Synthesis and Biological Evaluation of Novel N^6-[4-(Substituted)sulfonamidophenylcarbamoyl]adenosine-5′-uronamides as A_3 Adenosine Receptor Agonists

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A new series of 1-deoxy-1-[(6-(4-(substituted-aminosulfonyl)phenyl)amino)carbonylamino-9*H*-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamides (**83**−**102**) have been synthesized and tested at the human A_3 adenosine receptor subtype. All the derivatives described in this work displayed affinity versus this receptor in the nanomolar range and good selectivity versus A_1 adenosine receptor subtype, confirming that the *p*-sulfonamido moiety positively affected the activity of the molecules. The best substituents at the sulfonamido nucleus were found to be small alkyl groups, like methyl, isopropyl, ethyl, or allyl moieties (compounds **96**−**100**), whereas monosubstitution at the amino group led to a decrease in A_3 affinity values. The selectivity versus A_1 adenosine receptor subtype is increased when the amino group in the sulfonamido core is represented by a hydrogenated heterocyclic ring like piperidine, morpholine, or pyrroline. Bulky groups, like adamantane and alkyl chains with more than four carbon atoms, are detrimental for the affinity and the selectivity of the A_3 adenosine receptor agonists described here.

Introduction

Adenosine exerts a number of physiological functions through activation of four cell membrane receptors classified as A_1, A_{2A}, A_{2B}, and A_3.[1] Although all adenosine subclasses belong to the G protein-coupled receptors, they are associated with different second messenger systems.[1] The A_3 receptor has been cloned from a rat brain cDNA library,[2] and this subtype has a characteristic second messenger profile, in that it has been shown to mediate adenylyl cyclase inhibition and phospholipase C activation. Several studies indicate that the adenosine A_3 receptor may play a basic role in the modulation of cerebral ischemia,[3] inflammation,[4] hypotension,[5] ischemic heart preconditioning,[6] and asthma.[7] These and other findings make the A_3 receptor a promising therapeutic target for regulation of cell growth,[7] apoptosis, and study in leukemic Jurkat T cells,[8] the human malignant melanoma A375 cell line, and human neutrophils.[9]

The cloned human A_3 adenosine receptor was first characterized with N^6-(4-amino-3-[^{125}I]iodobenzyl)adenosine.[10] In a preliminary study, it was observed that the presence of the N^6-benzyl group in the adenosine structure determines a significant increase in A_3 receptor affinity and selectivity.[11] Additionally, it was shown that a methyl uronamide moiety at the 5′-position confers superior affinity and selectivity at the A_3 adenosine receptor. This combination of substitutions led to the discovery of N^6-(3-iodo-benzyl)adenosine-5′-*N*-methyluronamide (IB-MECA) as the first potent and selective agonist for rat A_3 adenosine receptors. IB-MECA is 50-fold selective for A_3 versus either A_1 or A_{2A} receptors. Starting from this observation, a selective radioligand, the [^{125}I]-N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide ([^{125}I]-AB-MECA), was developed.[12] This compound, endowed with high affinity but low selectivity, is still considered the standard agonist for the A_3 adenosine receptor. In an evolution of this work, the effect of substitution at the C-2 position was studied.[13] It was observed that substitutions at the 2-position with halogen, methylamino, or thiomethyl groups increase both affinity and selectivity at the A_3 receptor subtype. Other studies have demonstrated that substituents at the 3-position on the benzyl group are well tolerated. These studies suggest that both affinity and selectivity for the A_3 receptor subtype appears to be related more to the type of substitution on the phenyl ring than to the position of the substituent. A large series of purine and ribose-modified adenosine analogues have been studied for their affinity at rat A_3 adenosine receptors, but none of these compounds shows a better profile with respect to the reference compounds. Several modifications utilized[14] have shown that (i) deaza derivatives are well tolerated at the A_3 receptor; (ii) substitutions at the 8-position are detrimental, in terms of affinity; (iii) carbocyclic nucleosides exhibit, in general, weak affinity at all receptor subtypes; (iv) replacement of the 6-$NHCH_2$ linkage with hydroxylamino or hydrazino moieties is well tolerated; and (v) substitution of the 4′-hydrogen with a methyl group retains agonist activity and selectivity at rat A_3 adenos-

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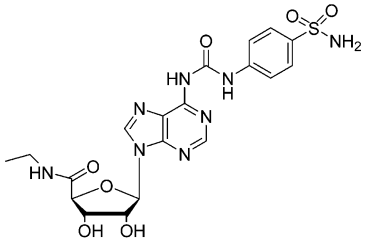
ine receptors. Recently, our group has proposed that the N^6-benzylamino chain can be successfully replaced by n urea moiety.[15,16] While N^6-alkylcarbamoyl derivatives have been reported to exhibit low affinity and selectivity at all adenosine receptor subtypes, interesting results can be obtained with the N^6-arylcarbamoyl substituted analogues. Among this new series of compounds, the 1-deoxy-1-[6-[[[4-sulfonamido-phenyl)-amino]carbonyl]-amino]-9*H*-purin-9-yl]-*N*-ethyl-*β*-D-ribofuranuronamide (compound **A**)[16] showed an interesting increase in its selectivity versus A_1 receptor [K_i (rA_1) = 453 nM; K_i (rA_3) = 9.73 nM], whereas substitution of the amino group of the sulfonamido radical with some heteroaryl groups, like pyridine, pyrimidine, or isoxazole, led to derivatives with modest affinity at rat A_3 adenosine receptor subtype.[16]



A

K_i (hA_3)= 14.8 nM

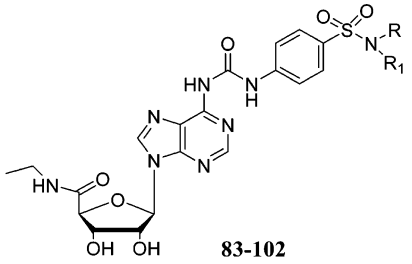
hA_1/hA_3= 2.03

Starting from these observations, our group designed an enlarged series of substituted-*p*-sulfonamido phenylcarbamoyl adenosine analogues with a conserved ethyl uronamide group at the 5′-position as A_3 adenosine receptor agonists. The new adenosine agonists were prepared (compounds **83**−**102**) as sulfonamido derivatives substituted with aliphatic groups (cyclic or linear) or aromatic radicals on the sulfonamido nitrogen in order to better evaluate the importance of this position and the structure−activity relationships of A_3 adenosine receptor agonists. The structures of these derivatives are indicated in Table 1. These compounds, including the parent compound **A**, have been pharmacologically characterized, determining their binding affinity and selectivity at CHO cells transfected with human A_1, A_{2A}, and A_3 adenosine receptors using agonists as radioligands that preferentially recognize the high affinity state in order to better evaluate the pharmacological characteristics of the compounds analyzed.

Chemistry

The preparation of the *N*-substituted-sulfonamidophenyl derivatives of NECA, compounds **83**−**102**, was performed following the general synthetic strategy depicted in Scheme 1. The amino group at the 6-position of NECA is not very reactive; therefore, it was necessary to protect the hydroxyl groups of the ribose moiety during the nucleophilic reaction with the appropriate isocyanate. This was achieved by protection of the 2′- and 3′-hydroxyl groups as the isopropylidene derivative (**1a**). The isocyanates were prepared by reacting the corresponding substituted anilines (**23**−**42**) using trichloromethylchloroformate as previously described[17] and reported in Scheme 2. Reaction of the 2′,3′-*O*-isopropylidene-protected NECA **1a** with the isocyanates in anhydrous dioxane as solvent at reflux afforded the adducts **63**−**82** in a quite good yield. These intermediates were converted into the final desired compounds **83**−**102** by deprotection of the ribosyl moiety in aqueous 1 N HCl and dioxane at 65 °C.

Table 1. New A_3 Adenosine Receptor Agonists Synthesized (**83**−**102**)



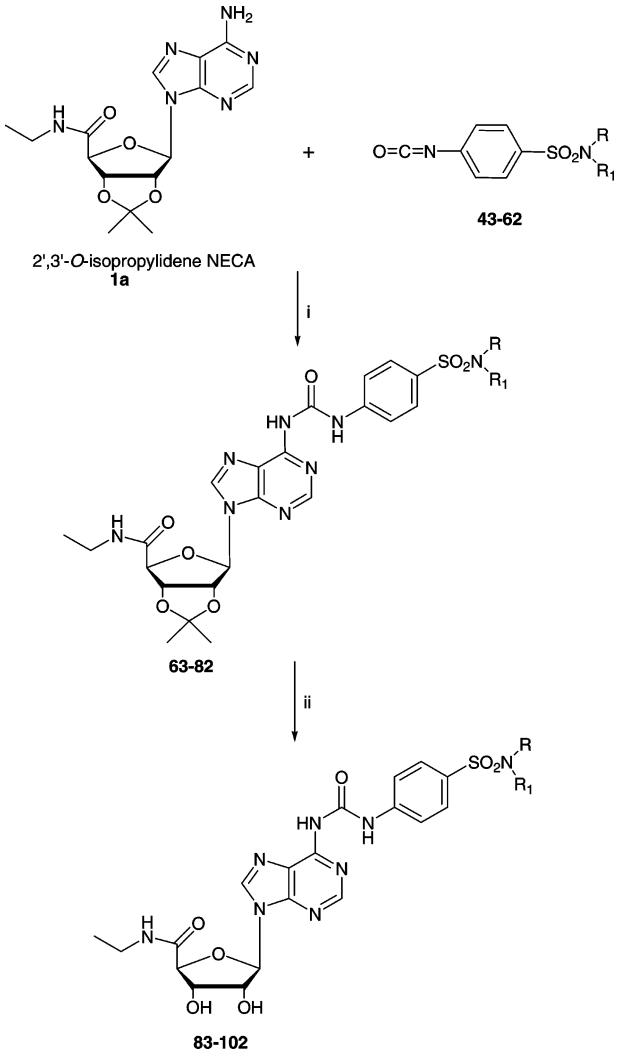
83-102

Compound	R	R_1
83	R=R1	
84	R=R1	
85	Et	H
86	Bn	H
87	n-C_5H_{11}	H
88	4-MeO-Ph	H
89	c-C_3H_7	H
90	R=R1	
91		
92	t-C_4H_9	H
93	Adamantyl	H
94	c-C_6H_{11}	H
95	c-C_5H_9	H
96	Me	
97	Me	i-C_3H_7
98	Me	Me
99		
100	Et	Et
101	R=R1	
102	n-C_3H_7	n-C_3H_7

Results and Discussion

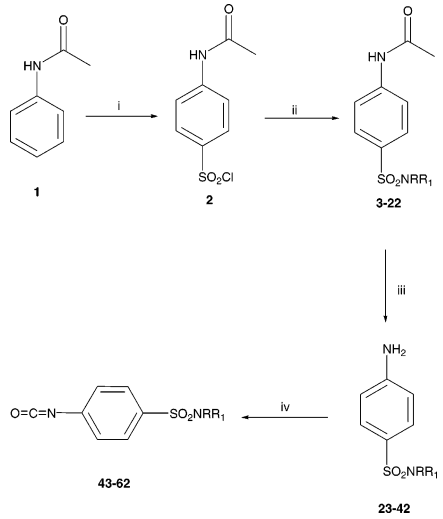
All of the synthesized compounds were evaluated in radioligand binding assays for affinity at the human A_1, A_{2A}, and A_3 adenosine receptors. The results are summarized in Table 2. The NECA derivative *N*-6 substituted with a simple phenylsulfonamido function (compound **A,** see Table 2) exhibits good affinity for the hA_3 adenosine receptor subtype but, differently from the data reported from the rat receptors, low selectivity (hA_1/hA_3 = 2.03 and hA_{2A}/hA_3 = 6.43). The new synthesized compounds reported in this work are all functionalized at the 6-position of the purine moiety with a mono- or bis-substituted sulfonamidopheny-

Scheme 1[a]



[a] Reagents: (i) dioxane, reflux, 18 h; (ii) 1 N hydrochloric acid, 65 °C.

Scheme 2[a]



[a] Reagents: (i) HSO_3Cl, 100 °C; (ii) substituted amines, dioxane; (iii) aqueous 20% HCl; (iv) $Cl_3COCOCl$, dioxane.[17]

lamino function. The substituents introduced are aromatic functions, small alkyl chains like methyl, ethyl, allyl, or isopropyl, cycloalkyl functions, or hydrogenated heterocyclic rings like morpholine, piperidine, or pyrroline. All of the newly synthesized compounds showed affinity at the human A_3 adenosine receptor in the nanomolar range and high selectivity versus the hA_1 adenosine receptor subtype demonstrating that the sulfonamido functions positively influence the affinity and substitutions at the sulfonamido moiety increase the selectivity versus the A_1 adenosine receptor subtype. From an analysis of the data, it seems that the presence of an *N*-hydrogen on the sulfonamido moiety is quite detrimental for the A_3 affinity. When the monosubstitution at the sulfonamido moiety is a carbon chain where the number of carbon atoms is more than four, affinity toward the A_3 adenosine receptor subtype is diminished (compound **87**). In general, disubstitution of the amino group provides better values in terms of A_3 interaction. The best results in terms of A_3 affinity are achieved when the two substituents on the sulfonamido nitrogen moiety are short alkyl chains (compounds **96**−**100**) like methyl, allyl, ethyl, and isopropyl groups. Selectivity versus the A_1 adenosine receptor subtype is increased when the amino group of the sulfonamido moiety is substituted by a reduced heterocycle like piperidine, morpholine, or pyrroline (compounds **83**, **84**, **90**) or when halogen atoms are present on the alkyl chains (compound **91**). The presence of a basic nitrogen at the sulfonamido group seems to be very important for the A_3 interaction. Compounds **83** and **90**, which respectively hold radicals like the piperidine and pyrroline moieties, showed a better affinity than the corresponding N-monosubstituted compounds **94** and **95**. The introduction of a sterically bulky substituent, such as the adamantyl group (compound **93**), proved detrimental for the interaction with the A_3 adenosine receptor binding site.

Table 2. Affinities of Synthesized Derivatives (**83**−**102**) in Radioligand Binding Assays at Human A_1, A_{2A}, and A_3 Adenosine Receptors

compound	K_i (A_1)[a]	K_i (A_{2A})[b] or % inhibition	K_i (A_3)[c]	A_1/A_3
IB-MECA	54 (49−59)	56 (48−64)	1.1 (0.8−1.4)	49
Cl-IB-MECA	823 (778−870)	448 (365−550)	0.33 (0.3−0.4)	2500
NECA	18.2 (14.2−23.4)	12.2 (8.4−17.7)	34.8 (26.7−45.3)	0.52
A	30.1 (22.9−39.6)	95.2 (86.4−105.0)	14.8 (10.2−21.5)	2.03
83	750 (664−849)	>1000 (15%)	17 (13−22)	44
84	725 (620−849)	>1000 (20%)	20 (12−34)	36.25
85	250 (179−348)	>1000 (34%)	23 (17−32)	10.86
86	300 (226−398)	>1000 (30%)	24 (18−33)	12.5
87	445 (369−537)	>1000 (32%)	80 (66−97)	5.56
88	280 (192−409)	>1000 (34%)	23 (15−34)	12.17
89	290 (272−309)	>1000 (31%)	25 (17−36)	11.6
90	700 (614−799)	>1000 (23%)	22 (17−29)	31.8
91	730 (602−886)	>1000 (27%)	23 (17−32)	31.74
92	250 (166−374)	>1000 (32%)	20 (11−36)	12.5
93	240 (193−297)	>1000 (31%)	35 (28−43)	7.5
94	350 (333−434)	>1000 (25%)	23 (18−28)	15.22
95	415 (304−568)	>1000 (19%)	24 (16−36)	17.3
96	206(179−238)	>1000 (10%)	10 (6−16)	20.6
97	325 (282−374)	>1000 (13%)	8 (6−11)	40.6
98	280 (230−342)	>1000 (21%)	12 (10−16)	23.33
99	380 (321−451)	>1000 (15%)	10 (5−17)	38
100	350 (307−398)	>1000 (28%)	9 (4−18)	38.89
101	250 (171−364)	>1000 (17%)	19 (14−26)	13.16
102	350 (288−426)	>1000 (15%)	14 (10−21)	25

[a] Displacement of [^{3}H]CHA binding to hA_1 adenosine receptors expressed in CHO cells. K_i values in nM. [b] Displacement of [^{3}H]CGS21680 binding to hA_{2A} adenosine receptors expressed in CHO cells. K_i values in nM. [c] Displacement of [^{125}I]-AB-MECA binding to hA_3 adenosine receptors expressed in CHO cells. K_i values in nM.

Table 3. Functional Assay. Inhibitory Effect of Selected Agonists on cAMP Production Stimulated by Forskolin 10 μM in hA_3 CHO Cells

compounds	IC_{50} (nM)
Cl-IB-MECA	2.2 (1.9−2.6)
A	81 (69−97)
96	42 (33−52)
97	55 (46−65)
99	65 (56−74)
100	50 (42−61)

The ability of some of the compounds examined to inhibit forskolin-stimulated 10 μM cAMP production via the adenosine A_3 receptor was also studied. In particular, a functional assay based on the evaluation of cyclic AMP levels in $hCHOA_3$ cells indicated that some of the new ligands were true A_3 adenosine agonists and were able to inhibit cyclic AMP accumulation showing IC_{50} values in the nanomolar range (Table 3). The compounds tested showed almost partial inhibition of the cAMP production comparable with the reference full agonist Cl-IB-MECA (2-chloro-N6-(3-iodobenzyl)-5′-methylcarboxamidoadenosine). All the adenosine derivatives showed IC_{50} values in the range 42−80 nM and a maximal inhibition in the range 55−65% at the concentration 1 μM. Under the same conditions, 1 μM Cl-IB-MECA caused an inhibition of cyclic AMP levels of 88%.

Conclusion

In the present study we have described the affinity at human A_1, A_{2A}, and A_3 adenosine receptor subtypes of a new series of N^6-[4-(substituted)sulfonamidophenylcarbamoyl]adenosine-5′-uronamides. All these molecules displayed high affinity versus the hA_3 receptor in the nanomolar range and an increase of selectivity versus the hA_1 subtype with respect to the parent compound **A**. Both affinity and selectivity were increased when the substituents on the sulfonamido moiety were represented by small alkyl groups or by hydrogenated heterocyclic rings.

Experimental Section

Chemistry. Reaction courses and product mixtures were routinely monitored by thin-layer chromatography (TLC) on silica gel (precoated F_{254} Merck plates) and visualized with aqueous potassium permanganate or ethanolic ninhydrin solutions. Infrared spectra (IR) were measured on a Perkin-Elmer 257 instrument. ^{1}H NMR were determined in CD_3OD or DMSO-d_6 solutions with a Bruker AC 200 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 with Merck 60−200 mesh silica gel. All products reported showed ^{1}H NMR spectra in agreement with the assigned structures. 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.

General Procedure for the Preparation of 1-Deoxy-1-[6-[[[(4-(substituted-aminosulfonyl)phenyl)amino]carbonyl]amino]-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide 63−82. 2′,3′-*O*-Isopropylidene-NECA **1a** (0.286 mmol) was dissolved in freshly distilled dioxane (5 mL), and then the appropriate isocyanate (0.572 mmol) and a catalytic amount of TEA (two drops) were added. The mixture was refluxed under argon for 18 h. Next the solvent was removed under reduced pressure, and the residue was purified by flash chromatography (EtOAc/light petroleum 8:2) to afford the desired compounds **63**−**82**.

1-Deoxy-1-[6-[[[(4-(piperidin-1-ylsulfonyl)phenyl)amino]carbonyl]amino]-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (63): yield 46%; white solid; mp 195−197 °C; ^{1}H NMR (CD_3OD) δ 0.62 (t, 3H, J = 7.2), 1.21 (s, 3H), 1.32 (s, 3H), 1.42 (m, 5H), 1.59 (m, 8H), 1.68 (s, 1H), 2.79−2.81 (m, 2H), 5.55 (m, 1H), 5.68 (d, 1H), 6.45 (s, 1H), 7.71−7.74 (d, 2H, J = 9), 7.86−7.89 (d, 2H, J = 9), 10.52 (bs, 1H), 12.10 (bs, 1H).

1-Deoxy-1-[6-[[[(4-(morpholin-1-yl-sulfonyl)phenyl)-amino]carbonyl]amino]-9*H*-purin-9-yl]-*N*-ethyl-2,3-*O*-(1-methylethylidene)-β-D-ribofuranuronamide (64): yield 49%; white solid; mp 211 °C; ^{1}H NMR (CD_3OD) δ 0.58−0.63 (t, 3H, J = 7.3), 1.42 (s, 3H), 1.59 (s, 3H), 2.80 (m, 3H), 2.95−2.98 (m, 4H, J = 5), 3.70−3.72 (m, 4H, J = 4), 4.68 (s, 1H), 5.53 (m, 1H), 5.68 (d, 1H, J = 4), 6.48 (s, 1H), 7.78 (m, 2H), 7.91 (m, 2H), 8.46 (s, 1H), 8.65 (s, 1H), 10.52 (bs, 1H), 12.10 (bs, 1H).

General Procedure for the Preparation of 1-Deoxy-1-[6-[[[(4-(substituted-aminosulfonyl)phenyl)amino]carbonyl]amino]-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide 83−102. A solution of the isopropylidene derivatives **63**−**82** (0.09 mmol) in aqueous 1 N HCl (5 mL) and dioxane (5 mL) was stirred at 65 °C for 1 h. The solvent was then removed at reduced pressure, and the residue was purified by crystallization from ethanol to afford the desired compounds **83**−**102** as solids.

1-Deoxy-1-[6-[[[(4-(piperidin-1-ylsulfonyl)phenyl)amino]carbonyl]amino]-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (83): yield 98%; white solid; mp 176−178 °C; ^{1}H NMR (DMSO-d_6) d 1.07 (t, 3H, J = 7), 1.35−1.37 (m, 2H), 1.48−1.52 (m, 4H), 2.85.2.88 (m, 4H), 3.16−3.26 (m, 2H), 4.23 (bs, 1H), 4.36 (s, 1H), 4.66−4.69 (m, 1H), 5.65−5.79 (m, 2H), 6.10 (m, 1H), 7.70 (d, 2H, J = 8), 7.88 (d, 2H, J = 8), 8.45 (m, 1H), 8.49 (s, 1H), 8.73 (s, 1H), 10.50 (bs, 1H), 12.05 (bs, 1H). Anal. ($C_{24}H_{30}N_8O_7S$) C, H, N.

1-Deoxy-1-[6-[[[(4-(morpholin-1-ylsulfonyl)phenyl)amino]carbonyl]amino]-9*H*-purin-9-yl]-*N*-ethyl-β-D-ribofuranuronamide (84): yield 97%; white solid; mp 204−205 °C; ^{1}H NMR (DMSO-d_6) d 1.06 (t, 3H, J = 7); 2.84−2.86 (m, 4H); 3.16−3.26 (m, 2H); 3.61−3.64 (m, 4H); 4.23 (bs, 1H); 4.36 (s, 1H); 4.66−4.69 (m, 1H); 5.65−5.79 (m, 2H); 6.09 (d, 1H, J = 7); 7.76 (d, 2H, J = 8); 7.88 (d, 2H, J = 8); 8.46−8.47 (m, 1H); 8.74 (s, 1H); 8.81 (s, 1H); 10.55 (bs, 1H); 12.1 (bs, 1H). Anal. ($C_{23}H_{28}N_8O_8S$) C, H, N.

Biology Experiments. All synthesized compounds have been tested for their affinity at human A_1, A_{2A}, and A_3 adenosine receptor subtypes cloned in CHO cells. Some of these compounds have been studied to evaluate their potency versus the hA_3 CHO cells.

Human Cloned A_1, A_{2A}, and A_3 Adenosine Receptor Binding Assay. The expression of the human A_1, A_{2A}, and A_3 receptors in CHO cells has been previously described.[18] The cells were grown adherently and mantained in Dulbecco's modified Eagles medium with nutrient mixture F12 (DMEM/F12) without nucleosides, containing 10% fetal calf serum, penicillin (100 units/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 2 or 3 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 with a Polytron, and the homogenate was spun for 10 min at 1000*g*. The supernatant was then centrifuged for 30 min at 100000*g*. The membrane pellet was resuspended in 50 mM Tris HCl buffer pH 7.4 (for A_3 adenosine receptors: 50 mM Tris HCl, 10 mM $MgCl_2$, 1 mM EDTA) and incubated with 3 IU/mL of adenosine deaminase for 30 min at 37 °C. Then the suspension was frozen at −80 °C. HEK 293 cells

transfected with the human recombinant A_{2A} adenosine receptor were obtained from Receptor Biology, Inc. (Beltsville, MD). Binding of [^{3}H]CHA to CHO cells transfected with the human recombinant A_1 adenosine receptor was performed according to the method previously described by Klotz et al.[19] Displacement experiments were performed for 120 min at 25 °C in 0.2 mL of 50 mM Tris HCl buffer pH 7.4 containing 1 nM [^{3}H]-CHA, diluted membranes (50 μg of protein/assay), and at least 6−8 different concentrations of agonists studied. Nonspecific binding was determined in the presence of 10 μM CHA, and this was always ≤10% of the total binding. Binding of [^{3}H]-CGS21680 to CHO cells transfected with the human recombinant A_{2A} adenosine receptors (50 μg of protein/assay) was performed using 0.2 mL of 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 30 min at 25 °C. Nonspecific binding was determined in the presence of 50 μM NECA and was about 20% of total binding. Binding of [^{125}I]-AB-MECA to CHO cells transfected with the human recombinant A_3 adenosine receptors was performed according to Varani et al.[8] Competition experiments were carried out in duplicate in a final volume of 100 μL in test tubes containing 1 nM [^{125}I]-AB-MECA, 50 mM Tris HCl buffer, 10 mM $MgCl_2$, 1 mM EDTA pH 7.4 and 100 μL of diluted membranes (50 μg of protein/assay) and at least 8−10 different concentrations of examined agonists. Incubation time was 120 min at 4 °C, according to the results of previous time-course experiments.

Bound and free radioactivity were separated by rapid filtration through Whatman GF/B glass-fiber filters which were washed three times with ice cold buffer. The filter bound radioactivity was counted in a Beckman LS-1800 spectrometer (efficiency 55%). The protein concentration was determined according to a Bio-Rad method[20] with bovine albumin as a standard reference.

Measurement of Cyclic AMP Levels in hCHOA_3 Cells. CHO cells transfected with human A_3 adenosine receptors were washed with phosphate-buffered saline and diluted tripsine and centrifuged for 10 min at 200*g*. The pellet containing 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 potencies of agonists studied were determined by their capability to inhibit cyclic AMP production stimulated by forskolin (10 μM). 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 pmol) 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 the samples were centrifuged at 2000*g* for 10 min. The clear supernatant was counted in a Beckman scintillation counter.

Data Analysis. Inhibitory binding constant, K_i, values were calculated from IC_{50} values according to the Cheng and Prusoff equation,[21] $K_i = IC_{50}/(1 + [C^*]/K_D^*)$, where [C*] is the concentration of the radioligand and K_D* its dissociation constant. A weighted nonlinear least-squares curve fitting program LIGAND[22] was used for computer analysis of saturation and inhibition experiments. IC_{50} values in the cAMP assay were calculated with the nonlinear least-squares curve fitting program Prism Graph PAD.

Data are expressed as geometric mean, with 95% or 99% confidence limits in parentheses.

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Supporting Information Available: Selected yields, mp, and ^{1}H NMR data of synthesized compounds. Elemental analyses of **83**−**102**. This material is available free of charge via Internet at http://pubs.acs.org.

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