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Synthesis and Biological Activity of New Potential Agonists for the Human Adenosine A_{2A} Receptor

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New adenosine derivatives have been synthesized and tested as putative agonists of adenosine receptors. Compounds **2**–**6** derive from the introduction of several types of substituents (electron donating, electron withdrawing, and halogens) in the para-position of the phenyl ring of the parent compound **1**, and compound **7** lacks the hydroxyl group of amino alcohol **1**. In radioligand binding assays using recombinant human A_1, A_{2A}, A_{2B}, and A_3 receptors, all compounds showed very low or negligible affinity for A_1 and A_{2B} receptors but compounds **3**, **5**, and **7** displayed a remarkably potent affinity for the A_{2A} receptor with K_i values of 1–5 nM. Bromo derivative **3** displayed a selectivity $A_1/A_{2A} = 62$ and $A_3/A_{2A} = 16$ whereas the presence of a hydroxyl group (compound **5**) improved the selectivity of A_1/A_{2A} and A_3/A_{2A} to 120- and 28-fold, respectively. When the methoxy derivative **4** lacks the hydroxyl group on the side chain (compound **7**), the binding affinity for A_{2A} is increased to 1 nM, improving selectivity ratios to 356- and 100-fold against A_1 and A_3, respectively. In Chinese hamster ovary cells transfected with human A_{2A} and A_{2B} receptors, most compounds showed a remarkable activity for the A_{2A} receptor, except chloro derivative **2**, with EC_{50} values ranging from 1.4 to 8.8 nM. The compounds behaved as good A_{2A} agonists, and all were more selective than 5′-(N-ethylcarboxamino)adenosine (NECA), with A_{2B}/A_{2A} ratios of cAMP accumulation ranging from 48 for compound **2** to 666 for compound **7** while the corresponding A_{2B}/A_{2A} ratio for NECA was only 9. Compounds **1**, **3**, **5**, and **7** also displayed higher selectivities than NECA up to 100-fold in isolated aortas of rat and guinea pig. In guinea pig tracheal rings precontracted by carbachol, compounds **2** and **4** were more potent than adenosine (100-fold) and NECA (10-fold), whereas compounds **1** and **7** displayed similar effects to NECA. Pretreatment of the tracheal rings with A_2, A_{2A}, and A_{2B} receptor antagonists 3,7-dimethyl-L-propargylxanthine, 8-(3-chlorostyryl)caffeine, and alloxazine produced a marked inhibition of the tracheal relaxations induced by compounds **1**, **2**, and **4**, but none of the compounds showed selectivity toward any of the adenosine receptors.

Introduction

Adenosine is an ubiquitous purine nucleoside, which affects a wide range of physiological functions, such as vasodilatation, vasoconstriction, inhibition of platelet aggregation, inhibition of lymphocyte functions, inhibition of insulin release, inhibition of lipolysis, cardiac depression, etc.[2–5] According to the chronological discovery, four major subclasses of adenosine receptors A_1, A_{2A}, A_{2B}, and A_3 have been identified and cloned from several species including rat, dog, mouse, and human.[6] All four classes are coupled to adenylyl cyclase; thus, activation of the A_1 and A_3 receptors leads to an inhibition of this enzyme diminishing the production of the second messenger cyclic AMP. On the contrary, A_{2A} and A_{2B} receptors stimulate adenylyl cyclase via G-protein coupling to produce cyclic AMP.[4] A_1 and A_{2A} receptors are considered "high affinity" receptors, and A_{2B} and A_3 are considered "low affinity" receptors.[7]

The fundamental nature of the processes by which agonist binding to cell membrane receptors promotes biological activation is a central issue in molecular pharmacology. All agonists are closely related in structure to the endogenous ligand adenosine. Substitution at C-8 of the adenine ring of adenosine resulted in agonists having decreased affinity for all adenosine receptors, presumably due to a conformational change of the nucleoside from the anti-conformation to the less favorable syn-conformation.[7,8] The presence of nitrogen atoms at 3- and 7-positions is required for the high affinity of adenosine in all subtypes.[7–9] 1-Deazaadenosine showed reduced adenosine receptor affinity, but 1-deaza-5′-(N-ethylcarboxamino)adenosine (NECA) retained activity. N^6-cycloalkyl-substituted 1-deazaadenosine compounds were ca. 10-fold less active than the corresponding adenosine derivatives.[10] The ribose ring is essential for high affinity and agonistic activity.[11,12] Replacement of the ribose furane by a cyclopentane ring

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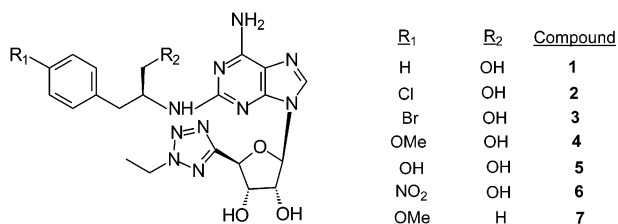
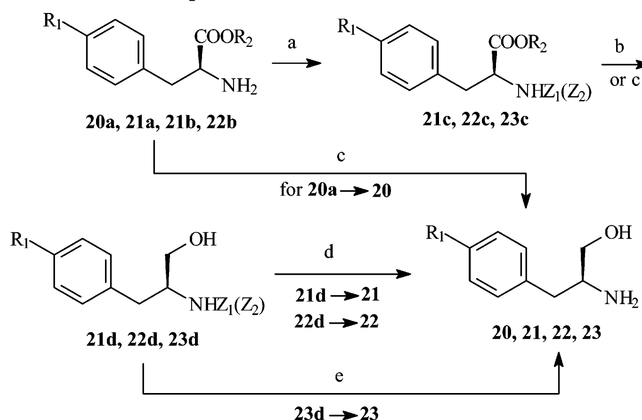
Chart 1. Adenosine Derivatives **1**−**7** Prepared and Evaluated



resulted in analogues with weakly A_{2A} selective ligands and poor affinity for A_3 receptors.[7] In this context, a carbocyclic analogue of 1-deazaadenosine (AMP 579) has been shown to be a good A_1/A_{2A} agonist with cardiovascular protective effects.[13,14] The receptor subtype selectivity can be achieved by substituting the adenosine molecule at different positions. For example, N^6-substituents, such as cyclopentyl, enhance adenosine A_1 receptor selectivity relative to the other subtypes,[15] while the introduction of a 3-iodobenzyl group induces A_3 receptor selectivity.[16] Bulky substituents at the C-2 position of the adenine moiety, such as (ar)alkylamino,[17] alkylidene hydrazine,[18] and alkynyl,[19] have been reported to induce selectivity for the A_{2A} receptor in comparison to A_1. Very recently, 2,5′-disubstituted adenosine derivatives displayed a modest A_3/A_{2A} selectivity ratio although the affinity for both receptors was in the nanomolar range.[20] The standard A_{2A} antagonist 2-[[[4-(2-carboxyethyl)phenyl]ethyl]amino]-5′-(N-ethylcarbamoyl)adenosine (CGS-21680), which is generally used as a "A_{2A} selective" adenosine receptor agonist, shows a 30-fold selectivity for A_{2A}/A_3 receptors in rat but virtually no selectivity in humans.[21] Other potent A_{2A} agonists are nonselective since they also exhibit a high affinity for A_3 and A_1 receptors. Therefore, so far, no satisfactory A_{2A} selective agonist is available.[21] This fact and the important applications of A_{2A} agonists as vasodilators, antihypertensive, platelet antiaggregatory, heart imaging, antiinflammatory, and antipsychotic agents led us to search for new potent and selective A_{2A} agonists. In this work, we present the synthesis and biological activity of adenosine derivatives **1**−**7** as potential agonists for human A_{2A} receptors (Chart 1). These compounds contain an ethyl-substituted tetrazole moiety at the 4′-position of the ribose and an amino alcohol at the 2-position of the adenine. Compound **1** has been described previously, and its activity as an A_{2A} agonist against human adenosine receptors using transfected Chinese hamster ovary (CHO) cells was determined.[1] We were interested in knowing the effect of the introduction of several types of substituents (electron donating, electron withdrawing, and halogens) in the para-position of the phenyl ring of the parent compound **1** (compounds **2**−**6**), as well as the relevance of the presence of the amino alcohol function by preparing compound **7**. The activity of these compounds has been evaluated in radioligand binding assays using cloned human A_1, A_{2A}, A_{2B}, and A_3 receptors. The compounds have also been profiled in cAMP assays using human receptors expressed on transfected CHO cells, as well as in functional assays using rat aorta, guinea pig aorta, and guinea pig tracheal rings.

Scheme 1. Preparation of Amino Alcohols **20**−**23**[a]



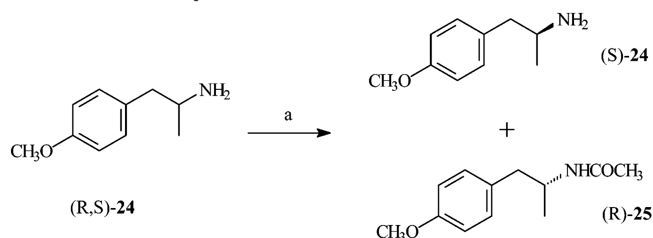
R_1	R_2	Z_1	Z_2	Compound
H	--	H	--	**18**
Cl	--	H	--	**19 (R,S)**
Br	H	--	--	**20a**
Br	--	H	--	**20**
CH_3O	H	--	--	**21a**
CH_3O	CH_3	--	--	**21b**
CH_3O	CH_3	BnOCO	--	**21c**
CH_3O	--	BnOCO	--	**21d**
CH_3	--	H	--	**21**
OH	CH_3	--	--	**22b**
OH	CH_3	BnOCO	--	**22c**
OH	--	BnOCO	--	**22d**
OH	--	H	--	**22**
NO_2	H	--	t-BuOCO	**23c**
NO_2	--	--	t-BuOCO	**23d**
NO_2	--	H	--	**23**

[a] Reagents and conditions: (a) BnOCOSu, CH_2Cl_2, 86% for **21c**, 99% for **22c**. (b) $LiBH_4$, THF, 85% for **21d**, 82% for **22d**. (c) BH_3, THF, THF, 81% for **20**, 75% for **23d**. (d) Pd/C, aqueous HCl, MeOH, 90% for **21**, 87% for **22**. (e) Aqueous HCl, dioxane, 70% for **23**.

Results and Discussion

Chemistry. For the synthesis of adenosine derivatives **1**−**7**, the corresponding amino alcohols **18**−**24** were obtained as follows. Compounds **18** (L-phenyl alaninol) and **19** were commercially available. Compounds **20**−**23** were prepared as described in Scheme 1. L-4-Bromophenyl alaninol [(*S*)-**20**] was obtained by direct reduction of 4-bromophenyl alanine (**20a**) with BH_3·THF (tetrahydrofuran) solution[22] in 81% yield (Scheme 1). Other reagents have also been used to reduce natural or nonnatural amino acids or their esters, such as $NaBH_4/LiCl$,[23] $NaBH_4/CaCl_2$,[24] and $LiBH_4$,[25] among others. (*S*)-4-Methoxyphenyl alaninol [(*S*)-**21**] was obtained from **21a** in a four step process: (i) esterification to **21b**, (ii) protection of **21b** as urethane **21c** with benzyloxycarbonyloxysuccinimide (ZOSu),[26] (iii) reduction with $LiBH_4$/THF to afford compound **21d**, and (iv) deprotection of the amino group with H_2/Pd/C (Scheme 1). The overall yield of the sequence was 58%. This compound had been previously obtained by $LiAlH_4$ reduction of the corresponding N-acetamido-protected ester followed by deprotection of the amino group.[27] Tyrosinol **22** has been described to proceed by $LiBH_4$ reduction of the methyl ester in quantitative yield.[28] However, in our hands, this procedure afforded a mixture of products from which the expected compound **22** was isolated in low yield. Therefore, a similar protocol as above was followed. Tyrosine methyl ester **22b** was protected as its (N)-benzyloxycarbonyl derivative **22c**; then, reduction with $LiBH_4$/THF provided **22d**,

Scheme 2. Enzymatic Resolution of Amine **24**[a]

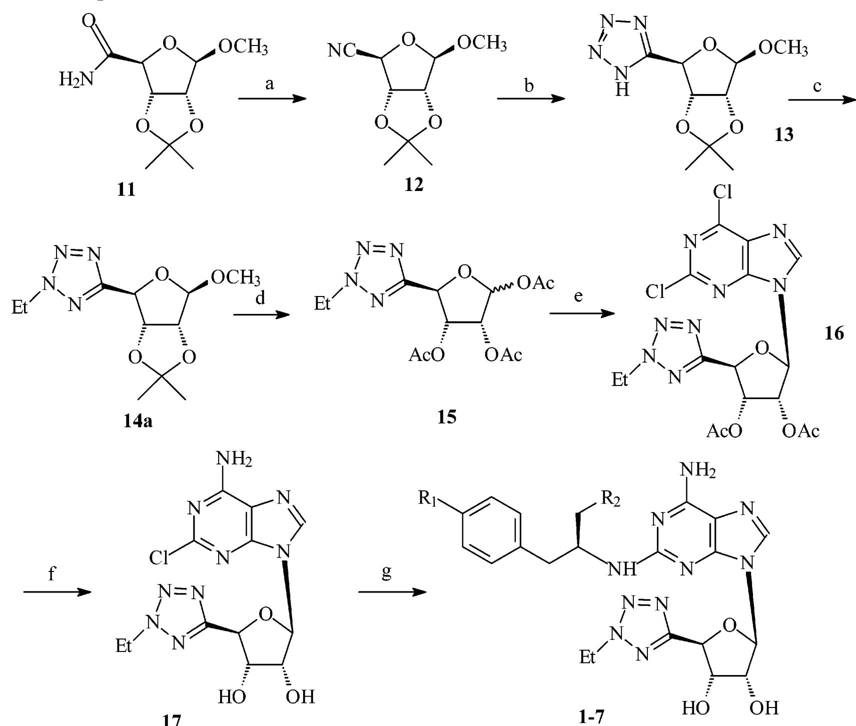


[a] Reagents and conditions: (a) CALB, AcOEt, 30 °C, 15 h.

which was finally hydrogenized over Pd/C to give **22** in 71% overall yield (Scheme 1). Nitroderivative **23** was obtained by reduction of Boc-4-nitrophenylalanine (**23c**) with BH_3·THF followed by deprotection with HCl/dioxane with a 52.5% overall yield. All of these processes occurred with complete retention of configuration. Amine **24**, a well-known amphetamine derivative,[29] was obtained in racemic form by $LiAlH_4$ reduction of the corresponding nitro derivative in 78% yield, as previously described by us.[30] Then, resolution of the racemic amine with *Candida antarctica* (CALB) lipase in ethyl acetate furnished acylamine (*R*)-**25** (32%) and unreactive amine (*S*)-**24** (47%)[30] (Scheme 2).

Synthesis of the adenosine derivatives **1**−**7** was carried out from ribose-protected **11** following a similar methodology previously described[1] (Scheme 3). Compound **11** was prepared from D-ribose (**8**) through protection as the corresponding acetonide (**9**)[31,32] and oxidation to the carboxylic acid **10**[32,33] with TEMPO/KBr/NaClO. Compound **10** was converted to amide **11**[32,34] by amination of the corresponding acid chloride in 92% yield. The treatment of **11** with $POCl_3/Et_3N$ in dimethyl formamide (DMF) provided nitrile **12** in 75% yield.[32,35] Introduction of the tetrazole moiety at the 5-position of the ribose skeleton was achieved by reaction of **12** with NaN_3/NH_4Cl in DMF (82% yield) to provide **13**.[34] At this stage, the relative difference in chemicals shifts of the two geminal methyls of the acetonide ($\Delta\delta = 0.19$ ppm) confirms the β-configuration of the compound.[36] Ethylation of **13** furnished a mixture of ethyl derivatives at N-2 (**14a**, 71%) and N-1 (**14b**), which were successfully separated by fractional crystallization. Trifluoroacetic acid-promoted hydrolysis of the hemiacetal and acetal groups followed by full acetylation provided the corresponding acetate **15** (52% overall)[1,35] as an inseparable mixture of the α- and β-anomers in a 29:71 ratio by gas chromatography analysis. Assignment of the relative stereochemistry to both compounds was based on the different J_{1-3} coupling constants of the hemiacetalic type protons in the ^1H NMR spectrum and double quantum correlation spectroscopy experiments. The α-anomer resonates at δ 6.58 and presents a $J_{1-3} = 4.2$ Hz corresponding to a quasi eclipsed $^1H-^1H$ conformation, while the β-anomer resonates at δ 6.27 and shows a J_{1-2} of only 1.2 Hz due to an almost 90° angle between the two hydrogens. The treatment of **15** with 2,6-dichloropurine/DBU in the presence of trimethylsilyl triflate in acetonitrile afforded the β-nucleoside **16** (88%)[1] as the only compound. Apparently and although the 2-acetyl group is β-directing, the possible formation of the α-nucleoside is also hampered because of the refluxing conditions to favor isomerization to the thermodynamically more stable β-nucleoside.[7] Amination with dry ammonia selectively produced replacement of the chlorine atom at the 6-position by an amino group to furnish **17**[1] after base hydrolysis (85% overall). Finally, reaction of **17** with amino alcohols **18**−**24** in ^{i}Pr$_2$EtN/dimethyl sulfoxide (DMSO) provided the ex-

Scheme 3. Synthesis of Compounds **1**−**7**[a]



[a] Reagents and conditions: (a) $POCl_3$, Et_3N, DMF, 75%. (b) NaN_3, NH_4Cl, DMF, 82%. (c) EtI, K_2CO_3, Me_2CO, 71%. (d) (i) TFA, H_2O; (ii) Ac_2O, Et_3N, DMAP, 52% overall. (e) 2,6-DCP, DBU, TMSOTf, 88%. (f) (i) NH_3, THF; (ii) NaMeO, MeOH, 85% overall. (g) Compounds **18**−**24**, *i*Pr_2EtN, DMSO, 145 °C, 31−62%.

Table 1. Binding Affinities (K_i) and Selectivity Ratios of Compounds **1**−**7** for Human Adenosine Receptors A_1, A_{2A}, A_{2B}, and A_3 Expressed in Transfected CHO (A_1), HeLa (A_{2A} and A_3), and HEK-293 (A_{2B}) Cells, in Comparison to NECA and CGS-21680 in Radioligand Assays[a]

compound	K_i (nM)[b]				selectivity ratios		
	A_1	A_{2A}	A_{2B}	A_3	A_1/A_{2A}	A_{2B}/A_{2A}	A_3/A_{2A}
NECA	5 ± 0.9	130 ± 8	760 ± 35	4 ± 0.5	0.04	5.8	0.03
CGS-21680	302 ± 27	50 ± 2.5	>10 000	68 ± 6	6	>200	1.3
1	369 ± 33	46 ± 3.2	1300 ± 110	92 ± 7.5	8	28.2	2
2[c]	670 ± 42	270 ± 15	2715 ± 180	288 ± 19	2.4	10	1
3	310 ± 18	5 ± 0.8	2261 ± 151	83 ± 7.8	62	452	16.6
4	>10 000	38 ± 2.7	1183 ± 210	130 ± 9	>263	31	3.4
5	600 ± 30	5 ± 0.1	624 ± 30	140 ± 12	120	124.8	28
6	>10 000	48 ± 3.1	3660 ± 230	400 ± 21	>208	76	8.3
7	356 ± 23	1 ± 0.2	2780 ± 195	100 ± 8.5	356	2780	100

[a] The binding affinities were determined using [^{3}H]DPCPX as the radioligand for A_1 and A_{2B}, [^{3}H]ZM241385 for A_{2A}, and [^{3}H]NECA for A_3. The experimental conditions used are summarized in Table 4. Values represent means ± SEM from two to three experiments. [b] K_i values were calculated by the expression $K_i = IC_{50}/[1 + (C/K_D)]$, where IC_{50} is the concentration of compound that displaces the binding of radioligand by 50%, C is the concentration of radioligand, and K_D is the apparent dissociation constant of each radioligand. [c] Mixture of diastereoisomers. Taking into account that the *R*-enantiomer is inactive, the actual K_i values for the *S*-enantiomer are half of the values cited.

pected adenosine derivatives **1**−**7** in variable yields (31−62%) (Scheme 3).

Biology. Carbocyclic nucleosides have found a wide application in the pharmaceutical and agricultural fields as antivirals,[37,38] but relatively few reports have appeared on the affinity of carbocyclic derivatives of adenosine toward the adenosine receptors.[7,19,20] From structure−activity relationship studies, some derivatives of adenosine have been revealed to be potent and selective A_1 and A_2 agonists[3] but few of them have entered clinical trials and none of them have been fully sucessful.[39]

In radioligand binding assays using recombinant human A_1, A_{2A}, A_{2B}, and A_3 receptors, our results compare favorably with those obtained with the known agonists NECA and CGS-21680, and some compounds display a potent agonist activity as well as a high selectivity toward the A_{2A} receptor (Tables 1 and 2). Although in binding experiments with radiolabeled antagonists agonists may show biphasic competition curves due to two affinity states, only one K_i was evaluated for our data. This value would appear to be the average of K_i high and K_i low affinity if we compare it with the EC_{50} in A_{2A} and A_{2B} receptors. To be able to discriminate between the two affinity states and observe fully biphasic curves, it would be necessary to analyze more points on the curve with smaller concentration intervals. However, in the particular experiments reported here, our goal was not to fully characterize the affinity states of the receptors but to verify that binding and functional studies were in the same qualitative range. Thus, by ensuring that all functional responses observed with our A_{2A} agonists were attributable to the activation of the A_{2A} receptor, we could discount the participation of other adenosine receptors or mechanisms, as reported by other authors.[19]

Most compounds displayed very low or negligible affinity for adenosine A_1 and A_{2B} receptors, and some of them exhibited a K_i in the nanomolar range for the A_{2A} receptor. We investigated the effects of changes at the para-position of the phenyl ring of reference compound **1**. The introduction of chlorine (compound **2**) reduces the binding affinity for the A_{2A} receptor (3-fold), without perceptibly affecting binding to A_1 or A_3 receptors, taking into account that compound **2** is a mixture of diastereoisomers and only the *S*-isomer is supposedly active (Table 1). The introduction of bromine (compound **3**), however, increases the binding affinity for the A_{2A} receptor (ca. 10-fold) without affecting binding to A_1, A_{2B}, or A_3, thus conferring to this compound greater selectivity toward A_1 and A_3 ($A_1/A_{2A} = 62$, $A_{2B}/A_{2A} = 452$, and $A_3/A_{2A} = 16$). This compound is 2-fold more selective in terms of the A_{2B}/A_{2A} ratio than the reference CGS-21680. These results suggest that the atomic volume does not appear to be responsible for the lack of activity of compound **2**. The presence of an OH group in **5** also improves the binding affinity (10-fold) for A_{2A} and slightly reduces the binding to A_1 and A_3, thus improving the selectivity of A_1/A_{2A} and A_3/A_{2A} 120- and 28-fold, respectively. The affinity for the A_{2B} receptor is also low ($K_i = 624$ nM). Introduction of a nitro group (compound **6**) or a methoxy group (compound **4**) eliminates observable binding to A_1, is also negligible for A_{2B}, but leaves A_{2A} affinity unaffected. Interestingly, when the methoxy derivative **4** lacks the hydroxyl group on the side chain (compound **7**), the binding affinity for A_{2A} is increased to 1 nM, improving the selectivity ratios 356× against A_1, 2780× against A_{2B}, and 100× against A_3. NECA exhibited a high affinity for A_1 and A_3 receptors but not for A_{2A} and A_{2B} receptors. CGS-21680, in turn, showed affinity for A_{2A} and A_3 receptors, a lower affinity for A_1 ($A_1/A_{2A} = 6$), and no activity for the A_{2B} receptor.

Subsequently, all compounds were tested in functional assays by determination of cAMP production in CHO cells transfected with the human A_{2A} receptor, which permitted direct comparison of A_{2A} and A_{2B} activity. Most compounds showed activity for the A_{2A} receptor, except chloro derivative **2**, with EC_{50} values ranging from 1.4 to 8.8 nM (Table 2). Practically all compounds produced similar amounts of cAMP than the full A_{2A} agonist NECA, suggesting that they all behaved as excellent A_{2A} agonists. Moreover, the compounds were all significantly more selective than NECA with the A_{2B}/A_{2A} ranging from 48 for compound **2** to 666 for compound **7** while for NECA it was only 9. These latter results imply that the adenosine derivatives assayed displayed modest or poor affinity for human A_{2B} receptor. The agonist nature of the compounds was confirmed by the activity of compounds **1**, **3**, **5**, and **7** in functional assays using isolated tissues (rat aorta for A_{2A} and guinea pig aorta for A_{2B}). In all cases, the activities

Table 2. Potency (EC_{50}) and Efficacy (% E_{max}) of Compounds **1**−**7** in Comparison to NECA Elicited by Measuring cAMP Formation in Recombinant Human A_{2A} and A_{2B} Receptors Expressed in Transfected CHO Cells and by Determination of Phenylephrine Precontracted Tissue Relaxation in Isolated Aorta from Rat (A_{2A}) and Guinea Pig (A_{2B})[a]

	cAMP				isolated aorta assay			
	human A_{2A}		human A_{2B}		rat A_{2A}		guinea pig A_{2B}	
compound	EC_{50} (nM)[b]	% E_{max} NECA[c]	EC_{50} (nM)[b]	% E_{max} NECA[c]	EC_{50} (nM)[b]	% E_{max} NECA[c]	EC_{50} (nM)[b]	% E_{max} NECA[c]
NECA	50.0 ± 3	100 ± 7	440 ± 32	100 ± 2	34.0 ± 2	100 ± 1	250 ± 13	100 ± 1
1	8.8 ± 0.4	78 ± 5	1000 ± 700	90 ± 3	10.0 ± 6	90 ± 3	950 ± 30	92 ± 3
2	97.0 ± 8.3	100 ± 8	4700 ± 700	70 ± 6	*d*	*d*	*d*	*d*
3	1.6 ± 0.1	100 ± 8	560 ± 60	146 ± 10	6.7 ± 0.9	100 ± 2	1300 ± 800	125 ± 5
4	1.4 ± 0.4	124 ± 10	680 ± 30	95 ± 5	*d*	*d*	*d*	*d*
5	8.0 ± 0.6	95 ± 5	1300 ± 600	85 ± 4	2.9 ± 0.3	110 ± 3	1140 ± 500	100 ± 3
6	3.1 ± 0.5	125 ± 9	1200 ± 200	77 ± 3	*d*	*d*	*d*	*d*
7	3.0 ± 0.6	90 ± 3	2000 ± 300	50 ± 2	4.0 ± 0.5	76 ± 5	3000 ± 500	80 ± 2

[a] Values represent means ± SEM from two to three experiments. [b] EC_{50}: concentration of compound that elicited 50% of maximal response. [c] E_{max}: percentage of maximal response obtained with respect to that observed with NECA. [d] Not tested.

Table 3. Pharmacodynamic Parameters (EC_{50}, EC_{50} Ratio, and E_{max} Ratio)[a] of Concentration−Relaxation Studies Exerted on Guinea Pig Tracheal Rings Precontracted by Carbachol by Adenosine, NECA, and Compounds **1**, **2**, **4**, and **7**[b]

	adenosine	NECA	**1**	**2**	**4**	**7**
			control			
EC_{50} (M)	3.2×10^{-4}	8.7×10^{-5}	2.1×10^{-5}	6.6×10^{-6}	8.3×10^{-6}	2.4×10^{-5}
			CSC (10^{-4} M)			
EC_{50} (M)	5.7×10^{-4}	8.4×10^{-5}	5.1×10^{-5}	1.7×10^{-5}	2.2×10^{-5}	2.6×10^{-5}
EC_{50} ratio	1.8	1.0	2.4	2.6	2.6	1.1
E_{max} ratio	98.3	100.7	79.4	105.1	127.3	89.1
			alloxazine (10^{-4} M)			
EC_{50} (M)	5.5×10^{-4}	8.8×10^{-5}	8.8×10^{-5}	3.8×10^{-5}	3.6×10^{-5}	1.5×10^{-5}
EC_{50} ratio	1.7	1.0	4.2	5.8	4.3	0.6
E_{max} ratio	119.7	97.5	85.2	89.7	83.3	89.1
			DMPX (10^{-4} M)			
EC_{50} (M)	3.4×10^{-4}	7.7×10^{-5}	4.6×10^{-5}	*c*	3.6×10^{-5}	2.3×10^{-5}
EC_{50} ratio	1.1	0.9	2.2		4.3	1.0
E_{max} ratio	96.1	98.7	76.7		110.4	67.2
			8-PT (10^{-4} M)			
EC_{50} (M)	4.6×10^{-4}	6.5×10^{-5}	6.4×10^{-5}	*c*	*c*	*c*
EC_{50} ratio	1.4	0.8	3.0			
E_{max} ratio	96.8	79.2	79.8			

[a] EC_{50}: concentration of agonist that produces 50% of the maximal response. EC_{50} ratio: ratio of the EC_{50} activity of the agonist when the antagonist is present relative to control (no antagonist). E_{max} ratio: ratio of the maximum relaxation values when the antagonist is present relative to control (no antagonist). [b] The activity of the compounds was determined in the absence (control) or presence of the adenosine antagonists CSC, alloxazine, DMPX, and 8-PT at 10^{-4} M concentration. The relaxant effects were evaluated from the results of 2−6 isolated tissues obtained from the same number of animals. Each experiment was carried out with 5−6 different concentrations. [c] Not determined.

observed on the corresponding receptors (A_{2A} in human and rat aorta and A_{2B} in human and guinea pig) were comparable to the activities displayed by the same compounds in the cAMP assays, despite the different species in which the tests were undertaken (Table 2).

Guinea pig tracheal rings precontracted by carbamylcholine chloride (carbachol) (5×10^{-7} M) were found to relax in a dose-dependent manner upon application of compound **1** ($EC_{50} = 2.1 \times 10^{-5}$ M), compound **2** ($EC_{50} = 6.6 \times 10^{-6}$ M), compound **4** ($EC_{50} = 8.3 \times 10^{-6}$ M), and compound **7** ($EC_{50} = 2.4 \times 10^{-5}$ M) (Table 3). Two of these compounds (**2** and **4**) were more potent (100-fold) than adenosine ($EC_{50} = 3.2 \times 10^{-4}$ M) and NECA ($EC_{50} = 8.7 \times 10^{-5}$ M) (10-fold), whereas compounds **1** and **7** displayed similar effects to NECA. Pretreatment of the tracheal rings with A_2, A_{2A}, and A_{2B} receptor antagonists 3,7-dimethyl-1-propargylxanthine (DMPX), 8-(3-chlorostyryl)caffeine (CSC), and alloxazine at 10^{-4} M concentration produced a marked inhibition of the tracheal relaxations induced by compounds **1**, **2**, and **4** but not that induced by compound **7** (Figure 1). Compound **1** was also tested in the presence of A_1 receptor antagonist 8-phenyltheophylline (8-PT), which produced a similar degree of inhibition than the other antagonists. In this experiment, none of the compounds tested showed selectivity toward any of the classical adenosine receptors. It has been postulated that the A_{2A} adenosine receptor mediates the relaxation of guinea pig trachea[40] and that in precontracted isolated tracheal rings the tracheal adenosine receptor is likely a further subtype of the A_2 receptor, distinct from the A_{2A} and A_{2B} receptors.[41] The precise adenosine receptor subtype involved in guinea pig trachea relaxation is still controversial.[42] Whatever subtype receptors are involved, our experiments show that compounds **2** and **4** elicited bronchodilation activity by interaction with adenosine receptors since their concentration−response curves are shifted to the right in the presence of adenosine selective antagonists.

Conclusions

The reference compound **1**,[1] despite having good selectivity for the A_{2A} over the A_{2B} receptor, is of limited

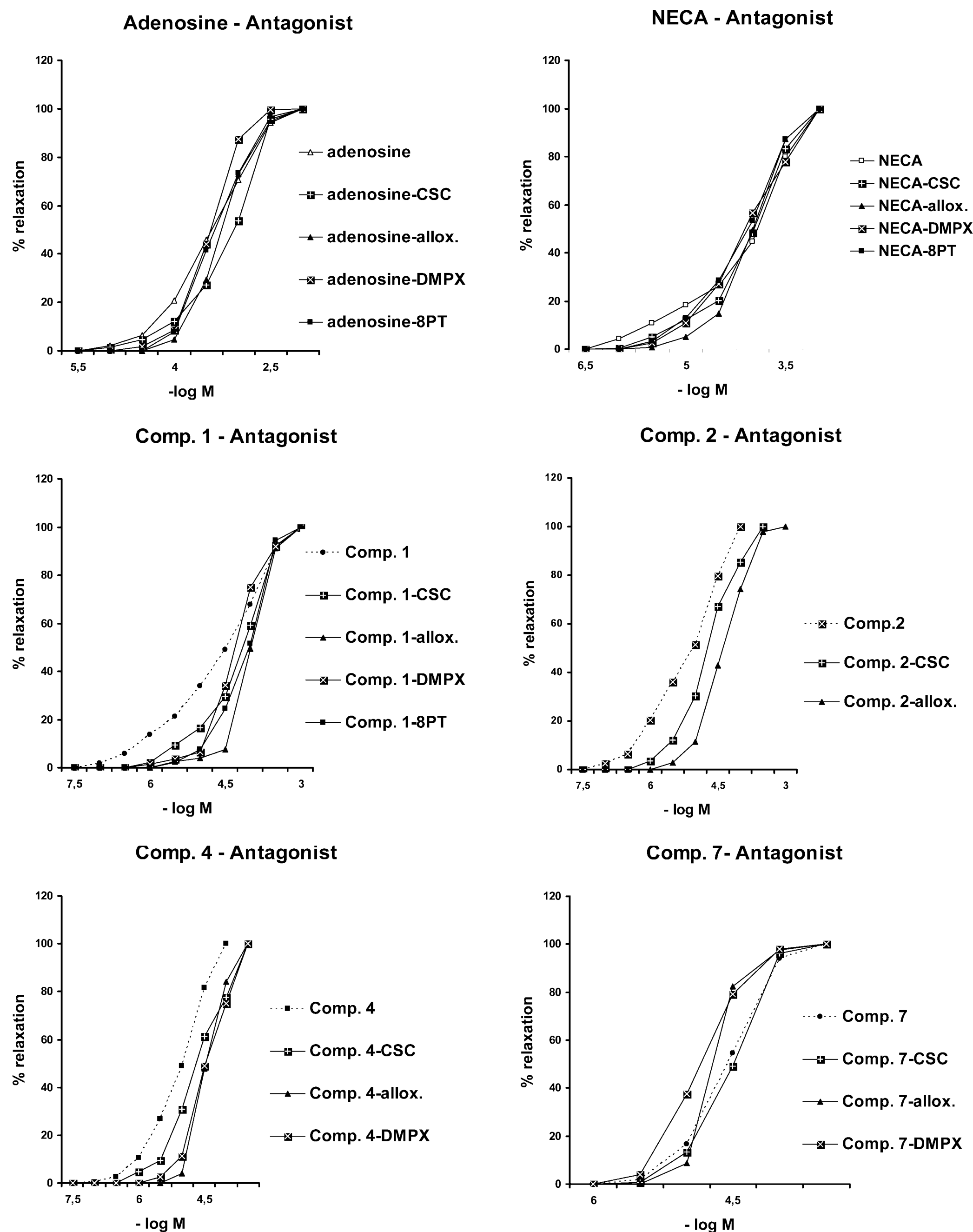


Figure 1. Relaxation effects vs concentration of compounds **1**, **2**, **4**, **7**, adenosine, and NECA on guinea pig tracheal rings precontracted with carbachol (5×10^{-7} M) in the presence or not (control) of receptor antagonists CSC, alloxazine, DMPX, and 8-PT at 10^{-4} M concentration. The ranges of concentrations of the agonists used were as follows: compound **1**, 3×10^{-8} to 10^{-3} M; compound **2**, 3×10^{-7} to 10^{-3} M; compound **4**, 3×10^{-8} to 3×10^{-4} M; compound **7**, 10^{-6} to 3×10^{-4} M; adenosine, 3×10^{-6} to 10^{-2} M; and NECA, 3×10^{-7} to 10^{-3}M. The relaxation values are expressed as a percentage of the maximal relaxation obtained in all cases.

use as a biochemical tool due to its poor selectivity with respect to A_1 and A_3 receptors. Our results show that substitution at the para-position of the phenyl ring of compound **1** by different groups greatly increases the binding affinity for the A_{2A} receptor. At the same time, the substituted derivatives tested (**2**−**7**) have reduced affinity for A_1 and A_3 receptors, thus remarkably improving the selectivity of A_1/A_{2A} and A_3/A_{2A}. Among the adenosine derivatives tested, compound **7** is a highly selective and potent biochemical tool to study the role of the A_{2A} receptor in important processes such as inflammation, bronchodilation, etc.

Experimental Section

Abbreviations. (*R*)-PIA, (*R*)-N6-(phenylisopropyl)adenosine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; ZM241385, 7-amino-2-(2-furyl)-5-[2-(4-hydroxyphenyl)ethyl]amino[1,2,4]-triazolo[1,5-a][1,3,5]triazine.

Melting points are uncorrected. Optical rotations were measured on a Perkin-Elmer PE-341 polarimeter. IR spectra were recorded on a Bonem MB-120 with Fourier transform spectrometer. ^{1}H and ^{13}C NMR spectra were obtained in $CDCl_3$ or CD_3OD solutions on a Varian Unity 300 instrument, operating at 300 MHz for ^{1}H and 75 MHz for ^{13}C or on a Varian Gemini operating at 200 MHz for ^{1}H and 50 MHz for ^{13}C. The values are expressed in δ relative to the $CHCl_3$ present in the solvent (δ 7.24 ppm for ^{1}H and δ 77.0 ppm for ^{13}C). Alterna-

tively, when CD_3OD was used, the values are expressed relative to CD_3OH signal at 3.31 ppm for ^1H and 49.0 ppm for ^{13}C. Coupling constants (*J*) are in Hz. ^{19}F NMR spectra were recorded on a Unity 300 (282 MHz) using $CFCl_3$ (0.5% solution in $CDCl_3$) as a reference. High-performance liquid chromatography (HPLC) analyses were performed on a Waters 510 instrument with a Rheodyne injector and a photodiode array detector set at 254 nm. Amino alcohols were run on a Symmetry-C18 (5 μm, 3.9 mm × 150 mm) reverse phase column eluting with mixtures of ammonium formate 0.015 M (pH 4.9) and MeOH. Compounds **1**−**7** were finally purified with the same column eluting with acetonitrile:water 25:75 containing 0.22% trifluoroacetic acid. LC-electrosparay ionization (ESI)-MS were recorded on a HPLC 1100-Agilent instrument coupled to1100 MSD-Agilent mass spectrometer with an electrospray interphase and using MeOH:H_2O 50:50 as a mobile phase. High-resolution mass spectra (HRMS) were run on a VG Auto Spec mass spectrometer. All reagents were purchased from Aldrich Chemical Co. and were used without further purification. THF was distilled from sodium-benzophenone ketyl; MeOH and DMF were distilled from CaH_2; *i*Pr_2NEt was distilled from KOH; and DBU and DMSO were distilled from CaH_2. The reactions were followed by thin-layer chromatography (TLC) using precoated Merck F_{254} silica gel plates. For flash column chromatography, 35−70 μm of silica gel from Solvents, Documentation, Syntheses (SDS) was used.

1-Methoxy-2,3-O-isopropylidene-*β*-D-ribofuranoside (9). In a 250 mL round-bottomed flask were placed D-ribose (**8**) (10.0 g, 0.066 mol), acetone (80 mL), 2,2-dimethoyxpropane (20 mL, 0.163 mol), and 60% $HClO_4$ (4.0 mL, 0.335 mol). The mixture was stirred at room temperature for 2.5 h, and MeOH (14 mL) was then added and further stirred for 2 h more. The mixture was cooled on an ice bath, and a 30% Na_2CO_3 solution (30 mL) was slowly added so that the temperature did not exceed 10 °C. The precipitate was filtered and washed with AcOEt (2 × 10 mL). The filtrate was concentrated and diluted with a mixture of AcOEt (80 mL) and water (40 mL). The phases were decanted, and the aqueous one was extracted with AcOEt (2 × 40 mL). After it was washed with brine and dried, compound **9**[31] (11.58 g, 85%) was obtained as a yellow oil. The crude was used in the next step without purification. IR (film, KBr): ν 3461, 2989, 2941, 1458 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 4.97 (s, 1H, C*H*OCH_3), 4.83 (d, 1H, *J* = 5.7 Hz, C*H*O), 4.58 (d, 1H, *J* = 6.0 Hz, C*H*O), 4.43 (dd, 1H, *J* = *J'* = 3.0 Hz, C*H*CH_2OH), 3.56−3.72 (sc, 2H, C*H*$_2$OH), 3.43 (s, 3H, OC*H*$_3$), 3.26 (dd, *J* = 10.2 Hz, *J'* = 3.0 Hz, 1H, CH_2O*H*), 1.48 (s, 3H, C*H*$_3$CCH_3), 1.31 (s, 3H, CH_3CC*H*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 112.05, 109.93, 88.32, 85.77, 81.43, 63.97, 55.50, 26.29, 24.63 ppm.

1-Methoxy-2,3-O-isopropylidene-*β*-D-ribofuranosyl-5′-carboxylic Acid (10). In a 250 mL three-necked round-bottomed flask were placed compound **9** (11.06 g, 54.2 mmol), AcOEt (88 mL), 6% $NaHCO_3$ solution (39 mL, 27.1 mmol), KBr (0.554 g, 4.7 mmol), and TEMPO (41 mg, 0.26 mmol). The mixture was cooled to 0 °C, and then, a solution of $NaHCO_3$ (1.66 g, 19.5 mmol) in 10% NaClO (98 mL, 132 mmol) was slowly added so that the temperature was maintained between 5 and 10 °C. When the addition was complete, the reaction was stirred at room temperature for 3 h. Then, 10% Na_2SO_3 solution (22 mL) and water (25 mL) were added, the phases separated, and the aqueous one was acidified with 3 M HCl and extracted with AcOEt (3 × 100 mL). The organic phase was dried and concentrated to provide a residue, which was washed with cold hexane (3 × 25 mL). After it was dried under vacuum, compound **10**[33] (9.08 g, 77%) was obtained as a white crystalline solid, which was directly used in the next step; mp 129−131 °C. IR (film, KBr): ν 3180, 2941, 1735, 1271 cm^{-1}.^1H NMR (300 MHz, $CDCl_3$): δ 8.0−9.2 (bs, 1H, COO*H*), 5.20 (dd, 1H, *J* = 5.7 Hz, *J'* = 0.9 Hz, C*H*O), 5.07 (s, 1H, C*H*OCH_3), 4.68 (dd, 1H, *J* = 0.9 Hz, *J'* = 0.9 Hz, C*H*COOH), 4.58 (dd, 1H, *J* = 5.7 Hz, *J'* = 0.6 Hz, C*H*O), 3.43 (s, 3H, OC*H*$_3$), 1.49 (s, 3H, C*H*$_3$CCH_3), 1.33 (s, 3H, CH_3CC*H*$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 175.05, 112.94, 109.62, 84.15, 83.50, 82.10, 55.70, 26.30, 24.91 ppm.

1-Methoxy-2,3-O-isopropylidene-*β*-D-ribofuranosyl-5′-carboxamide (11). A 250 mL three-necked round-bottomed flask with a thermometer and an argon inlet was charged with carboxylic acid **10** (9.07 g, 0.041 mol) in anhydrous AcOEt (72 mL) and $SOCl_2$ (4.3 mL, 0.058 mol). The reaction mixture was warmed to 60 °C for 2 h and cooled to room temperature, and then, dry NH_3 was bubbled for 15 min. Water (55 mL) and AcOEt (20 mL) were added, the phases separated, and the aqueous phase was extracted with AcOEt (3 × 40 mL). The organic phase was washed with brine, dried, and concentrated to give a solid, which was washed with cold hexane (2 × 25 mL). Compound **11**[32] (8.30 g, 92% yield) was thus obtained as a white yellowish solid; mp 134.5−137 °C. IR (film): ν 3419, 3199, 1660, 1209 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 6.55 (bs, 1H, CON*H*$_2$), 6.07 (bs, 1H, CON*H*$_2$), 5.13 (dd, 1H, *J* = 6.0 Hz, *J'* = 1.2 Hz, C*H*O), 5.08 (s, 1H, C*H*OCH_3), 4.60 (d, 1H, *J* = 1.2 Hz, C*H*$CONH_2$), 4.58 (d, *J* = 6.0 Hz, 1H, C*H*O), 3.46 (s, 3H, OC*H*$_3$), 1.50 (s, 3H, C*H*$_3$CCH_3), 1.33 (s, 3H, CH_3CC*H*$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.10, 112.83, 111.28, 86.16, 84.47, 82.40, 56.25, 26.48, 24.92 ppm.

1-Methoxy-2,3-O-isopropylidene-*β*-D-ribofuranosyl-5′-nitrile (12). To a 500 mL three-necked round-bottomed flask with an argon inlet and thermometer were added **11** (8.29 g, 0.038 mol), anhydrous AcOEt (100 mL), anhydrous DMF (16.2 mL), and anhydrous Et_3N (27.6 mL, 0.198 mol). The mixture was cooled to 0 °C, and then, $POCl_3$ (17.5 mL, 0.191 mol) was added at such a rate that the temperature did not exceed 40 °C. The reaction was stirred for 2 h, cooled to 0 °C, and quenched with $NaHCO_3$-saturated solution (108 mL). The layers were separated, the aqueous layer was extracted with AcOEt (2 × 70 mL), and the combined organic layers were washed with $NaHCO_3$-saturated solution (2 × 80 mL) and brine. After the solution was dried, removal of the solvent provided a crude, which was purified by flash distillation to provide compound **12**[32] (5.73 g, 75% yield) as a yellow oil (bp 100 °C/0.03 Torr). $[\alpha]_D^{20}$ −127.7° ($CHCl_3$, *c* = 0.96). IR (film): ν 2940, 1747, 1639, 1211 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ 5.12 (d, *J* = 0.3 Hz, 1H, C*H*OCH_3), 5.06 (d, *J* = 5.7 Hz, 1H, C*H*O), 4.78 (dd, *J* = *J'* = 0.8 Hz, 1H, C*H*CN), 4.71 (dd, *J* = 5.7 Hz, *J'* = 0.3 Hz, 1H, C*H*O), 3.45 (s, 3H, OC*H*$_3$), 1.46 (d, 3H, *J* = 0.6 Hz, 3H, C*H*$_3$CCH_3), 1.32 (d, *J* = 0.6 Hz, 3H, CH_3-CC*H*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 117.17, 113.74, 109.61, 84.21, 83.31, 71.70, 55.14, 26.14, 24.83 ppm. Anal. calcd for $C_9H_{13}NO_4$: C, 54.26; H, 6.58; N, 7.03. Found: C, 54.07; H, 6.69; N, 7.06.

5-(1-Methoxy-2,3-O-isopropylidene-*β*-D-ribofuranosyl)-1H-tetrazole (13). To a 250 mL three-necked round-bottomed flask with an argon inlet was added compound **12** (5.71 g, 0.028 mol) in anhydrous DMF (144 mL) and NH_4Cl (3.22 g, 0.060 mol). The mixture was cooled to 0 °C, and NaN_3 (3.73 g, 0.057 mol) was added portionwise over 6−8 min. The reaction mixture was stirred at 0 °C for 5 min and at room temperature for 15 min. The mixture was heated at 40 °C for 1 h and then slowly increased to 90 °C over a period of 3 h. The reaction mixture was stirred overnight at 90 °C and then cooled to 0 °C. The reaction was quenched with 6% aqueous solution of $NaNO_2$ (47 mL) and water (130 mL), and the mixture was stirred at 0 °C for 1 h. The pH of the solution was then adjusted to pH 2 with 2 M H_2SO_4 (ca. 55 mL) and extracted with AcOEt (3 × 150 mL). The combined organic phases were washed with H_2O (5 × 120 mL), dried, filtered off, and concentrated to give a gum, which was thoroughly dried under vacuum (<0.1 Torr) for 10 h to afford compound **13**[34] (5.73 g, 82% yield) as a yellow amorphous solid; mp 123−126 °C. IR (film): ν 3525, 2993, 2941, 1548, 1211 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 12−14 (bs, 1H), 5.70 (s, 1H, C*H*CN), 5.18 (s, 1H, C*H*O), 5.18 (d, *J* = 5.6 Hz, 1H, C*H*OCH_3), 4.74 (d, *J* = 5.8 Hz, 1H, C*H*O), 3.41 (s, 3H, OC*H*$_3$), 1.53 (s, 3H, C*H*$_3$CCH_3), 1.34 (s, 3H, CH_3CC*H*$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 155.63, 113.52, 111.32, 84.70, 83.68, 79.10, 56.14, 26.27, 24.81 ppm.

2-Ethyl-5-(1-methoxy-2,3-O-isopropylidene-*β*-D-ribofuranosyl)-2H-tetrazole (14a). To a 250 mL three-necked round-bottomed flask with an argon inlet were added anhydrous K_2CO_3 (4.25 g, 0.031 mol), compound **13** (5.73 g, 0.024

mol) in anhydrous acetone (40 mL), and freshly distilled ethyl iodide (2.5 mL, 0.031 mol). The reaction mixture was stirred at 40–45 °C for 3.5 h and then cooled to room temperature, and cyclohexane (40 mL) was added. The precipitate was thoroughly washed with cyclohexane (3 × 10 mL). The filtrate was concentrated, and the solid was dissolved with cyclohexane (63 mL), warmed to 65 °C, and allowed to cool to room temperature. The resulting crystalline yellow solid was allowed to crystallize further for 3 days at 5 °C. The alkylation product at N-1 (isomer **14b**) (1.86 g) was filtered and thoroughly washed with cyclohexane (4 × 50 mL); mp 103–109 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.88 (d, *J* = 5.7 Hz, 1H, C*H*O), 5.30 (dd, *J* = *J'* = 0.9 Hz, 1H, C*H*CN), 5.06 (s, 1H, C*H*OCH$_3$), 4.79 (d, *J* = 6.0 Hz, 1H, C*H*O), 4.54 (dq, *J* = 7.2 Hz, *J'* = 3.9 Hz, 2H, C*H*$_2$CH$_3$), 3.01 (s, 3H, OC*H*$_3$), 1.61 (t, *J* = 7.4 Hz, 3H, CH$_2$C*H*$_3$), 1.54 (s, 3H, C*H*$_3$CCH$_3$), 1.39 (s, 3H, CH$_3$CC*H*$_3$) ppm. The filtrate was concentrated to provide **14a**, the alkylation product at N-2 (4.56 g, 71% yield). IR (film): ν 3531, 2989, 1461, 1211 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.57 (dd, *J* = 5.7 Hz, *J'* = 0.6 Hz, 1H, C*H*O), 5.42 (dd, *J* = *J'* = 0.5 Hz, 1H, C*H*CN), 5.10 (s, 1H, C*H*OCH$_3$), 4.72 (d, *J* = 5.7 Hz, 1H, C*H*O), 4.65 (dq, *J* = 7.5 Hz, *J'* = 2.0 Hz, 2H, C*H*$_2$CH$_3$), 3.12 (s, 3H, OC*H*$_3$), 1.63 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$), 1.56 (s, 3H, C*H*$_3$CCH$_3$), 1.39 (s, 3H, CH$_3$CC*H*$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.36, 112.77, 109.88, 85.33, 82.86, 79.43, 54.62, 48.33, 26.40, 24.97, 14.51 ppm.

2-Ethyl-5-(1,2,3-triacetoxy-2,3-O-isopropylidene-β-D-ribofuranosyl)-2H-tetrazole (15). A mixture of compound **14a** (4.54 g, 0.017 mol), H_2O (1.2 mL, 0.065 mol), and trifluoroacetic acid (TFA, 11.1 mL, 0.14mol) was stirred for 6 h at room temperature. After this time, the mixture was concentrated in vacuo, and the residue was diluted with anhydrous CH_2Cl_2 (10 mL) and concentrated again. This operation was repeated until no odor of TFA was detected. Then, the residue was again diluted with CH_2Cl_2 (60 mL), and DMAP (123 mg, 1.0 mmol) was added. The solution was cooled to 0 °C, and anhydrous Et_3N (8.4 mL, 0.060 mol) and Ac_2O (9.5 mL, 0.10 mol) were subsequently added. The reaction mixture was stirred for 16 h at room temperature, diluted with CH_2Cl_2 (30 mL), and washed with 1 M HCl (2 × 60 mL). The aqueous phase was extracted with CH_2Cl_2 (3 × 20 mL), the organic phase was washed with $NaHCO_3$ saturated solution (1 × 60 mL) and H_2O (1 × 60 mL), dried, and concentrated. The residue was purified by flash column chromatography eluting with hexane–AcOEt mixtures to afford the title compound **15** (2.99 g, 52% yield) as a gum formed by an inseparable mixture of the α- and β-anomers.[1] IR (film): ν 3531, 2989, 1448, 1211 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ 6.58 (d, *J* = 4.2 Hz, 1H, OC*H*OAc, α-anomer), 6.27 (d, *J* = 1.2 Hz, 1H, OC*H*OAc, β-anomer), 5.92 (dd, *J* = 6.9 Hz, *J'* = 4.5 Hz, 1H, C*H*OAc), 5.67 (dd, *J* = 6.6 Hz, *J'* = 3 Hz, *J''* = 2.7 Hz, 1H, C*H*OAc), 5.63 (dd, *J* = 6.3 Hz, *J'* = 4.5 Hz, 1H, C*H*OAc), 5.57 (dd, *J* = 4.5 Hz, *J'* = 1.2 Hz, 1H, C*H*OAc), 5.55 (d, *J* = 2.4 Hz, 1H, C*H*CN), 5.46 (d, *J* = 7.2 Hz, 1H, C*H*CN), 4.67 (dq, 2H, *J* = 7.5 Hz, *J'* = 1.5 Hz, 2H, C*H*$_2$CH$_3$), 1.65 (t, *J* = 7.5 Hz, *J'* = 0.6 Hz, 3H, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 169.67, 169.362, 169.11, 163.52, 162.88, 98.23, 93.78, 76.30, 74.49, 74.00, 73.63, 72.13, 69.82, 48.50, 20.88, 20.41, 20.29, 14.30 ppm.

2,6-Dichloro-9H-[2,3-di-O-acetyl-5-(2′-ethyl-2H-tetrazolyl)-β-D-ribofuranosyl]purine (16). To a 50 mL three-necked round-bottomed flask with an argon inlet were added 2,6-dichloropurine (DCP, 0.96 g, 5.1 mmol), **15** (1.34 g, 3.9 mmol) in anhydrous. MeCN (17.3 mL), and DBU (881 μL, 5.9 mmol). To the mixture was added dropwise trimethylsilyl triflate (1.3 mL, 7.1 mmol) in a 5 min period. The mixture was stirred at room temperature for 21 h and then heated to reflux for 3.5 h. The reaction was cooled, quenched with H_2O (100 mL), and extracted with AcOEt (3 × 60 mL). The combined organic layers were dried, concentrated, and purified by flash column chromatography eluting with hexane–AcOEt mixtures to afford compound **16**[1] (1.45 g, yield 88%) as a white foamy solid and recovering 148 mg of starting **15**; mp 50–55 °C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.82 (s, 1H, NC*H*N), 6.56 (d, *J* = 6.9 Hz, 1H, C*H*O), 6.20 (dd, *J* = 6.9 Hz, *J'* = 4.8 Hz, 1H, C*H*OAc), 5.77 (ddd, *J* = 4.8 Hz, *J'* = 2.1 Hz, *J''* = 0.3 Hz, 1H, C*H*OAc), 5.63 (d, *J* = 2.1 Hz, 1H, C*H*CN), 4.73 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 2.24 (s, 3H, OCOCH$_3$), 2.09 (s, 3H, OCOCH$_3$), 1.70 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 169.35, 169.22, 162.96, 153.29, 153.13, 152.09, 144.34, 131.06, 85.57, 76.70, 73.93, 73.72, 48.95, 20.58, 20.29, 14.40 ppm.

2-Chloro-6-amino-9H-[5-(2′-ethyl-2H-tetrazolyl)-β-D-ribofuranosyl]purine (17). To a three-necked round-bottomed flask was added compound **16** (1.43 g, 3.0 mmol) in anhydrous THF (34 mL). The solution was cooled to 0 °C, and dry ammonia was slowly bubbled for 90 min. The reaction mixture was stirred for 48 h at room temperature, the solvent was then removed in vacuo, and the residue was dissolved with anhydrous methanol (84 mL). Then, NaOMe (806 μL of a 25% w/w in MeOH) was added slowly in a 3 h period while stirring. The mixture was further stirred for 1 h at room temperature, the solvent was stripped off, and the residue was purified by flash column chromatography eluting with mixtures of CH_2Cl_2 and MeOH to give compound **17**[1] (0.95 g, 85% yield). ^{1}H NMR (300 MHz, DMSO): δ 8.40 (s, 1H, NC*H*N,), 7.81 (bs, 2H, N*H*$_2$), 6.04 (d, *J* = 5.4 Hz, 1H, C*H*O), 5.78 (dd, *J* = 6.9 Hz, *J'* = 6 Hz, 1H, C*H*OH), 5.21 (d, *J* = 4.2 Hz, 1H, C*H*CN), 4.79 (q, *J* = 5.1 Hz, 1H, OH), 4.72 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 4.57 (q, *J* = 4.2 Hz, 1H, OH), 1.29 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (50 MHz, DMSO): δ 164.31, 156.99, 153.44, 150.75, 139.65, 118.17, 87.79, 77.40, 73.97, 73.69, 48.41, 14.25 ppm.

(*S*)-2-Amino-3-(4-bromophenyl)-1-propanol (20). To a cooled solution (0 °C) of **20a** (0.556 g, 2.3 mmol) in anhydrous THF (12.5 mL) was added under nitrogen a 1 M BH_3·THF solution (5.8 mL, 6.16 mmol) in several portions. The mixture was stirred overnight, and then, EtOH was added and stirred till no evolution of hydrogen occurred. The mixture was acidified with 3 N HCl (7 mL), 2-propanol (30 mL) was added, and the solvent was evaporated off. This treatment with 2-propanol was repeated (5 × 30 mL), and after removal of the solvent, the solid was taken in H_2O (30 mL) and filtered, and the solution was made basic with 2 N NaOH. The resulting suspension was treated with 2-propanol (10 mL), evaporated, and lyophilized. The solid was washed with $CHCl_3$ (3 × 20 mL), filtered, and concentrated to give a crude, which was purified by flash column chromatography to yield compound **20**[22] (0.450 g, 81% yield); mp 105–106 °C (85–87 °C[22]). $[\alpha]_D^{20}$ −19.7 ° (MeOH, *c* = 1). IR (KBr): ν 3343, 3278, 1591, 1488, 950, 844, 621 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.40 (d, *J* = 8.1 Hz, 2H, C$_6$*H*$_4$), 7.08 (d, *J* = 8.4 Hz, 2H, C$_6$*H*$_4$), 3.60 (dd, *J* = 14.4 Hz, *J'* = 10.8 Hz, 1H, C*H*$_2$), 3.34 (dd, *J* = 14.4 Hz, *J'* = 8.2 Hz, 1H, C*H*$_2$), 3.10 (m, 1H, C*H*), 2.72 (dd, *J* = 18.9 Hz, *J'* = 13.5 Hz, 1H, C*H*$_2$), 2.46 (dd, *J* = 18.9 Hz, *J'* = 11.7 Hz, 1H, C*H*$_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 137.6, 131.6, 130.9, 120.2, 66.2, 53.9, 40.2 ppm. HPLC-UV: λ_{max} = 228 nm.

(*S*)-2-Amino-3-(4-methoxyphenyl)-1-propionic Acid Methyl Ester (21b). To a stirred solution of **21a**[43] (0.99 g, 5.05 mmol) in MeOH (8.75 mL) at 0 °C was added $SOCl_2$ (2.15 mL, 29.7 mmol). The mixture was stirred for 12 h, and the solvent was stripped off. The residue was taken up with AcOEt (30 mL), washed with $NaHCO_3$ saturated solution (3 × 20 mL), and dried. After the solution was filtered, removal of the solvent afforded the methyl ester **21b**[44] (0.93 g, 88% yield). $[\alpha]_D^{20}$ −7.2° (MeOH, *c* = 0.06). IR (film): ν 3648, 3585, 3210, 1737, 1688, 1652, 1492, 821, cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.07 (d, *J* = 8.4 Hz, 2H, C$_6$*H*$_4$), 6.82 (d, *J* = 8.4 Hz, 2H, C$_6$*H*$_4$), 3.74 (s, 3H, OC*H*$_3$), 3.67 (s, 3H, OC*H*$_3$), 2.99 (dd, *J* = 12.9 Hz, *J'* = 5.4 Hz, 1H, C*H*$_2$), 2.79 (dd, *J* = 12 Hz, *J'* = 7.5 Hz, 1H, C*H*$_2$), 2.76 (dd, *J* = 12 Hz, *J'* = 7.5 Hz, 1H, C*H*$_2$), 1.49 (bs, 2H, N*H*$_2$) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 175.02, 157.99, 129.76, 128.69, 113.43, 55.44, 54.63, 51.33, 39.69 ppm.

(*S*)-2-(Benzyloxycarbonylamino)-3-(4-methoxyphenyl)-1-propionic Acid Methyl Ester (21c). To a 100 mL round-bottomed flask containing **21b** (0.9 g, 4.3 mmol) in CH_2Cl_2 (22 mL) was added N-(benzyloxycarbonyloxy)succinimide[26] (1.1 g, 4.3 mmol) in CH_2Cl_2 (8.5 mL). The mixture was stirred for 12 h. The solvent was stripped off, AcOEt (30 mL) was added,

and the organic phase was washed with 5% citric acid (3 × 25 mL), 5% $NaHCO_3$ solution (3 × 25 mL), and brine (3 × 25 mL). After the mixture was dried, evaporation of the solvent afforded **21c**[45] (1.3 g, 86% yield). $[\alpha]_D^{20}$ −18° (MeOH, *c* = 0.9). IR (film): ν 3624, 3353, 1736, 1668, 1601, 1575, 808 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (s, 5H, C_6H_5), 6.99 (d, *J* = 8.7 Hz, 2H, C_6H_4), 6.79 (d, *J* = 8.7 Hz, 2H C_6H_4), 5.21 (d, *J* = 8.1 Hz, 1H, N*H*), 5.08 (d, *J* = 3 Hz, 2H, CH_2), 4.61 (m, 1H, C*H*), 3.76 (s, 3H, OCH_3), 3.70 (s, 3H, OCH_3), 3.05 (dd, *J* = 15.9 Hz, *J'* = 6.6 Hz, 1H, CH_2), 3.02 (dd, *J* = 15.9 Hz, *J'* = 6.6 Hz, 1H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.02, 158.67, 155.59, 136.21, 130.23, 128.48, 128.14, 128.05, 127.53, 113.99, 66.90, 55.16, 54.87, 52.27, 37.28 ppm.

(*S*)-2-(Benzyloxycarbonylamino)-3-(4-methoxyphenyl)-1-propanol (21d). To a stirred solution of **21c** (1.2 g, 3.53 mmol) in anhydrous THF (12 mL) were slowly added at −10 °C a 2 M $LiBH_4$ solution in THF (5.5 mL, 11.0 mmol) and MeOH (3.3 mL). The solution was allowed to warm to room temperature and was stirred for 30 min more, and the solvent was evaporated. AcOEt (20 mL) and H_2O (10 mL) were added and decanted, and the organic phase was washed with brine, dried, and filtered. The solvent was removed, and the residue was purified by a flash column chromatography eluting with hexane−AcOEt mixtures to provide **21d**[45] (0.49 g, 85% yield); mp 98−101 °C. $[\alpha]_D^{20}$ −46° (MeOH, *c* = 0.07). IR (KBr): ν 3434, 3189, 3044, 1688, 1555, 887, 775 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 7.31 (s, 5H, C_6H_5), 7.09 (d, *J* = 8.1 Hz, 2H, C_6H_4), 6.80 (d, *J* = 8.4 Hz, 2H, C_6H_4), 5.15 (bs, 1H, N*H*), 5.05 (s, 2H, CH_2), 3.88 (m, 1H, C*H*), 3.76 (s, 3H, OCH_3), 3.62 (dd, *J* = 14.7 Hz, *J'* = 3.9 Hz, 1H, CH_2), 3.52 (dd, *J* = 15.6 Hz, *J'* = 4.8 Hz, 1H, CH_2), 2.77 (dd, *J* = 6.6 Hz, *J'* = 2.2, 1H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.20, 156.48, 136.28, 130.16, 129.49, 128.44, 128.05, 127.95, 113.90, 66.70, 63.74, 55.14, 54.15, 36.32 ppm.

(*S*)-2-Amino-3-(4-methoxyphenyl)propan-1-ol (21). To a 100 mL three-necked round-bottomed flask were added **21d** (0.81 g, 2.57 mmol) in MeOH (40 mL), 20% HCl (4 droplets), and 10% Pd/C (0.189 g). The mixture was stirred under an H_2 atmosphere and stirred for 2 h. The residue was filtered and washed with MeOH, the solvent was evaporated, and the residue was purified by flash column chromatography affording **21**[46] (0.42 g, 90% yield); mp 111−112.5 °C (96−97 °C[46]). $[\alpha]_D^{20}$ −13.2° (MeOH, *c* = 0.05). IR (KBr): ν 3276, 3240, 1589, 1501, 906, 799 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.06 (d, *J* = 8.7 Hz, 2H, C_6H_4), 6.80 (d, *J* = 8.7 Hz, 2H, C_6H_4), 3.75 (s, 3H, OCH_3), 3.58 (dd, *J* = 9 Hz, *J'* = 3.9 Hz, 1H, CH_2), 3.34 (dd, *J* = 12 Hz, *J'* = 7.2 Hz, 1H, CH_2), 3.02 (m, 1H, C*H*), 2.68 (dd, *J* = 15 Hz, *J'* = 5.1 Hz, 1H, CH_2), 2.42 (dd, *J* = 15 Hz, *J'* = 8.7 Hz, 1H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 158.07, 130.56, 130.02, 113.86, 65.91, 55.13, 54.20, 39.58 ppm.

(*S*)-2-Benzyloxycarbonylamino-3-(4-hydroxyphenyl)-1-propionic Acid Methyl Ester (22c). The same procedure described for **21c** was used. Thus, starting from tyrosine methyl ester hydrochloride **22b**[47] (3 g, 0.013 mol) in CH_2Cl_2 (50 mL), Na_2CO_3 (0.84 g) in H_2O (5 mL), and N-(benzyloxycarbonyloxy)succinimide (3.23 g, 0.013 mol) in H_2O (20 mL), compound **22c**[48] was obtained in 99% yield (4.26 g); mp 93−94 °C (91−92 °C [48]). $[\alpha]_D^{20}$ −7.9° (MeOH, *c* = 0.5). IR (KBr): ν 3325, 3049, 2220, 1688, 1676, 1514 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.33 (m, 5H, C_6H_5), 6.90 (d, *J* = 8.4 Hz, 2H, C_6H_4), 6.70 (d, *J* = 8.4 Hz, 2H, C_6H_4), 5.10 (s, 2H, CH_2), 4.60 (m, 1H, C*H*), 3.70 (s, 3H, OCH_3), 3.0 (m, 2H, CH_2) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ 172.21, 155.78, 155.06, 136.11, 130.36, 128.52, 128.20, 128.06, 127.27, 115.53, 67.08, 54.96, 52.37, 37.43 ppm.

(*S*)-2-Benzyloxycarbonylamino-3-(4-hydroxyphenyl)-1-propanol (22d). The same procedure described for **21d** was used. Starting from **22c** (2.17 g, 6.6 mmol), anhydrous THF (23 mL), and $LiBH_4$ in THF (3.9 mL, 7.8 mmol), compound **22d**[49] was obtained in 82% yield (1.64 g); mp 112−114 °C. $[\alpha]_D^{20}$ −8.2° (MeOH, *c* = 1). IR (KBr): ν 3321, 3099, 1686, 1523, 665 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 7.10 (m, 5H, C_6H_5), 6.92 (d, *J* = 8.4 Hz, 2H, C_6H_4), 6.58 (d, *J* = 8.4 Hz, 2H, C_6H_4), 4.90 (m, 2H, CH_2), 3.67 (m, 1H, C*H*), 3.42 (dd, *J* = 11.1 Hz, *J'* = 8.4 Hz, 1H, CH_2), 3.37 (dd, *J* = 11.1 Hz, *J'* = 8.1 Hz, 1H, CH_2), 2.68 (dd, *J* = 13.8 Hz, *J'* = 6 Hz, 1H, CH_2), 2.49 (dd, *J* = 13.8 Hz, *J'* = 8.1 Hz, 1H, CH_2) ppm. ^{13}C NMR (75 MHz, MeOD): δ 158.5, 156.8, 138.4, 131.3, 130.6, 129.4, 128.8, 128.5, 116.0, 67.1, 64.5, 56.2, 37.4 ppm.

(*S*)-2-Amino-3-(4-hydroxyphenyl)-1-propanol (22). The same procedure described for **21** was applied. Thus, treatment of **22d** (0.78 g, 2.9 mmol) in MeOH (40 mL) with 2 N HCl (4 droplets) and 10% Pd/C (0.182 g) for 2 h afforded **22**[50] (0.58 g, 87% yield). $[\alpha]_D^{20}$ −0.4° (MeOH, *c* = 1). IR (film): ν 3355, 3223, 1602, 1480, 1449, 656, cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 7.03 (d, *J* = 8.4 Hz, 2H, C_6H_4), 6.70 (d, *J* = 8.7 Hz, 2H, C_6H_4), 3.53 (dd, *J* = 10.8 Hz, *J'* = 4.5 Hz, 1H, CH_2), 3.35 (dd, *J* = 10.8 Hz, *J'* = 6.9 Hz, 1H, CH_2), 2.90 (m, 1H, C*H*), 2.67 (dd, *J* = 13.5 Hz, *J'* = 6.0 Hz, 1H, CH_2), 2.47 (dd, *J* = 13.8 Hz, *J'* = 7.8 Hz, 1H, CH_2) ppm. ^{13}C NMR (75 MHz, MeOD): δ 157.1, 131.2, 129.5, 116.3, 61.7, 61.1, 35.2 ppm. HPLC-UV: λ_{max} 223 and 270 nm.

(*S*)-2-*tert*-Butoxycarbonylamino-3-(4-nitrophenyl)-1-propanol (23d). A solution of the protected amino acid **23c**[51] (2.05 g, 6.45 mmol) in THF (6.4 mL) was added dropwise to a 1 M solution of BH_3·THF in THF (12.8 mL, 12.8 mmol) at 0 °C. The addition occurred over 30 min, and the reaction was allowed to proceed 6 h more at 0 °C. The reaction was quenched by using a 10% AcOH solution in MeOH (25.6 mL). After the solvent was evaporated, the crude product was dissolved in EtOAc and washed with 1 M HCl, H_2O, and 1 M NH_4HCO_3. The organic layer was dried, filtered, and evaporated to give compound **23d**[52] (1.47 g, 75% yield); mp 130−33 °C. $[\alpha]_D^{20}$ −25.3° (MeOH, *c* = 2.0). IR (KBr): ν 3358, 2962, 2494, 1679, 1603, 1523, 1348, 1166, 1008, 857, 699 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 8.14 (d, *J* = 8.7 Hz, 2H, C_6H_4), 7.47 (d, *J* = 8.7 Hz, 2H, C_6H_4), 3.82 (m, 1 H, C*H*), 3.52 (dd, *J* = 5.1 Hz, *J'* = 2.4 Hz, 2H, CH_2), 3.05 (dd, *J* = 13.8 Hz, *J'* = 5.4 Hz, 1H, CH_2), 2.76 (dd, *J* = 13.5 Hz, *J'* = 9.3 Hz, 1H, CH_2), 133 (s, 9H, CH_3) ppm. ^{13}C NMR (75 MHz, MeOD): δ 157.88, 148.49, 147.95, 131.52, 124.26, 80.03, 64.70, 54.95, 38.39, 28.64 ppm.

(*S*)-2-Amino-3-(4-nitrophenyl)-1-propanol (23). A solution of **23d** (1.06 g, 3.57 mmol) in dioxane (86 mL) and 1 N HCl (13 mL) was heated at 100 °C for 1 h. The mixture was cooled to room temperature, and 2 N NaOH (26 mL) was added. The product was extracted with AcOEt (20 mL × 3), and the organic layer was washed with brine and dried. Solvent removal furnished **23**[53] as a solid, which was purified by column chromatography (0.49 g, 70% yield); mp 144−46 °C (141−42 °C[53]). $[\alpha]_D^{20}$ −26.67° (MeOH, *c* = 2.28). IR (KBr): ν 3440, 3351, 3106, 2907, 1600, 1515 1344, 1112, 1059, 701 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 8.17 (d, *J* = 8.7 Hz, 2H, C_6H_4), 7.48 (d, *J* = 8.7 Hz, 2H, C_6H_4), 3.52 (dd, *J* = 10.8 Hz, *J'* = 4.8 Hz, 1H, CH_2), 3.39 (dd, *J* = 9.9 Hz, *J'* = 6.3 Hz, 1H, CH_2), 3.09 (m, 1H, C*H*), 2.93 (dd, *J* = 13.2 Hz, *J'* = 6 Hz, 1H, CH_2), 2.70 (dd, *J* = 13.2 Hz, *J'* = 7.8 Hz, 1H, CH_2) ppm. ^{13}C NMR (75 MHz, MeOD): δ 148.46, 148.07, 131.44, 124.54, 66.35, 55.24, 40.48 ppm.

2-[1-(*S*)-Hydroxymethyl-2-phenyl-ethylamino]-6-amino-9H-[5-(2′-ethyl-2H-tetrazolyl)-β-D-ribofuranosyl]purine (1). A mixture of (*S*)-2-amino-3-phenyl-1-propanol (**18**) (0.548 g, 3.62 mmol), compound **17** (608 mg, 1.65 mmol), anhydrous DMSO (1.5 mL), and ^{i}Pr$_2$NEt (4.5 mL, 25.8 mmol) fluxed with argon was heated under Ar at 145 °C for 22 h. The mixture was cooled to room temperature and diluted with AcOEt (40 mL), and all of the volatile material was removed under vacuum. Then, AcOEt (25 mL) was again added and the organic phase was washed with NaCl (5 × 20 mL), dried, and filtered, and the solvent was evaporated. The crude was purified by flash column chromatography eluting with AcOEt: MeOH 95:5 to obtain a brown semisolid, which was repurified with a reverse phase Isolute column (1 g) eluting with H_2O: MeCN:AcOH mixtures (77:23:0.1). Evaporation of the solvent and lyophilization afforded compound **1** (311 mg, 55% yield) as a white foamy solid; mp 104−107 °C. $[\alpha]_D^{20}$ −11.2° (MeOH, *c* = 0.109). IR (KBr): ν 3349, 1637, 1604, 1480, 1039, 702 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 8.11 (s, 1H, NC*H*N), 7.27 (m, 5H, C_6H_5), 7.13 (m, 2H, C_6H_5), 6.11 (d, *J* = 4.5 Hz, 1H, C*H*O),

5.31 (d, *J* = 4.8 Hz, 1H, NCC*H*O), 4.86 (m, 1H, C*H*O), 4.73 (m, 1H, C*H*O), 4.71 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 4.27 (m, 1H, C*H*), 3.61 (dd, *J* = 18.3 Hz, *J'* = 5.1 Hz, 1H, C*H*$_2$OH), 3.60 (dd, *J* = 13.2 Hz, *J'* = 5.1 Hz, 1H, C*H*$_2$OH), 2.93 (dd, *J* = 13.5 Hz, *J'* = 6.9 Hz, 1H, C*H*$_2$), 2.89 (dd, *J* = 13.5 Hz, *J'* = 7.2 Hz, 1H, C*H*$_2$), 1.61 (t, 3H, *J* = 7.5 Hz, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (75 MHz, MeOD): δ 165.91, 160.78, 157.27, 152.96, 140.39, 137.66, 130.51, 129.27, 127.10, 114.22, 89.90, 78.60, 75.62, 64.19, 55.51, 49.72, 38.40, 14.69 ppm. HPLC-UV: λ_{max} = 222.6, 259.1, and 300.5 nm. LC-ESI-MS: 505 (M$^+$ + 23, 100%), 483 (M$^+$ + 1, 22%), 481 (M$^+$ − 1, 35%). Anal. calcd for $C_{21}H_{26}N_{10}O_4 \cdot H_2O$: C, 50.39; H, 5.64; N, 27.98. Found: C, 50.92; H, 5.55; N, 27.43.

2-[1-(*R,S*)-Hydroxymethyl-2-(4-chlorophenyl)ethylamino]-6-amino-9H-[5-(2′-ethyl-2H-tetrazolyl)-*β*-D-ribofuranosyl]purine (2). The procedure was the same as described for **1**. Thus, from **17** (0.060 g, 0.16 mmol), (*R,S*)-2-amino-3-*p*-chlorophenyl-1-propanol (**19**) (0.072 g, 0.35 mmol), anhydrous DMSO (0.150 mL), and ^{i}Pr$_2$NEt (0.44 mL, 2.56 mmol), compound **2** was obtained as two diastereoisomers as a white solid (0.015 g, 39% yield); mp 115−117 °C. [α]$_D^{20}$ −18° (MeOH). IR (KBr): ν 3362, 3220, 1684, 1602, 1545, 1496, 825 cm^{-1}. ^{1}H NMR (300 MHz, CDCl$_3$): δ 8.11 (s, 1H, NC*H*N), 8.10 (s, 1H, NC*H*N), 7.25 (ca, 4H, C$_6$*H*$_4$), 6.12 (pt, *J* = 4.5 Hz, 1H, C*H*O), 5.32 (d, *J* = 4.8 Hz, 1H, NCC*H*O), 4.87 (m, 1H, NCC*H*O), 4.74 (m, 1H, C*H*O), 4.71 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 4.69 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 4.28 (m, 1H, C*H*), 3.73 (s, 3H, OC*H*$_3$), 3.60 (d, 2H, *J* = 4.8 Hz, C*H*$_2$OH), 2.90 (m, 1H, C*H*$_2$), 1.61 (t, 3H, *J* = 7.5 Hz, CH$_2$C*H*$_3$), 1.60 (t, 3H, *J* = 7.5 Hz, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (75 MHz, MeOD): δ 165.82, 160.69, 157.23, 152.89, 151.24, 139.17, 139.06, 133.84, 132.06, 132.03, 129.22, 114.26, 89.85, 78,53, 78.48, 75.67, 75.57, 64.08, 55.34, 55.17, 49.67, 37.64, 37.56, 14.67 ppm. HPLC-UV: λ_{max} = 215.6, 257.9, and 300.5 nm. LC-ESI-MS: 539 (M$^+$ + 23, 35%), 517 (M$^+$ + 1, 100%). HRMS (*m/z*) calcd for $C_{21}H_{25}ClN_{10}O_4$, 539.164877 (M$^+$ + Na); found, 539.164647.

2-[1-(*S*)-Hydroxymethyl-2-(4-bromophenyl)ethylamino]-6-amino-9H-[5-(2′-ethyl-2H-tetrazolyl)-*β*-D-ribofuranosyl]purine (3). Following the same procedure as described for **1**, from compound **17** (0.060 g, 0.165 mmol), (*S*)-**20** (0.072 g, 0.353 mmol), anhydrous DMSO (0.150 mL), and ^{i}Pr$_2$NEt (0.44 mL, 2.56 mmol), compound **3** (0.020 g, 52% yield) was obtained as a white solid; mp 122−125 °C. [α]$_D^{20}$ −11.6° (MeOH, *c* = 0.21). IR (KBr): ν 3354, 3217, 2937, 1636, 1603, 1534, 1482, 1042, 794 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 8.1 (s, 1H, NC*H*N), 7.38 (d, 2H, *J* = 8.7 Hz, C$_6$*H*$_4$), 7.23 (d, 2H, *J* = 8.4 Hz, C$_6$*H*$_4$), 6.11 (d, 1H, *J* = 4.8 Hz, C*H*O), 5.32 (d, 1H, *J* = 4.8 Hz, NCC*H*O), 4.87 (m, 1H, C*H*O), 4.74 (m, 1H, C*H*O), 4.70 (q, 2H, *J* = 7.2 Hz, C*H*$_2$CH$_3$), 4.27 (m, 1H, C*H*), 3.61 (dd, *J* = 14.7 Hz, *J'* = 6 Hz, 2H, C*H*$_2$OH), 3.59 (dd, *J* = 15.9 Hz, *J'* = 6.3 Hz, 2H, C*H*$_2$OH), 2.91 (dd, *J* = 13.5 Hz, *J'* = 6.6 Hz, 1H, C*H*$_2$), 2.86 (dd, *J* = 13.5 Hz, *J'* = 7.2 Hz, 1H, C*H*$_2$), 1.60 (t, 3H, *J* = 7.2 Hz, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (75 MHz, MeOD): δ 165.91, 160.81, 157.33, 152.99, 139.81, 137.72, 132.50, 132.27, 120.80, 114.32, 89.93, 78.65, 75.63, 64.17, 55.39, 49.73, 37.80, 14.66 ppm. HPLC-UV: λ_{max} = 215.6, 257.9, and 300.5 nm. LC-ESI-MS: 583, 585 (M$^+$ + 23, 95%, 100%), 561, 563 (M$^+$ + 1, 43%, 41%), 559, 561 (M$^+$ − 1, 100%, 98%). HRMS *m/z* calcd for $C_{21}H_{25}BrN_{10}O_4$, 561.133467 (M$^+$ + H); found, 561.132186.

2-[1-(*S*)-Hydroxymethyl-2-(4-methoxyphenyl)ethylamino]-6-amino-9H-[5-(2′-ethyl-2H-tetrazolyl)-*β*-D-ribofuranosyl]purine (4). A similar procedure to that described for **1** was applied. Starting from compound **17** (71 mg; 0.19 mmol), **21** (77 mg, 0.43 mmol), anhydrous DMSO (0.20 mL), and ^{i}Pr$_2$NEt$_2$ (0.52 mL, 3.03 mmol), compound **4** (43 mg; 43% yield) was obtained as a white solid; mp 108−110 °C. [α]$_D^{20}$ −13.0°, (MeOH, *c* = 1.0). IR (KBr): ν 3355, 3217, 2941, 1638, 1606, 1512, 1479, 1246, 1038, 789 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 8.12 (s, 1H, NC*H*N), 7.21 (d, *J* = 8.7 Hz, 2H, C$_6$*H*$_4$), 6.81 (d, *J* = 8.7 Hz, 2H, C$_6$*H*$_4$), 6.12 (d, 1H, *J* = 4.5 Hz, C*H*O), 5.32 (d, 1H, *J* = 4.8 Hz, NCC*H*O), 4.87 (m, 1H, C*H*O), 4.73 (m, 1H, C*H*O), 4.71 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 4.24 (m, 1H, C*H*), 3.72 (s, 3H, OC*H*$_3$), 3.73 (s, 3H, OC*H*$_3$), 3.60 (d, *J* = 5.1 Hz, 2H, C*H*$_2$OH), 2.86 (dd, *J* = 13.8 Hz, *J'* = 6.9 Hz, 1H, C*H*$_2$), 2.82 (dd, *J* = 13.5 Hz, *J'* = 6.9 Hz, 1H, C*H*$_2$), 1.61 (t, 3H, *J* = 7.5 Hz, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (75 MHz, MeOD): δ 165.91, 160.86, 159.53, 157.27, 152.96, 137.65, 132.27, 131.43, 114.68, 114.19, 89.86, 78.59, 75.63, 64.19, 55.65, 55.60, 49.72, 37.49, 14.68 ppm. HPLC-UV: λ_{max} = 222.6, 259.1, and 300.5 nm. LC-ESI-MS: 535 (M$^+$ + 23, 100%), 513 (M$^+$ + 1, 18%). HRMS *m/z* calcd for $C_{22}H_{28}N_{10}O_5$, 513.224414 (M$^+$ + H); found, 513.232239.

2-[1-(*S*)-Hydroxymethyl-2-(4-hydroxyphenyl)ethylamino]-6-amino-9H-[5-(2′-ethyl-2H-tetrazolyl)-*β*-D-ribofuranosyl]purine (5). Following the same procedure as described for **1**, from compound **17** (0.070 g, 0.165 mmol), **22** (0.072 g 0.165 mmol), anhydrous DMSO (0.150 mL), and ^{i}Pr$_2$NEt (0.439 mL, 2.56 mmol), compound **5** (0.020 g, 52% yield) was obtained as a white solid; mp 123−125 °C. [α]$_D^{20}$ −8.7 (MeOH, *c* = 0.160). IR (KBr): ν 3349, 3040, 1639, 1604, 1560, 1487, 765 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 8.11 (s, 1H, NC*H*N), 7.12 (d, *J* = 8.7 Hz, 2H, C$_6$*H*$_4$), 6.69 (d, *J* = 8.4 Hz, 2H, C$_6$*H*$_4$), 6.12 (d, *J* = 4.5 Hz, 1H, C*H*O), 5.32 (d, *J* = 4.8 Hz, 1H, NCC*H*O), 4.89 (m, 1H, C*H*O), 4.75 (m, 1H, C*H*O), 4.70 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 4.22 (m, 1H, C*H*), 3.59 (dd *J* = 22.5 Hz, *J'* = 4.8 Hz, 1H, C*H*$_2$OH), 2.83 (dd, *J* = 20.7 Hz, Hz, *J'* = 6.9 Hz, 1H, C*H*$_2$OH), 2.83 (dd, *J* = 20.7 Hz, *J'* = 6.9 Hz, 1H, C*H*$_2$), 2.80 (dd, *J* = 20.7 Hz, *J'* = 6.9 Hz, C*H*$_2$), 1.60 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (75 MHz, MeOD): δ 165.91, 160.87, 157.30, 156.71, 152.99, 137.70, 131.45, 131.04, 116.08, 114.23, 89.92, 78.62, 75.63, 64.15, 55.64, 49.72, 37.47, 14.68 ppm. HPLC−UV: λ_{max} = 215.6, 257.9, and 300.5 nm. LC-ESI-MS: 498.2 (M$^+$, 37.2%), 497.1 (M$^+$ − 1, 100%). HRMS *m/z* calcd for $C_{21}H_{26}N_{10}O_5$, 499.216263 (M$^+$ + H); found, 499.216589.

2-[1-(*S*)-Hydroxymethyl-2-(4-nitrophenyl)ethylamino]-6-amino-9H-[5-(2′-ethyl-2H-tetrazolyl)-*β*-D-ribofuranosyl]purine (6). Following a similar procedure as described for **1**, starting from compound **17** (35 mg, 0.0097 mmol), **23** (41 mg, 0.213 mmol), anhydrous DMSO (0.120 mL), and ^{i}Pr$_2$NEt$_2$ (0.260 mL, 1.51 mmol), compound **6** (15 mg, 31% yield) was obtained as a yellowish solid; mp 115−118 °C. [α]$_D^{20}$ −16.0, (MeOH, *c* = 1). IR (KBr): ν 3450, 2927, 1638, 1602, 1519, 1347, 1042 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 8.11 (d, *J* = 8.7 Hz, C$_6$*H*$_4$), 8.09 (s, 1H, NC*H*N), 7.55 (d, *J* = 8.7 Hz, C$_6$*H*$_4$), 6.07 (d, 1H, *J* = 4.5 Hz, C*H*O), 5.32 (d, 1H, *J* = 4.8 Hz, NCC*H*O), 4.93 (m, 1H, C*H*O), 4.85 (m, 1H, C*H*O), 4.72 (q, *J* = 7.2 Hz, 2H, C*H*$_2$CH$_3$), 4.38 (m, 1H, C*H*), 3.64 (dd, *J* = 4.8 Hz, *J'* = 1.8 Hz, 1H, C*H*$_2$OH), 3.10 (dd, *J* = 13.5 Hz, *J'* = 6 Hz, 1H, C*H*$_2$), 3.00 (dd, *J* = 13.2 Hz, *J'* = 7.5 Hz, 1H, C*H*$_2$), 1.61 (t, 3H, *J* = 7.2 Hz, CH$_2$C*H*$_3$) ppm. ^{13}C NMR (75 MHz, MeOD): δ 165.93, 160.74, 157.28, 152.94, 149.07, 147.85, 131.61, 124.24, 114.22, 89.67, 78.65, 75.66, 64.39, 55.37, 49.74, 38.59, 14.69 ppm. LC-ESI-MS: 550 (M$^+$ + 23, 28%), 528 (M$^+$ + 1, 100%). HRMS *m/z* calcd for $C_{21}H_{25}N_{11}O_6$, 528.2091 (M$^+$ + H); found, 528.2094.

2-[1-(*S*)-Methyl-2-(4-methoxyphenyl)ethylamino]-6-amino-9H-[5-(2′-ethyl-2H-tetrazolyl)-*β*-D-ribofuranosyl]purine (7). The same procedure as described for **1** was applied. From compound **17** (50 mg, 0.14 mmol), (*S*)-**24**[30] (49 mg, 0.030 mmol), anhydrous DMSO (0.120 mL), and ^{i}Pr$_2$NEt (0.370 mL, 2.12 mmol), compound **7** (43 mg, 62% yield) was obtained as a white solid; mp 112−113 °C. [α]$_D^{20}$ −1° (MeOH, *c* = 1.24). IR (KBr): ν 3359, 3277, 1635, 1512, 1426, 789 cm^{-1}. ^{1}H NMR (300 MHz, MeOD): δ 8.11 (s, 1H, NC*H*N), 7.15 (d, *J* = 8.7 Hz, 2H, C$_6$*H*$_4$), 6.80 (d, *J* = 9 Hz, 2H, C$_6$*H*$_4$), 6.13 (d, *J* = 4.5 Hz, 1H, C*H*O), 5.33 (d, *J* = 4.5 Hz, 1H, NCC*H*O), 4.86 (m, 1H, C*H*O), 4.74 (m, 1H, C*H*O), 4.69 (q, 2H, *J* = 7.5 Hz, C*H*$_2$CH$_3$), 4.22 (m, 1H, C*H*), 2.89 (dd, *J* = 13.5 Hz, *J'* = 5.7 Hz, 1H, C*H*$_2$), 2.63 (dd, *J* = 13.2 Hz, *J'* = 7.2 Hz, 1H, C*H*$_2$), 1.60 (t, 3H, *J* = 7.5 Hz, CH$_2$C*H*$_3$), 1.13 (d, *J* = 6.6 Hz, C*H*$_3$) ppm. ^{13}C NMR (75 MHz, MeOD): δ 165.90, 160.48, 159.48, 157.20, 153.09, 137.64, 132.55, 131.47, 114.59, 89.89, 78.80, 78.65, 75.63, 55.60, 49.69, 42.87, 20.30, 14.68 ppm. HPLC-UV: λ_{max} = 215.6, 257.9, and 300.5 nm. LC-ESI-MS: 519 (M$^+$ + 23, 72%), 497 (M$^+$ + 1, 100%), 495 (M$^+$ − 1, 50%). HRMS *m/z* calcd for $C_{21}H_{25}N_{10}O_4$, 497.238205 (M$^+$ + H); found, 497.237325.

Drugs and Reagents. Recombinant A$_{2A}$ and A$_3$ human receptors were cloned and expressed in transfected HeLa cells

Table 4. Conditions Used for Radioligand Binding Assays Using A_1, A_{2A}, A_{2B}, and A_3 Human Receptors

	A_1	A_{2A}	A_{2B}	A_3
buffer A	20 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$, 2 units/mL adenosine deaminase (pH 7.4)	50 mM Tris-HCl, 1 mM EDTA, 10 mM $MgCl_2$, 2 units/mL adenosine deaminase (pH 7.4)	50 mM Tris-HCl, 1 mM EDTA, 10 mM $MgCl_2$, 0.1 mM benzamidine, 2 units/mL adenosine deaminase (pH 6.5)	50 mM Tris-HCl, 1 mM EDTA 5 mM $MgCl_2$, 2 units/mL adenosine deaminase (pH 7.4)
buffer B	20 mM Hepes, 100 mM NaCl, 10 mM $MgCl_2$ (pH 7.4)	50 mM Tris-HCl, 1 mM EDTA, 10 mM $MgCl_2$ (pH 7.4)	50 mM Tris-HCl (pH 6.5)	50 mM Tris-HCl (pH 7.4)
plate	GF/C	GF/C	GF/B	GF/B
radioligand	[^{3}H]DPCPX (2 nM)	[^{3}H]ZM241385 (3 nM)	[^{3}H]DPCPX (35 nM)	[^{3}H]NECA (30 nM)
nonspecific binding	10 μM (*R*)-PIA	50 μM NECA	400 μM NECA	100 μM (*R*)-PIA
incubation	25 °C/60 min	25 °C/30 min	25 °C/30 min	25 °C/180 min

as part of a collaboration between the University of Santiago and Almirall Prodesfarma. Transfected CHO and HEK-293 cell lines expressing human recombinant A_1 and A_{2B} receptors, respectively, were licensed from Euroscreen (Belgium). All pharmacological and cell culture reagents (including adenosine receptor agonists and antagonists such as NECA, CSC, alloxazine, DMPX, and 8-PT) were purchased, unless otherwise stated, from Sigma-R.B.I. or Sigma-Aldrich (Alcobendas, Spain).

Radioligand Binding Assays. Radioligand binding competition assays were performed in vitro using A_1, A_{2A}, A_{2B}, and A_3 human receptors expressed in transfected CHO (A_1), HeLa (A_{2A} and A_3), and HEK-293 (A_{2B}) cells. The experimental conditions used are summarized in Table 4. In each instance, aliquots of membranes (15 μg for A_1, 10 μg for A_{2A}, 18 μg for A_{2B}, and 90 μg for A_3) in buffer A (see Table 4) were incubated for the specified period at 25 °C with the radioligand (2−35 nM) and six different concentrations (ranging from 0.1 nM to 1 μM) of the test molecule or standard in a final volume of 200 μL. The binding reaction was stopped by rapid filtration in a multiscreen manifold system (Millipore Ibérica, Madrid, Spain). Unbound radioligand was removed by washing 4× with 250 μL of ice-cold buffer B for A_1 and A_{2A} receptors and 6 × 250 μL of ice-cold buffer B for A_{2B} and A_3 receptors (see Table 4). Nonspecific binding was determined using a 50−400 μM NECA solution for A_{2A} and A_{2B} receptors and 10−100 μM (*R*)-PIA solution for A_1 and A_3. Radioactivity retained on filters was determined by liquid scintillation counting using Universol (ICN Biochemicals, Inc.). The binding affinities were determined using [^{3}H]DPCPX (130 Ci/mmol; Amersham Biosciences, Barcelona, Spain) as the radioligand for A_1 and A_{2B}, [^{3}H]ZM241385 (21 Ci/mmol; Tocris, Madrid, Spain) for A_{2A}, and [^{3}H]NECA (15.3 Ci/mmol; NEN-Perkin-Elmer Life Sciences, Madrid, Spain) for A_3. The inhibition constant (K_i) of each compound was calculated by the expression $K_i = IC_{50}/[1 + (C/K_D)]$, where IC_{50} is the concentration of compound that displaces the binding of radioligand by 50%, C is the free concentration of radioligand, and K_D is the apparent dissociation constant of each radioligand.

cAMP Assays. These assays were performed using A_{2A} and A_{2B} receptors by using the method described by Salomon.[54] Briefly, cells were seeded in 12 well culture plates and incubated at 37 °C in an atmosphere with 5% CO_2 in Dulbeco's modified Eagle's medium nutrient mixture F-12 (DMEM F-12), containing 10% fetal calf serum (FCS) and 1% L-glutamine, and this medium was replaced 24 h before the assays by medium containing dialyzed FCS. Prior to the assay, 2,8-[^{3}H]-adenine (21 Ci/mmol, Moravek Biochemicals, United States) was added to the medium (3 μCi/mL) and cells were incubated for 2 h in 5% CO_2 atmosphere at 37 °C. The cells were washed 3× with 1 mL of assay medium (DMEM-F12 and 25 mM HEPES pH 7.4) and preincubated with assay medium containing 30 μM Rolipram at 37 °C for 15 min. Compounds were incubated for 15 min at 37 °C. The reaction was stopped by adding ice-cold 300 mM perchloric acid containing [^{14}C]cAMP (56 mCi/mmol, Moravek Biochemicals), and the cells were maintained at 4 °C for 30 min. The [^{3}H]cAMP elicited in each well was isolated by chromatographic methods, and [^{14}C]cAMP allowed calculation of the isolation yield. The potency of the compounds was expressed as EC_{50} (concentration of compound that elicited 50% of maximal response), and the efficacy was expressed as E_{max} (maximal response observed with respect to that observed with NECA).

Isolated Organ Assays. A_{2A} Receptors. These assays were performed in A_{2A} receptors[55] from isolated aortas of 200−250 g male Sprague−Dawley rats. The aorta was rapidly excised and placed in modified Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.18; $NaHCO_3$, 25; and glucose, 11. The solution was maintained at 37 °C with aeration by carbogen (95% CO_2 + 5% O_2, pH 7.4 ± 0.1). The vessels were cleaned to remove connective tissue, cut into rings 4 mm in length, and suspended between stainless steel wires in organ baths containing 20 mL of Krebs solution, under a basal tension of 2 g (maintained throughout the experiment). The aorta rings were stabilized for 60 min in the modified Krebs solution at 37 ± 0.2 °C continuously saturated with carbogen before the start of the assay, and during the stabilization time, they were washed with new Krebs solution at least three times (15 min each time). All aorta rings were initially exposed to 0.1 μM phenylephrine to elicit a contractile response, and after this, the presence of endothelium was confirmed by the addition of acetylcholine (10 μM). Tissues giving less than 25% relaxation of phenylephrine contraction were discarded. After a recovery time of 60 min with successive washes with fresh Krebs solution, a new phenylephrine contraction was elicited and responses to NECA or the test compound were measured and used to construct cumulative relaxant−response curves. The potency of the compounds was expressed as EC_{50} (concentration of compound that elicited 50% of maximal response), and efficacy was expressed as E_{max} (maximal response observed with respect to that observed with NECA).

A_{2B} Receptors. These assays were performed in A_{2B} receptors[56] from isolated aortas of 300−350 g male guinea pigs. The aorta was rapidly excised and placed in modified Krebs solution of the following composition (mM): NaCl, 118; KCl, 4.7; $MgSO_4 \cdot 7H_2O$, 1.2; $CaCl_2 \cdot 2H_2O$, 2.5; KH_2PO_4, 1.18; $NaHCO_3$, 25; glucose, 11; and indomethacin, 0.01. The solution was maintained at 37 °C with aeration by carbogen. The vessels were cleaned to remove connective tissue and rubbed to remove the endothelium. They were cut into rings, 4 mm in length, that were suspended between stainless steel wires in organ baths containing 20 mL of Krebs solution, under a basal tension of 1 g (maintained throughout the experiment). The aorta rings were stabilized for 60 min in the modified Krebs solution at 37 ± 0.2 °C continuously saturated with carbogen before the start of the assay, and during the stabilization time, they were washed with new Krebs solution at least three times (15 min each time). All aorta rings were initially exposed to 4 μM phenylephrine to elicit a contractile response, and after this, the absence of endothelium was confirmed by the addition of acetylcholine (10 μM). Tissues giving any relaxation of phenylephrine contraction were discarded. After a recovery

time (60 min) with successive washes with fresh Krebs solution, a new phenylephrine contraction was elicited and responses to NECA or the test compound were measured as above. The potency of compounds and efficacy were expressed as EC_{50} and E_{max} as for A_{2A} receptors.

Isolated Guinea Pig Tracheal Ring Assays. Dunkin Hartley guinea pigs, weighing 250−300 g, were sacrificed by a blow to the head. The trachea was dissected out, transferred to a dish containing Krebs solution, and cut transversally between the segments of cartilage. Five of the tracheal rings were tied together and mounted in a 15 mL organ bath containing a modified Krebs solution maintained at 37 °C and gassed with 95% O_2−5% CO_2 throughout the whole experiment. An initial basal tension of 1 g was applied to each tracheal chain, and the tissue was allowed to stabilize for 90 min. Isometric force was recorded from the preparations by a force−displacement transducer coupled to an Omni-Scribe recorder. A constant level of tone was induced by the addition of a 5×10^{-7} M carbachol chloride solution to the bath to obtain after 15 min a control concentration−response curve for each agonist.[42,57] The tracheal chain was washed thoroughly with the Krebs solution for 30 min, and then, a 10^{-4}M solution of the antagonist (8-PT, DMPX, CSC, and alloxazine) was added to the bath and allowed to act for 30 min. During the last 15 min of the antagonist incubation, the carbachol chloride solution was added to the bath and the cumulative concentration−response curve for each agonist was determined by measuring the maximal relaxation produced by different concentrations of the agonist (compound **1**, 3×10^{-8} to 10^{-3} M; compound **2**, 3×10^{-7} to 10^{-3} M; compound **4**, 3×10^{-8} to 3×10^{-4} M; compound **7**, 10^{-6} to 3×10^{-4} M; adenosine, 3×10^{-6} to 10^{-2} M; and NECA, 3×10^{-7} to 10^{-3}M). The relaxation values were expressed as a percentage of the maximal relaxation obtained in all cases. The relaxant effects are evaluated from the results of 2−6 isolated tissues obtained from the same number of animals, and each experiment was carried out with 5−6 different concentrations within the range cited above. E_{max} is the theoretical maximal effect, and the EC_{50} is the concentration of agonist required to elicit 50% of the maximal response.[58] EC_{50} values were calculated by regression analysis of at least four points in the linear region of the curves ($r^2 \geq 0.91$ for all curves).

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Supporting Information Available: Table of the purity of the target compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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