 1H), 8.36 (s, 1H), 9.04 (br t, 1H), 9.57 (s, 1H), 9.62 (s, 1H). Anal. ($C_{28}H_{31}N_7O_8$) C, H, N.

5.3.10. 1-Deoxy-1-[6-{4-[(Benzo[1,3]dioxol-5-ylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (33). The product was purified by column chromatography on silica gel eluting with a mixture of CH_2Cl_2/CH_3OH 9.5:0.5: yield 75%; white solid; mp 270–272 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.11 (t, 3H, J = 7.2), 3.25 (m, 2H), 4.17 (m, 1H), 4.35 (s, 1H), 4.59 (m, 3H), 5.48 (m, 1H), 5.71 (m, 1H), 5.93 (s, 2H), 6.02 (d, 1H, J = 7.4), 6.74 (d, 1H, J = 8), 6.92–7.05 (m, 3H), 7.31 (m, 1H), 7.81 (d, 2H, J = 8), 8.32 (s, 1H), 8.41 (s, 1H), 8.86 (br t, 1H), 9.81 (s, 1H), 9.85 (s, 1H). Anal. ($C_{27}H_{27}N_7O_8$) C, H, N.

5.3.11. 1-Deoxy-1-[6-{4-[(4-*tert*-butyl-phenylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (34). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 9.5:0.5: yield 78%; white solid; mp 155–156 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.08 (t, 3H, J = 7.2), 1.26 (s, 9H), 3.20 (m, 2H), 4.20 (m, 1H), 4.32 (m, 1H), 4.67 (br m, 3H), 5.60 (br d, 1H), 5.80 (br d, 1H), 6.02 (d, 1H, J = 7.2), 6.99 (d, 2H, J = 9.0), 7.33 (d, 2H, J = 8.4), 7.56 (d, 2H, J = 8.8), 7.81 (d, 2H, J = 9.0), 8.39 (s, 1H), 8.57 (s, 1H), 8.80 (br t, 1H), 9.89 (s, 1H), 10.10 (s, 1H). Anal. ($C_{30}H_{35}N_7O_6$) C, H, N.

5.3.12. 1-Deoxy-1-[6-{4-[(4-morpholin-4-yl-phenylcarbamoyl)-methoxy]- phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (35). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 9:1: yield 50%; pale yellow solid; mp 274 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.08 (t, 3H, J = 7.4), 3.02–3.07 (m, 4H), 3.20 (m, 2H), 3.70–3.75 (m, 4H), 4.20 (m, 1H), 4.32 (m, 1H), 4.64 (br m, 3H), 5.60 (br m, 1H), 5.80 (br m, 1H), 6.03 (d, 1H, J = 7.2), 6.90 (d, 2H, J = 8.8), 6.99 (d, 2H, J = 8.8), 7.51 (d, 2H, J = 9.2), 7.81 (d, 2H, J = 9.0), 8.39 (s, 1H), 8.57 (s, 1H), 8.80 (br m, 1H), 9.90 (br s, 1H), 10.00 (br s, 1H). Anal. ($C_{30}H_{34}N_8O_7$) C, H, N.

5.3.13. 1-Deoxy-1-[6-{4-[(pyridin-4-yl-carbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (36). The product was purified by crystallization from EtOH: yield 90%; pale yellow solid; mp 145 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.08 (t, 3H, J = 7.4), 3.20 (m, 2H), 4.16 (m, 1H), 4.33 (m, 1H), 4.62 (m, 1H), 4.76 (s, 2H), 5.60 (br m, 1H), 5.80 (br m, 1H), 6.02 (d, 1H, J = 7.6), 6.99 (d, 2H, J = 9), 7.69–7.84 (m, 4H), 8.39 (s, 1H), 8.48 (m, 2H), 8.58 (s, 1H), 8.77 (br t, 1H), 10.00 (s, 1H), 10.60 (s, 1H). Anal. ($C_{25}H_{26}N_8O_6$) C, H, N.

5.3.14. 1-Deoxy-1-[6-{4-[(benzylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin- 9-yl]-*N*-β-D-ribofuranuronamide (37). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 9:1: yield 82%; white; mp 129–130 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.08 (t, 3H, J = 7.2), 3.21 (m, 2H), 4.18 (br t, 1H), 4.36 (br d, 3H), 4.54 (s, 2H), 4.62 (m, 1H), 5.61 (d, 1H), 5.79 (d, 1H), 6.05 (d, 1H), 6.96 (d, 2H, J = 8.00), 7.28 (m, 5H), 7.80 (d, 2H, J = 8.00), 8.39 (s, 1H), 8.57 (s, 1H), 8.65 (br t, 1H), 8.8 (br t, 1H), 9.94 (s,1H). Anal. ($C_{27}H_{29}N_7O_6$) C, H, N.

5.3.15. 1-Deoxy-1-[6-{4-[(4-methoxy-benzylcarbamoyl)-methoxy]-phenylamino}- 9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (38). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 9:1: yield 80%; white solid; mp 115–116 °C; ^{1}H NMR

(200 MHz, DMSO-d_6): δ (ppm) 1.08 (t, 3H, J = 7.2), 3.22 (m, 2H), 3.71 (s, 3H), 4.16 (br d, 1H), 4.28 (d, 2H, J = 5.8), 4.33 (m, 1H), 4.52 (s, 2H), 4.65 (m, 1H), 5.60 (br d, 1H), 5.80 (br d, 1H), 6.03 (d, 1H, J = 7.4), 6.84–6.98 (m, 4H), 7.18 (d, 2H, J = 8.6), 7.78 (d, 2H, J = 8.4), 8.39 (s, 1H), 8.57 (br m, 2H), 8.78 (br t, 1H), 10.00 (s, 1H). Anal. ($C_{28}H_{31}N_7O_7$) C, H, N.

5.3.16. 1-Deoxy-1-[6-{4-[(cyclohexylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (39). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 9:1: yield 88%; white solid; mp 211–212 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.02–1.27 (m, 9H), 1.51–1.70 (br m, 4H), 3.16 (m, 2H), 3.59 (br s, 1H), 4.20 (br m, 1H), 4.30 (s, 1H), 4.40 (s, 2H), 4.62 (br m, 1H), 5.58 (d, 1H, J = 6.4), 5.75 (d, 1H, J = 4.2), 5.99 (d, 1H, J = 7.40), 6.90 (d, 2H, J = 8.00), 7.73–7.87 (m, 3H), 8.36 (s, 1H), 8.54 (s, 1H), 8.75 (br t, 1H), 9.90 (s, 1H). Anal. ($C_{26}H_{33}N_7O_6$) C, H, N.

5.3.17. 1-Deoxy-1-[2-chloro-6-{4-[(phenylcarbamoyl)-methoxy]-phenylamino}- 9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (40). The product was purified by crystallization from EtOAc: yield 82%; white solid; mp 261–262 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.05 (t, 3H, J = 7.2), 3.21 (m, 2H), 4.17 (m, 1H), 4.32 (m, 1H), 4.58 (m, 1H), 4.70 (s, 2H), 5.63 (d, 1H, J = 6.00), 5.75 (d, 1H, J = 4.6), 5.97 (d, 1H, J = 7.2), 7.00–7.12 (m, 3H), 7.29–7.37 (m, 2H), 7.63–7.78 (m, 4H), 8.34 (br t, 1H), 8.62 (s, 1H), 10.10 (s, 1H), 10.36 (s, 1H). Anal. ($C_{26}H_{26}ClN_7O_6$) C, H, N.

5.3.18. 1-Deoxy-1-[2-chloro-6-{4-[(4-methoxy-phenylcarbamoyl)-methoxy]- phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (41). The product was purified by column chromatography on silica gel eluting with a mixture EtOAc/CH_3OH 9.5:0.5: yield 73%; white solid; mp 264–265 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.05 (t, 3H, J = 7.2), 3.23 (m, 2H), 3.72 (s, 3H), 4.14 (br m, 2H), 4.33 (m, 1H), 4.62 (br m, 2H), 5.60–5.80 (br m, 2H), 5.97 (d, 1H, J = 6.4), 6.88–7.05 (m, 4H), 7.54–7.73 (m, 4H), 8.34 (br t, 1H), 8.62 (s, 1H), 9.96 (s, 1H), 10.35 (s, 1H). Anal. ($C_{27}H_{28}ClN_7O_7$) C, H, N.

5.3.19. 1-Deoxy-1-[2-chloro-6-{4-[(benzylcarbamoyl)-methoxy]-phenylamino}- 9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (42). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 9:1: yield 87%; white solid; mp 235–236 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.05 (t, 3H, J = 7.2), 3.22 (m, 2H), 4.18 (br s, 1H), 4.35 (m, 3H), 4.56 (s, 3H), 5.68 (br d, 1H), 5.80 (br d, 1H), 5.98 (d, 1H, J = 6), 7.00 (d, 2H, J = 9.2), 7.22–7.32 (m, 5H), 7.70 (d, 2H, J = 8.8), 8.35 (t, 1H, J = 6.00), 8.68 (m, 2H), 10.35 (s, 1H). Anal. ($C_{27}H_{28}ClN_7O_6$) C, H, N.

5.3.20. 1-Deoxy-1-[2-chloro-6-{4-[(cyclohexylcarbamoyl)-methoxy]-phenylamino}-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (43). The product was purified by column chromatography on silica gel eluting with EtOAc/CH_3OH 4:1: yield 93%; white solid; mp 275–276 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 1.02–1.25 (m, 9H), 1.54–1.80 (br m, 4H), 3.21 (m, 2H), 3.61 (br s, 1H), 4.18 (br s, 1H), 4.33 (s, 1H), 4.44 (s, 2H), 4.59 (br m, 1H), 5.66 (br d, 1H), 5.79 (br d, 1H), 5.97 (d, 1H, J = 7.00), 6.96 (d, 2H, J = 8.8), 7.67 (d, 2H, J = 9.2), 7.88 (d, 1H, J = 8.00), 8.35 (br t, 1H), 8.62 (s, 1H), 10.32 (br s, 1H). Anal. ($C_{26}H_{32}ClN_7O_6$) C, H, N.

5.4. General procedure for the preparation of 2-chloro-N-substituted-acetamides 44–59[26]

Chloroacetyl chloride (33 mmol) was added dropwise to a mixture of the appropriate amine (28 mmol) and K_2CO_3 (33 mmol) in CH_2Cl_2 (50 mL) at room temperature. The reaction mixture was refluxed for 4h, then, after cooling to room temperature, it was slowly poured into 100 mL of ice water. The aqueous solution was extracted with CH_2Cl_2 (2 × 100 mL), the organic phase was dried over Na_2SO_4, filtered and the solvent was evaporated to furnish a solid residue which was purified by crystallization from a mixture of Et_2O/light petroleum.

5.4.1. 2-Chloro-*N*-phenyl-acetamide (44). Yield 90%; white solid; mp 134-137 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.21 (s, 2H), 7.20 (m, 1H), 7.39 (m, 2H), 7.60 (m, 2H), 8.55 (br s, 1H).

5.4.2. 2-Chloro-*N*-(4-fluoro-phenyl)-acetamide (45). Yield 85%; white solid; mp 130–131 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.19 (s, 2H), 7.01–7.09 (m, 2H), 7.47–7.54 (m, 2H), 8.21 (br s, 1H).

5.4.3. 2-Chloro-*N*-(4-chloro-phenyl)-acetamide (46). Yield 60%; pale yellow solid; mp 170–171 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.26 (s, 2H), 7.39 (d, 2 H, J = 8), 7.61 (d, 2H, J = 8), 10.45 (br s, 1H).

5.4.4. 2-Chloro-*N*-(4-bromo-phenyl)-acetamide (47). Yield 90%; grey solid; mp 180 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.19 (s, 2H), 7. 47 (m, 4H), 8.24 (br s, 1H).

5.4.5. 2-Chloro-*N*-(4-iodo-phenyl)-acetamide (48). Yield 65%; white solid; mp 194–195 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.25 (s, 2H), 7.42 (d, 2 H, J = 8), 7.68 (d, 2H, J = 8), 10.41 (br s, 1H).

5.4.6. 2-Chloro-*N*-(2-trifluoromethyl-phenyl)-acetamide (49). Yield 80%; white solid; mp 94–95 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.24 (s, 2H), 7.26–7.68 (m, 3H), 8.22 (d, 1H, J = 8), 8.76 (br s, 1H).

5.4.7. 2-Chloro-*N*-(4-methoxy-phenyl)-acetamide (50). Yield 82%; white solid; mp 124 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.80 (s, 3H), 4.17 (s, 2H), 6.90 (d, 2H, J = 8), 7.43 (d, 2H, J = 8), 8.17 (br s, 1H).

5.4.8. 2-Chloro-*N*-(3,4-dimethoxy-phenyl)-acetamide (51). Yield 73%; white solid; mp 134 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.87 (s, 3H), 3.89 (s, 3H),

4.18 (s, 2H), 6.80–6.98 (m, 2H), 7.27 (m, 1H), 8.17 (br s, 1H).

5.4.9. 2-Chloro-*N*-(3,5-dimethoxy-phenyl)-acetamide (52). Yield 83%; white solid; mp 97–98 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.77 (s, 6H), 4.14 (s, 2H), 6.23 (m, 1H), 6.85 (m, 2H), 9.48 (br s, 1 H).

5.4.10. *N*-Benzo[1,3]dioxol-5-yl-2-chloro-acetamide (53). Yield 74%; white solid; mp 157–158 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.18 (s, 2H), 5.97 (s, 2H), 6.74–6.87 (m, 2H), 7.23 (m, 1H), 8.14 (br s, 1H).

5.4.11. 2-Chloro-*N*-(4-*tert*-butyl-phenyl)-acetamide (54). Yield 79%; white solid; mp 87–90 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.31 (s, 9H), 4.18 (s, 2H), 7.34–7.48 (m, 4H), 8.18 (br s, 1H).

5.4.12. 2-Chloro-*N*-(4-morpholin-4-yl-phenyl)-acetamide (55). Yield 70%; white solid; mp 170–172 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.07 (br t, 4H), 3.80 (br t, 4H), 4.12 (s, 2H), 6.84 (d, 2H, J = 8.8), 7.37 (d, 2H, J = 8.2), 8.06 (br s, 1H).

5.4.13. 2-Chloro-*N*-pyridin-4-yl-acetamide (56). Yield 58%; white solid; mp 125–127 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.19 (s, 2H), 8.03 (d, 2H, J = 7.2), 8.49 (d, 2H, J = 7.00), 11.53 (s, 1H).

5.4.14. *N*-Benzyl-2-chloro-acetamide (57). Yield 90%; white solid; mp 95–96 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.04 (s, 2H), 4.43 (d, 2H, J = 6), 6.90 (br s, 1H), 7.26 (m, 5H).

5.4.15. 2-Bromo-*N*-(4-methoxy-benzyl)-acetamide (58). Yield 85%; white solid; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 3.72 (s, 3H), 3.88 (s, 2H), 4.20 (d, 2H, J = 5.8), 6.86–6.92 (d, 2H, J = 8.00), 7.16–7.21 (d, 2H, J = 8.00), 8.70 (br s, 1H).,

5.4.16. 2-Chloro-*N*-cyclohexyl-acetamide (59). Yield 84%; white solid; mp 97–98 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.10–1.88 (m, 10H), 3.72 (m, 1H), 3.96 (s, 2H), 6.40 (br s, 1H).

5.5. General procedure for the preparation of 2-(4-nitrophenoxy)-N-substituted-acetamides 60–75

A mixture of 4-nitrophenol (10 mmol) and K_2CO_3 (12 mmol) in anhydrous acetone (60 mL) was stirred at room temperature for 5 min., then a solution of the appropriate 2-chloro-N-substituted-acetamide (**44**–**59**, 10 mmol) was added dropwise. After 12 h at reflux, the solvent was evaporated to give a residue which was suspended in H_2O (50 mL) and extracted with EtOAc (3×50 mL). After drying over Na_2SO_4, the organic layer was filtered and the solvent evaporated to dryness. The desired products **60**–**75** were finally isolated as solids by crystallization from a mixture of EtOAc/Et_2O.

5.5.1. 2-(4-Nitrophenoxy)-*N*-phenyl-acetamide (60). Yield 77%; pale yellow solid; mp 170–171 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.90 (s, 2H), 7.08–7.37 (m, 5H), 7.60 (m, 2H), 8.23 (d, 2H, J = 8), 10.20 (br s, 1H).

5.5.2. *N*-(4-Fluoro-phenyl)-2-(4-nitro-phenoxy)-acetamide (61). Yield 73%; pale yellow solid; mp 159–160 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.71 (s, 2H), 7.02–7.12 (m, 4H), 7.51–7.58 (m, 2H), 8.11 (br s, 1H), 8.28 (m, 2H).

5.5.3. *N*-(4-Chloro-phenyl)-2-(4-nitro-phenoxy)-acetamide (62). Yield 65%; pale yellow solid; mp 181 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.75 (s, 2 H), 7.06 (d, 2H, J = 8), 7.20 (d, 2H, J = 8), 7.58 (d, 2H, J = 8), 8.16 (d, 2H, J = 8), 10.03 (br s, 1H).

5.5.4. *N*-(4-Bromo-phenyl)-2-(4-nitro-phenoxy)-acetamide (63). Yield 70%; pale yellow solid; mp 120–121 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.70 (s, 2 H), 7.10 (d, 2H, J = 8), 7. 49 (m, 4H), 8.26 (br s, 1H), 8.30 (d, 2H, J = 8).

5.5.5. *N*-(4-Iodo-phenyl)-2-(4-nitro-phenoxy)-acetamide (64). Yield 62%; pale yellow solid; mp 155 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.90 (s, 2H), 7.19 (d, 2H, J = 8), 7.46 (d, 2H, J = 8), 7.68 (d, 2H, J = 8), 8.23 (d, 2H, J = 8), 10.32 (br s, 1H).

5.5.6. 2-(4-Nitro-phenoxy)-*N*-(2-trifluoromethyl-phenyl)-acetamide (65). Yield 68%; pale yellow solid; mp 136-137 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.75 (s, 2H), 7.04–7.84 (m, 5H), 8.25–8.40 (m, 3H), 8.79 (br s, 1H).

5.5.7. *N*-(4-Methoxy-phenyl)-2-(4-nitro-phenoxy)-acetamide (66). Yield 85%; pale yellow solid; mp 179–180 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.81 (s, 3H), 4.70 (s, 2H), 6.90 (d, 2H, J = 8), 7.00 (d, 2H, J = 8), 7.50 (d, 2H, J = 8), 8.05 (br s, 1H), 8.28 (d, 2H, J = 8).

5.5.8. *N*-(3,4-Dimethoxy-phenyl)-2-(4-nitro-phenoxy)-acetamide (67). Yield 65%; pale yellow solid; mp 145–146 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.88 (s, 3H), 3.90 (s, 3H), 4.70 (s, 2H), 6.82–7.34 (m, 5H), 8.08 (br s, 1H), 8.27 (d, 2H, J = 8).

5.5.9. *N*-(3,5-Dimethoxy-phenyl)-2-(4-nitro-phenoxy)-acetamide (68). Yield 68%; pale yellow solid; mp 152 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.80 (s, 6H), 4.68 (s, 2H), 6.29 (m, 1H), 6.80 (m, 2H), 7.08 (d, 2H, J = 8), 8.06 (br s, 1H), 8.28 (d, 2H, J = 8).

5.5.10. *N*-Benzo[1,3]dioxol-5-yl-2-(4-nitro-phenoxy)-acetamide (69). Yield 55%; pale yellow solid; mp 171–172 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.76 (s, 2H), 5.91 (s, 2H), 6.69–7.26 (m, 5H), 8.15 (d, 2H, J = 10), 9.92 (br s, 1H).

5.5.11. *N*-(4-*tert*-Butyl-phenyl)-2-(4-nitro-phenoxy)-acetamide (70). Yield 76%; pale yellow solid; mp 146–148 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.31 (s, 9H), 4.70 (s, 2H), 7.10 (m, 2H), 7.35–7.51 (m, 4H), 8.07 (br s, 1H), 8.26 (m, 2H).

5.5.12. *N*-(4-Morpholin-4-yl-phenyl)-2-(4-nitro-phenoxy)-acetamide (71). Yield 61%; pale yellow solid; mp 170–172 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.08 (br t, 4H), 3.81 (br t, 4H), 4.66 (s, 2H), 6.85 (d, 2H, J = 9.00), 7.06 (m, 2H), 7.42 (m, 2H), 8.19–8.28 (m, 3H).

5.5.13. 2-(4-Nitro-phenoxy)-*N*-pyridin-4-yl-acetamide (72). Yield 65%; pale yellow solid; mp 154–156 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.97 (s, 2H), 7.18 (m, 2H), 7.59 (m, 2H), 8.23 (m, 2H), 8.44 (m, 2H), 10.80 (br s, 1H).

5.5.14. *N*-Benzyl-2-(4-nitro-phenoxy)-acetamide (73). Yield 85%; pale yellow solid; mp 124–125 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 4.50 (d, 2H, J = 6.2), 4.57 (s, 2H), 6.80 (br s, 1H), 6.91–6.98 (m, 2H), 7.19–7.31 (m, 5H), 8.12–8.20 (m, 2H).

5.5.15. *N*-(4-Methoxy-benzyl)-2-(4-nitro-phenoxy)-acetamide (74). Yield 68%; pale yellow solid; mp 137–138 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.72 (s, 3H), 4.26 (d, 2H, J = 6.0), 4.72 (s, 2H), 6.86 (m, 2H), 7.11–7.21 (m, 4H), 8.22 (m, 2H), 8.70 (br t, 1H).

5.5.16. *N*-Cyclohexyl-2-(4-nitro-phenoxy)-acetamide (75). Yield 68%; pale yellow solid; mp 119–120 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.09–1.90 (m, 10H), 3.81 (m, 1H), 4.49 (s, 2H), 6.22 (br d, 1H), 6.92-6.98 (m, 2H), 8.14–8.22 (m, 2H).

5.6. General procedure for the preparation of 2-(4-amino-phenoxy)-N-substituted-acetamides 76–91[29]

A solution of the appropriate 2-(4-nitro-phenoxy)-N-substituted-acetamide (**60**–**75**, 1 mmol) in the minimum amount of CH_3OH was added dropwise at 0 °C to a mixture previously prepared by adding an aqueous solution (1.5 mL) of $NaBH_4$ (2 mmol) to a suspension of 10% Pd/C (5 mg) in water (1 mL). After stirring at room temperature for 2–3 h, the reaction mixture was filtered through Celite and the solvents were removed from the filtrate under vacuum. The residue was suspended in water (20 mL) and extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was dried on Na_2SO_4, filtered and the solvent was removed under vacuum.

5.6.1. 2-(4-Amino-phenoxy)-*N*-phenyl-acetamide (76). The product was purified by crystallization with CH_2Cl_2/light petroleum 1:1: yield 80%; white solid; mp 104–105 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.50 (s, 2H), 4.68 (br s, 2H), 6.50 (m, 2H), 6.72 (m, 2H), 7.06 (m, 1H), 7.31 (m, 2H), 7.66 (m, 2H), 9.95 (br s, 1H).

5.6.2. 2-(4-Amino-phenoxy)-*N*-(4-fluoro-phenyl)-acetamide (77). The product was purified by crystallization with Et_2O/light petroleum 1:1: yield 85%; pale yellow solid; mp 106–107°C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.40 (br s, 2H), 4.52 (s, 2H), 6.63–6.68 (m, 2H), 6.68–6.83 (m, 2H), 6.99–7.08 (m, 2H), 7.50–7.57 (m, 2H), 8.28 (br s, 1H).

5.6.3. 2-(4-Amino-phenoxy)-*N*-(4-chloro-phenyl)-acetamide (78). The product was purified by column chromatography on silica gel eluting with EtOAc/light petroleum 1:1: yield 80%; white solid; mp 195–196 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 3.58 (br s, 2 H), 4.39 (s, 2H), 6.53 (d, 2H, J = 8), 6.68 (d, 2H, J = 8), 7.15 (d, 2H, J = 8), 7.45 (d, 2H, J = 8), 8.60 (br s, 1H).

5.6.4. 2-(4-Amino-phenoxy)-*N*-(4-bromo-phenyl)-acetamide (79). The product was purified by column chromatography on silica gel eluting with EtOAc/light petroleum 3:2: yield 78%; white solid; mp 189–190 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.50 (s, 2H), 4.69 (br s, 2H), 6.50 (d, 2H, J = 8), 6.72 (d, 2H, J = 8), 7.50 (d, 2H, J = 8), 7.62 (d, 2H, J = 8), 10.11 (br s, 1H).

5.6.5. 2-(4-Amino-phenoxy)-*N*-(4-iodo-phenyl)-acetamide (80). The product was purified by column chromatography on silica gel eluting with EtOAc/light petroleum 3:2: yield 85%; white solid; mp 190–191 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.50 (br s, 2 H), 4.52 (s, 2H), 6.68 (d, 2H, J = 8), 6.81 (d, 2H, J = 8), 7.40 (d, 2H, J = 8), 7.64 (d, 2H, J = 8), 8.30 (b s, 1H).

5.6.6. 2-(4-Amino-phenoxy)-*N*-(2-trifluoromethyl-phenyl)-acetamide (81). The product was purified by column chromatography on silica gel eluting with EtOAc/light petroleum 3:2: yield 69%; white solid; mp 187–188 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.50 (br s, 2H), 4.57 (s, 2H), 6.68 (d, 2H, J = 8), 6.83 (d, 2H, J = 8), 7.25-7.65 (m, 3H), 8.40 (d, 1H, J = 10), 8.99 (br s, 1H).

5.6.7. 2-(4-Amino-phenoxy)-*N*-(4-methoxy-phenyl)-acetamide (82). The product was purified by crystallization from CH_2Cl_2/light petroleum: yield 67%; white solid; mp 225–228 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.50 (br s, 2H), 3.79 (s, 3H), 4.75 (s, 2H), 6.50 (d, 2H, J = 8), 6.80 (d, 2H, J = 8), 7.56 (d, 2H, J = 8), 7.99 (br s, 1H), 8.15 (d, 2H, J = 8).

5.6.8. 2-(4-Amino-phenoxy)-*N*-(3,4-dimethoxy-phenyl)-acetamide (83). The product was purified by column chromatography on silica gel eluting with EtOAc/light petroleum 3:2: yield 83%; white solid; mp 221–222 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.53 (br s, 2H), 3.80 (s, 3H), 3.84 (s, 3H), 4.75 (s, 2 H), 6.50–7.44 (m, 5H), 8.12 (br s, 1H), 8.17 (d, 2H, J = 8).

5.6.9. 2-(4-Amino-phenoxy)-*N*-(3,5-dimethoxy-phenyl)-acetamide (84). The product was purified by column chromatography on silica gel eluting with EtOAc/light petroleum 3:2: yield 72%; white solid; mp 215–217 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.50 (br s, 2H), 3.79 (s, 6H), 4.51 (s, 2H), 6.27 (m, 1H), 6.65–6.84 (m, 6H), 8.25 (br s, 1H).

5.6.10. 2-(4-Amino-phenoxy)-*N*-benzo[1,3]dioxol-5-yl-acetamide (85). The product was purified by column chromatography on silica gel eluting with EtOAc/light petroleum 1:1: yield 76%; pale yellow solid; mp 210–211 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.41 (br s, 4H), 5.92 (s, 2H), 6.50 (d, 2H, J = 8), 6.70 (m, 2H), 7.00 (d, 2H, J = 8), 7.30 (m, 1H), 9.68 (b s, 1H).

5.6.11. 2-(4-Amino-phenoxy)-*N*-(4-*tert*-butyl-phenyl)-acetamide (86). The product was purified by crystallization with Et_2O/light petroleum: yield 84%; white solid; mp 119–120 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.30 (s, 9H), 4.52 (s, 2H), 6.66 (d, 2H, J = 8.8), 6.81 (d, 2H, J = 8.8), 7.35 (d, 2H, J = 8.8), 7.48 (d, 2H, J = 8.6), 8.30 (br s, 1H).

5.6.12. 2-(4-Amino-phenoxy)-*N*-(4-morpholin-4-yl-phenyl)-acetamide (87). The product was purified by crystallization with Et_2O/light petroleum 1:1: yield 72%; white solid; mp 188–190 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 3.09–3.14 (br m, 4H), 3.83–3.88 (br m, 4H), 4.51 (s, 2H), 6.63–6.68 (m, 2H), 6.79–6.91 (m, 4H), 7.46 (d, 2H, J = 9), 8.19 (br s, 1H).

5.6.13. 2-(4-Amino-phenoxy)-*N*-pyridin-4-yl-acetamide (88). The product was purified by crystallization with EtOAc/Et_2O: yield 45%; white solid; mp 148–149 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.56 (s, 2H), 4.70 (br s, 2H), 6.49–6.55 (m, 2H), 6.69–6.75 (m, 2H), 7.65 (m, 2H), 8.43 (m, 2H), 10.39 (br s, 1H).

5.6.14. 2-(4-Amino-phenoxy)-*N*-benzyl-acetamide (89). The product was purified by crystallization from CH_2Cl_2/light petroleum: yield 72%; white solid; mp 108–111 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 4.30–4.35 (m, 4H), 4.66 (br s, 2H), 6.48 (d, 2H, J = 8.4), 6.68 (d, 2H, J = 8.8) 7.19–7.32 (m, 5H), 8.52 (br t, 1H).

5.6.15. 2-(4-Amino-phenoxy)-*N*-(4-methoxy-benzyl)-acetamide (90). The product was purified by crystallization with Et_2O/light petroleum: yield 73%; white solid; mp 103–107 °C; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 3.72 (s, 3H), 4.25 (d, 2H, J = 6.00), 4.34 (s, 2H), 4.65 (br s, 2H), 6.47–6.52 (m, 2H), 6.67–6.71 (m, 2H), 6.87–6.82 (m, 2H), 7.18–7.14 (m, 2H), 8.46 (br t, 1H).

5.6.16. 2-(4-Amino-phenoxy)-*N*-cyclohexyl-acetamide (91). The product was purified by crystallization with EtOAc/Et_2O/light petroleum 1:1:1: yield 55%; white solid; mp 168–169°C ; ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 1.13–1.94 (m, 10H), 3.80 (m, 1H), 4.37 (s, 2H), 6.40 (br s, 1H), 6.61–6.77 (m, 4H).

5.7. Biology experiments

All synthesised compounds have been tested, by radioligand binding assay, for their affinity to human A_1, A_{2A} and A_3 adenosine receptors and for their potency, in a cAMP assay, to human A_{2B} subtypes.

5.7.1. Binding assays. The expression of the human A_1, A_{2A}, A_{2B} and A_3 receptors in CHO cells has been previously described.[30] The cells were grown adherently and maintained in Dulbecco's Modified Eagle's Medium with nutrient mixture F12 (DMEM/F12) without nucleosides, containing 10% fotal 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. Cells were split two or three times weekly at a ratio between 1:5 and 1:20. 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 and centrifuged for 30 min at 100,000*g*. The membrane pellet was resuspended in 50 mM Tris–HCl buffer, pH 7.4, and incubated with 2 IU/mL of adenosine deaminase for 30 min at 37 °C. Then the suspension was frozen at −80 °C and the protein concentration was determined according to a Bio-Rad method[31] with bovine albumin as a standard reference. Binding of 1 nM [^{3}H]-CHA to hA_1CHO cells (50 μg of protein/assay) was performed using 50 mM Tris–HCl buffer, pH 7.4, and at least 6–8 different concentrations of agonists studied for an incubation time of 150 min at 25 °C.[32] Non-specific binding was determined in the presence of 10 μM CHA and was about 20% of the total binding. Inhibition binding experiments of [^{3}H]-CGS 21680 to hA_{2A}CHO cells (100 μg of protein/assay) were performed using 50 mM Tris–HCl buffer, 10 mM $MgCl_2$, pH 7.4, and at least 6–8 different concentrations of agonists studied for an incubation time of 180 min at 25 °C.[33] Non-specific binding was determined in the presence of 10 μM CGS 21680 and was about 25% of the total binding. Binding of [^{125}I]-ABMECA to hA_3CHO cells (50 μg of protein/assay) was performed using 0.2 mL of 50 mM Tris–HCl buffer, 10 mM $MgCl_2$, EDTA 1 mM, pH 7.4, and at least 6–8 different concentrations of tested compounds for an incubation time of 60 min at 37 °C.[27] Non-specific binding was determined in the presence of 1 μM ABMECA and was about 20% of total binding. Separation of bound from free radioligand was performed by rapid filtration through Whatman GF/B filters which were washed three times with ice-cold buffer. Filter bound radioactivity was measured by scintillation spectrometry after addition of 5 mL of Aquassure.

5.7.2. Cyclic AMP assays. For cell preparation CHO cells transfected with human A_{2B} receptors were washed with PBS, diluted trypsin and centrifuged for 10 min at 200 g. The pellet containing the CHO cells (1×10^6 cells/assay) was resuspended in incubation mixture (mM): NaCl 15, KCl 0.27, NaH_2PO_4 0.037, 2 IU/mL adenosine deaminase and 4-(3-butoxy-4methoxy-benzyl)-2-imidazolidone (Ro 20-1724) as phosphodiesterase inhibitor and preincubated for 10 min in a shaking bath at 37 °C. After the preincubation time at 37 °C the examined ligands (1 nM–10 μM) were added to the mixture and incubated for a further 5 min. 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.[34] Samples of cyclic AMP standard (0–10 pmol) were added to each test tube containing the incubation buffer (trizma base 0.1 mM, aminophylline 8.0 mM, 2-mercaptoethanol 6.0 mM, pH 7.4) and [^{3}H]cAMP in a total volume of 0.5 mL. The binding protein, previously prepared from beef adrenals, was incubated at 4 °C for 150 min, and after the addition of charcoal was centrifuged at 2000*g* for

10 min. The clear supernatant was counted in a Beckman scintillation counter.

Inhibitory binding constant, K_i, values were calculated from the IC_{50} values according to Cheng and Prusoff equation[35] $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where $[C^*]$ is the concentration of the radioligand and K_D^* its dissociation constant. A weighted non-linear least-squares curve fitting program LIGAND[36] was used for computer analysis of inhibition binding experiments. The EC_{50} values obtained in the 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). All experimental data are expressed as means ± standard error of the mean (Se mean) of three or four independent experiments performed in duplicate.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2007.01.055.

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