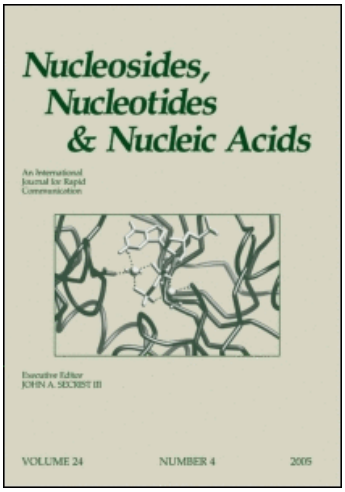

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A_{2B} Adenosine Receptor Agonists: Synthesis and Biological Evaluation of 2-Phenylhydroxypropynyl Adenosine and NECA Derivatives

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A_{2B} Adenosine Receptor Agonists: Synthesis and Biological Evaluation of 2-Phenylhydroxypropynyl Adenosine and NECA Derivatives[†]

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ABSTRACT

In the search for agonists for the elusive A_{2B} adenosine receptor subtypes, 2-phenylhydroxypropynyl-5′-N-methylcarboxamido adenosine (PHPMECA, **14**), 2-phenylhydroxypropynyl-5′-N-propylcarboxamido adenosine (PHPPECA, **15**), and N^6-ethyl-2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine (**19**) were synthesized on the basis that introduction of alkynyl chains in 2-position of adenosine derivatives resulted in reasonably good A_{2B} potency compared to NECA [see N^6-ethyl-2-phenylhydroxypropynyl adenosine (**5**) EC_{50} = 1,700 nM and 2-phenylhydroxypropynyl-5′-N-ethylcarboxamido adenosine (PHPNECA, **8**) EC_{50} = 1,100 nM, respectively]. Radioligand binding studies and adenylyl cyclase assays, performed with recently cloned human A_1, A_{2A}, A_{2B}, and A_3 adenosine receptors, showed that these modifications produced a decrease in potency at A_{2B} receptor, as well as a general reduction in affinity at the other receptor subtypes. On the other hand, the

[†]In honor and celebration of the 70th birthday of Professor Leroy B. Townsend.

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contemporary presence of an ethyl substituent in N^6-position and of a 4′-ethylcarboxamido group in the same compounds led to (*R*,*S*)-N^6-ethyl-2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine and (*S*)-N^6-ethyl-2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine, which did not show the expected increase in potency at A_{2B} subtype. Hence, (*S*)-2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine [(*S*)-PHPNECA] with EC_{50} A_{2B} = 220 nM remains the most potent agonist at A_{2B} receptor reported so far.

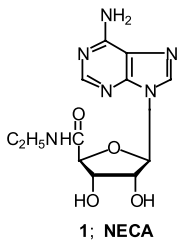
Key Words: Adenosine receptor; A_{2B} adenosine receptor; 2-alkynyladenosine derivatives; 2-alkynylNECA derivatives.

INTRODUCTION

Adenosine and adenosine related molecules regulate many aspects of cellular metabolism mainly through the interaction with extracellular receptors. Some of the physiological actions of adenosine include effects on heart rate and atrial contractility, vascular smooth muscle tone, release of neurotransmitters, platelet function, lipolysis, renal function and white blood cell function.[1] Although purinergic mechanism is an expanding therapeutic target for the discovery of novel drugs, to date only adenosine itself is approved for human use although over the last years many efforts have been directed toward the discovery of potent and selective adenosine agonists.[2,3] The diverse actions of adenosine are mediated by at least four human adenosine receptors (P1), belonging to the superfamily of G protein-coupled receptors, which have been recently cloned[4,5] and classified as A_1, A_{2A}, A_{2B}, and A_3.[6]

Although several potent adenosine receptor ligands have been developed over the past two decades there is still no complete panel of subtype selective agonists and antagonists.[7,8] In the case of the elusive low-affinity A_{2B} subtype, coupled to stimulation of adenylyl cyclase, the search for potent and selective agonists is a major challenge for medicinal chemists. To date, 5′-N-ethylcarboxamidoadenosine (NECA, **1**) is one of the most active nucleoside at this subtype with an EC_{50} of 2.4 μM[5] (Figure 1).

However, introduction of alkynyl chains in the 2-position of adenosine derivatives resulted in reasonably good A_{2B} potency compared to NECA. In particular, racemic 2-phenylhydroxypropynyladenosine [(*R*,*S*)-PHPAdo, **2** (Figure 1)] showed activity at A_{2B} receptor similar to that of NECA, while the corresponding (*R*, **3**) and (*S*, **4**) diastereomers resulted to be about 2-fold less potent and 3-fold more potent than the



1; NECA

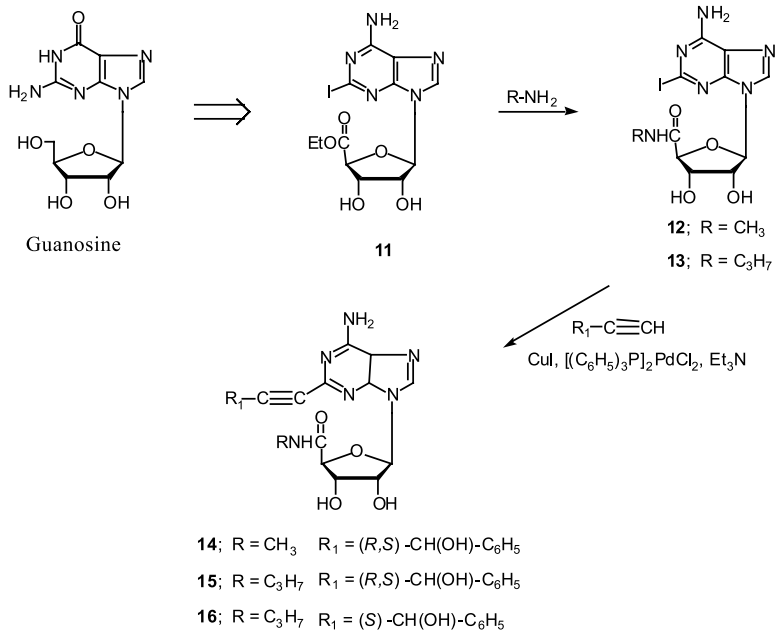
Figure 1. Structure of 5′-N-ethylcarboxamidoadenosine.

racemate, respectively[9,10] (Figure 1). Introduction of an ethyl group in N^6-position of PHPAdo[9] or substitution of the 4′-hydroxymethyl group with a 4′-ethylcarboxamido group in the same compound[11–13] enhanced A_{2B} potency [N^6-ethyl-2-phenylhydroxypropynyladenosine (**5**) EC_{50} = 1,700 nM and 2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine (PHPNECA, **8**) EC_{50} = 1,100 nM]. Also in these cases, the (*S*) diastereomers (**7** and **10**, respectively) were more potent than the (*R*) ones (**6** and **9**, respectively), hence (*S*)-PHPNECA (**10**) with an EC_{50} = 220 nM is the most potent A_{2B} agonist reported so far.[10]

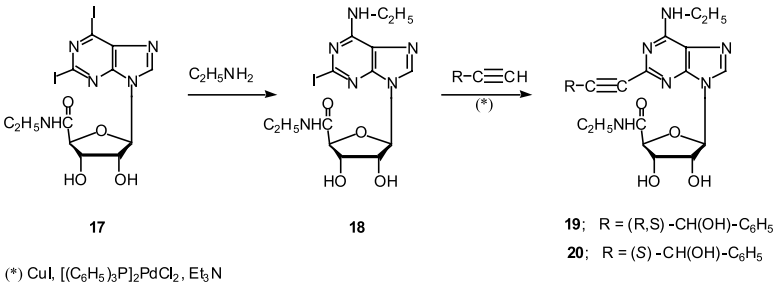
In the present study, the influence on A_{2B} affinity of modifying the N-ethylcarboxamido substituent of PHPNECA was investigated by synthesizing 2-phenylhydroxypropynyl-5′-N-methylcarboxamidoadenosine (PHPMECA, **14**) and 2-phenylhydroxypropynyl-5′-N-propylcarboxamidoadenosine (PHPPECA, **15**). Furthermore, a combination of the N^6- and 4′-substitutions was undertaken through the synthesis of N^6-ethyl-2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine (**19**).

CHEMISTRY

The synthesis of the desired compounds was achieved starting from compound **11**, prepared from commercially available guanosine in eight steps,[14] in which the 5′-position of ribose was functionalised with an ethyl ester and the 2-position of adenine moiety with an iodine. Hence, 5′-alkylcarboxamido nucleosides **12** and **13** were obtained by reacting the corresponding ethyl ester with the appropriate amines. Furthermore, compounds **14**–**16** were prepared in moderate yield by introducing at the



Scheme 1. Synthesis of 5′-N-alkylcarboxamido2-phenylhydroxypropynyladenosine.



Scheme 2. Synthesis of trisubstituted adenine nucleosides.

2-position the (*R*,*S*)-, and (*S*)-phenylhydroxypropynyl chains through a modification of the palladium catalyzed cross-coupling reaction (Scheme 1).

The synthesis of the trisubstituted compounds was achieved starting from 9-(*N*-ethyl-β-D-ribofuranuronamide)-2,6-diiodopurine (**17**),[15] which was treated with ethylamine to give compound **18** in good yield. The (*R*,*S*) and (*S*) phenylhydroxypropynyl chains were introduced in the 2-position of **18** through the palladium catalyzed cross-coupling reaction, to afford compounds **19** and **20** (Scheme 2).

BIOLOGICAL RESULTS AND DISCUSSION

All the compounds were evaluated at the human recombinant adenosine receptors, stably transfected into Chinese hamster ovary (CHO) cells, utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assay (A_{2B}). Receptor binding affinity was determined using [^{3}H]CCPA (2-chloro-N^6-cyclopentyladenosine) as radioligand for A_1 receptors, whereas [^{3}H]NECA was used for the A_{2A} and A_3 subtypes (K_i; nM).[5] The results are shown in Table 1.

The relative potencies of these compounds for the A_{2B} subtype were measured by evaluating the receptor-stimulated adenylyl cyclase activity expressed as EC_{50}, nM.

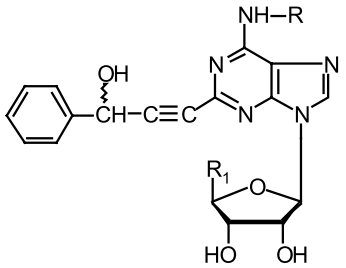
The reference compound NECA (**1**) showed high affinity at A_1, A_{2A}, and A_3 receptors and was rather unselective (K_i A_1 = 14 nM, K_i A_{2A} = 20 nM, and K_i A_3 = 6.2 nM). The potency for the A_{2B} receptor, in the low micromolar range, characterized NECA as one of the most active nucleosides at this subtype (EC_{50} A_{2B} = 2,400 nM).[5]

However, introduction of alkynyl chains in the 2-position of adenosine derivatives resulted in reasonably good A_{2B} potency compared to NECA.

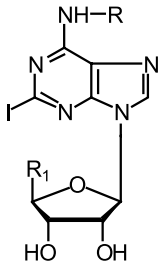
In particular, the introduction of phenylhydroxypropynyl chains (both the racemate and the *S* and *R* forms) led to compounds which showed activity at A_{2B} receptor similar to that of NECA [(*R*,*S*)-PHPAdo, **2** EC_{50} A_{2B} = 2,400 nM, (*R*)-PHPAdo, **3** EC_{50} A_{2B} = 6,200 nM, and (*S*)-PHPAdo, **4** EC_{50} A_{2B} = 920 nM]. Hence the *S* diastereomer **4** was found to be about 3-fold more potent than the racemate and than NECA.[12,13] However, the increase of affinity at the other adenosine receptor subtypes was even more relevant (Table 1).

Introduction of an ethyl group in the N^6-position of PHPAdo[9] or substitution of the 4′-hydroxymethyl group with a 4′-ethylcarboxamido group in the same compound[11–13] enhanced A_{2B} potency [N^6-ethyl-2-phenylhydroxypropynyladenosine,

Table 1. Affinities of adenosine and NECA derivatives in radioligand binding assays at human A_1, A_{2A}, and A_3 adenosine receptors and effects on adenylate cyclase activity at the human A_{2B} adenosine receptor.



2-10, 14-16, 19, 20



12, 13, 18

			K_i or EC_{50}, nM			
Cpd	R	R_1	K_i (A_1)[a]	K_i (A_{2A})[b]	EC_{50} (A_{2B})[c]	K_i (A_3)[d]
1 NECA	–	–	14 (16–28)	20 (12–35)	2,400 (1,900–3,000)	6.2 (3.5–11)
2 (*R,S*)-PHPAdo	H	CH_2–OH	0.67 (0.55–0.80)	7.0 (3.7–13)	2,400 (1,500–3,700)	3.3 (2.3–4.8)
3 (*R*)-PHPAdo	H	CH_2–OH	0.44 (0.38–0.52)	29 (19–35)	6,200 (1,900–3,000)	6.2 (5.0–7.7)
4 (*S*)-PHPAdo	H	CH_2–OH	0.67 (0.47–0.96)	1.8 (1.1–3.0)	920 (710–1,200)	1.4 (0.78–2.4)
5 (*R,S*)	C_2H_5	CH_2–OH	2.7 (2.4–2.9)	94 (72–123)	1,700 (973–3,000)	0.97 (0.58–1.6)
6 (*R*)	C_2H_5	CH_2–OH	2.2 (2.0–2.4)	1,200 (864–1,614)	9,100 (6,900–12,000)	1.4 (0.70–2.9)
7 (*S*)	C_2H_5	CH_2–OH	3.4 (2.7–4.2)	64 (34–120)	730 (610–890)	0.20 (0.14–0.28)
8 (*R,S*)-PHPNECA	H	CO–NH–C_2H_5	2.7 (1.7–4.1)	3.1 (2.4–3.9)	1,100 (471–2,600)	0.42 (0.17–1.0)
9 (*R*)-PHPNECA	H	CO–NH–C_2H_5	1.9 (1.8–2.1)	39 (25–59)	2,400 (1.500–3,800)	5.5 (3.6–8.5)
10 (*S*)-PHPNECA	H	CO–NH–C_2H_5	2.1 (1.2–3.7)	2.0 (1.2–3.5)	220 (220–230)	0.75 (0.52–1.1)
12	H	CO–NH–CH_3	140 (120–160)	1,000 (670–1.500)	>100 μM	16 (8.5–32)
13	H	CO–NH–C_3H_7	74 (70–79)	1,000 (940–1,100)	>100 μM	120 (91–160)

(*continued*)

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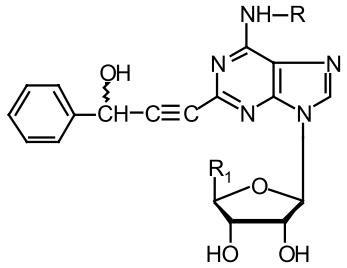
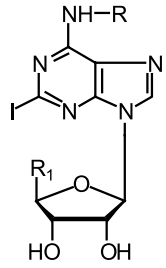
Table 1. Continued.



2-10, 14-16, 19, 20



12, 13, 18

			K_i or EC_{50}, nM			
Cpd	R	R_1	K_i (A_1)[a]	K_i (A_{2A})[b]	EC_{50} (A_{2B})[c]	K_i (A_3)[d]
14 (*R,S*)-PHPMECA	H	CO–NH–CH_3	14 (8.7–22)	3.1 (1.7–5.4)	5,000 (3,500–7,200)	1.7 (1.0–2.7)
15 (*R,S*)-PHPPECA	H	CO–NH–C_3H_7	13 (10–15)	19 (14–26)	> 100 μM	7.1 (4.7–11)
16 (*S*)-PHPPECA	H	CO–NH–C_3H_7	11 (10–12)	8.9 (5.8–14)	≥100 μM	4.2 (2.2–8.1)
18	C_2H_5	CO–NH–C_2H_5	43 (34–53)	360 (290–460)	> 100 μM	16 (13–19)
19 (*R,S*)	C_2H_5	CO–NH–C_2H_5	15 (8.0–29)	90 (48–170)	2,000 (1,300–3,200)	2.1 (1.3–3.4)
20 (*S*)	C_2H_5	CO–NH–C_2H_5	28 (22–35)	71 (39–130)	3,300 (1,900–5,700)	1.5 (1.2–1.8)

[a]Displacement of specific [^{3}H]CCPA binding in CHO cells, stably transfected with human recombinant A_1 adenosine receptor, expressed as K_i (nM).
[b]Displacement of specific [^{3}H]NECA binding in CHO cells, stably transfected with human recombinant A_{2A} adenosine receptor, expressed as K_i (nM).
[c]Measurement of receptor-stimulated adenylyl cyclase activity in CHO cells, stably transfected with human recombinant A_{2B} adenosine receptor, expressed as EC_{50} (nM).
[d]Displacement of specific [^{3}H]NECA binding in CHO cells, stably transfected with human recombinant A_3 adenosine receptor, expressed as K_i (nM).

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(**5**) EC_{50} = 1,700 nM and 2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine (PHPNECA, **8**) EC_{50} = 1,100 nM, respectively]. Also in these cases, the (*S*) diastereomers (**7** and **10**, respectively) were more potent than the (*R*) ones (**6** and **9**, respectively), hence (*S*)-PHPNECA (**10**) with an EC_{50} = 220 nM was the most potent A_{2B} agonist reported so far.[10]

In order to assess the influence on A_{2B} affinity of modifying the N-ethylcarboxamido substituent of PHPNECA, 2-phenylhydroxypropynyl-5′-N-methylcarboxamidoadenosine [(*R*,*S*)-PHPMECA, **14**] and 2-phenylhydroxypropynyl-5′-N-propylcarboxamidoadenosines [(*R*,*S*)- and (*S*)-PHPPECA, **15** and **16**, respectively] were tested at the cloned human adenosine receptors. The data reported in Table 1 showed that these modifications produced a decrease in potency at A_{2B} receptor, as well as a general reduction in affinity at the other receptor subtypes. It is worthwhile to note that the presence of a propyl substituent in the 4′-carboxamido group is detrimental for the A_{2B} potency (**14**, EC_{50} A_{2B} = 5,000 nM, **15** and **16** EC_{50} A_{2B} > 100 μM).

On the other hand, the presence of an ethyl substituent in N^6-position and of a 4′-ethylcarboxamido group in the same compounds led to (*R*,*S*)-N^6-ethyl-2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine (**19**) and (*S*)-N^6-ethyl-2-phenylhydroxypropynyl-5′-N-ethylcarboxamidoadenosine (**20**), which did not show the expected increase in potency at A_{2B} subtype (**19**, EC_{50} A_{2B} = 2,000 nM versus **5**, EC_{50} A_{2B} = 1,700 nM and **8** EC_{50} A_{2B} = 1,100 nM).

The intermediate compounds **12**, **13**, and **18**, bearing a iodine atom at C-2, were also tested and found to be inactive toward the A_{2B} receptor subtype.

These data confirmed the previously reported considerations that substitution of the 2-position of adenosine derivatives is in general not well tolerated by the A_{2B} receptor,[8,16] with the exception of compounds bearing alkynyl chains in this position; in particular the 2-phenylhydroxypropynyl group seems to have a favourable interaction with the A_{2B} receptor binding site.[10]

EXPERIMENTAL SECTION

Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with Varian VXR 300 MHz spectrometer; ∂ in ppm, *J* in Hz. TLC were carried out on pre-coated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Elemental analyses were determined on Carlo Erba model 1106 analyser and are within ± 0.4% of theoretical values.

A: General procedure for the preparation of 9-(*N*-alkyl-β-D-ribofuranuronamide)-2-iodoadenine (12 and 13). To 600 mg (1.38 mmol) of 2-iodoadenosine-5′-N-ethyluronate (**11**),[14] cooled at −20°C, the appropriate amine (15 mL) was added and the mixture was stirred at room temperature for 16 h. The excess amine was removed in vacuo and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents to give **12** or **13** as pure solids.

2-Iodo-9-(*N*-methyl-β-D-ribofuranuronamide)adenine (12). The reaction of **11** with methylamine, followed by chromatography on a silica gel column eluting with $CHCl_3$-MeOH (90:10), gave **12** (493 mg; 85%); mp 220–222°C; ^{1}H NMR (DMSO-d_6):

∂ 2.78 (d, 3H, J = 4.6 Hz, CONHCH_3), 4.16 (m, 1H, H-3′), 4.33 (m, 1H, H-4′), 4.61 (m, 1H, H-2′), 5.62 (d, 1H, J = 6.2 Hz, OH), 5.75 (d, 1H, J = 4.1 Hz, OH), 5.92 (d, 1H, J = 7.4 Hz, H-1′), 7.82 (s, 2H, NH_2), 8.11 (m, 1H, NH-CH_3), 8.41 (s, 1H, H-8). Anal. calcd. for $C_{11}H_{13}IN_6O_4$ (420.2): C 31.44; H 3.12; N 20.00. Found: C 31.22; H 3.08; N 20.86.

2-Iodo-9-(*N*-propyl-β-D-ribofuranuronamide)adenine (13). The reaction of **11** with propylamine, followed by chromatography on a silica gel column eluting with $CHCl_3$-MeOH (97:3), gave **13** (470 mg; 76%); mp 155–157°C; ^{1}H NMR (DMSO-d_6): ∂ 0.85 (t, 3H, J = 7.2 Hz, CONHCH$_2$CH$_2$CH_3), 1.47 (m, 2H, CONHCH$_2$CH_2CH$_3$), 3.22 (m, 2H, CONHCH_2), 4.15 (m, 1H, H-3′), 4.34 (m, 1H, H-4′), 4.60 (m, 1H, H-2′), 5.63 (d, 1H, J = 4.1 Hz, OH), 5.75 (d, 1H, J = 4.2 Hz, OH), 5.91 (d, 1H, J = 7.3 Hz, H-1′), 7.81 (s, 2H, NH_2), 8.16 (m, 1H, NH–CH_2), 8.41 (s, 1H, H-8).

Anal. calcd. for $C_{13}H_{17}IN_6O_4$ (448.2): C 34.84; H 3.82; N 18.75. Found: C 34.61; H 3.64; N 19.01.

N^6-Ethyl-9-(*N*-ethyl-β-D-ribofuranuronamide)-2-iodoadenine (18). A mixture of **17** (330 mg, 0.60 mmol) and ethylamine (15 mL) cooled at −20°C was stirred at room temperature for 4 h. The excess amine was evaporated and the residue purified on a flash silica gel column eluted with $CHCl_3$-MeOH (98:2) to obtain **18** as a white amorphous solid (255 mg; 92%); ^{1}H NMR (DMSO-d_6): ∂ 1.06 (t, 3H, J = 7.2 Hz, CONHCH$_2$CH_3), 1.18 (t, 3H, J = 7.1 Hz, NHCH$_2$CH_3), 3.26 (m, 2H, CONHCH_2), 3.46 (m, 2H, NHCH_2), 4.17 (m, 1H, H-3′), 4.31 (s, 1H, H-4′), 4.58 (m, 1H, H-2′), 5.60 (d, 1H, J = 6.3 Hz, OH), 5.72 (d, 1H, J = 4.8 Hz, OH), 5.92 (d, 1H, J = 7.0 Hz, H-1′), 8.15 (bt, 1H, NHCO), 8.28 (bt, 1H, NH), 8.40 (s, 1H, H-8). Anal. calcd. for $C_{14}H_{19}IN_6O_4$ (462.2): C 36.38; H 4.14; N 18.18. Found: C 36.09; H 4.10; N 18.46.

B: General procedure for the preparation of uronamides 14–16, 19, 20. To a solution of 0.36 mmol of the appropriate 2-iodoadenosine-5′-N-alkyluronamide (**12**, **13** or **18**) in dimethylformamide (5 mL), and triethylamine (1.5 mL) under an atmosphere of N_2 was added bis(triphenylphosphine)palladium dichloride (6.0 mg, 0.0089 mmol) and cuprous iodide (0.36 mg, 0.0018 mmol). 1-Phenyl-2-propyn-1-ol (2.16 mmol) was added to the mixture, which was stirred at room temperature for 8 h under an atmosphere of N_2. The solvent was removed in vacuo and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents to give compounds **14–16**, **19** and **20** as chromatographically pure solids.

2-[(*R,S*)-3-Hydroxy-3-phenyl-1-propyn-1-yl)-9-(*N*-methyl-β-D-ribofuranuronamide)adenine (14). The reaction of **12** with (*R,S*)-1-phenyl-2-propyn-1-ol for, followed by chromatography on a silica gel column eluting with $CHCl_3$-MeOH-CH_3CN (75:15:10), gave **14** as a chromatographically pure amorphous solid (86 mg; 56%); ^{1}H NMR (DMSO-d_6): ∂ 2.79 (m, 3H, CONHCH_3), 4.13 (m, 1H, H-3′), 4.32 (m, 1H, H-4′), 4.56 (m, 1H, H-2′), 5.60 (m, 2H, CHC≡C and OH), 5.77 (bs, 1H, OH), 5.93 (d, 1H, J = 5.0 Hz, H-1′), 6.30 (d, 1H, J = 4.0 Hz, OH), 7.31–7.60 (m, 7H, H–Ph and NH_2), 8.46 (s, 1H, H-8), 8.63 (m, 1H, NH–CH_3). Anal. calcd. for $C_{20}H_{20}N_6O_5$ (424.4): C 56.60; H 4.75; N 19.80. Found: C 56.33; H 4.47; N 19.98.

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2-[(*R,S*)-3-Hydroxy-3-phenyl-1-propyn-1-yl)-9-(*N*-propyl-β-D-ribofuranuronamide)adenine (15). The reaction of **13** with (*R,S*)-1-phenyl-2-propyn-1-ol, followed by chromatography on a silica gel column eluting with $CHCl_3$-MeOH (93:7), gave **15** (63 mg; 39%), mp 152–155°C; ^{1}H NMR (DMSO-d_6): ∂ 0.78 (m, 3H, *J* = 7.2 Hz, $CONHCH_2CH_2CH_3$), 1.40 (m, 2H, $CONHCH_2CH_2CH_3$), 3.17 (m, 2H, $CONHCH_2$), 4.13 (m, 1H, H-3′), 4.27 (m, 1H, H-4′), 4.58 (m, 1H, H-2′), 5.60 (m, 2H, C*H*C≡C and O*H*), 5.75 (bs, 1H, OH), 5.95 (d, 1H, *J* = 7.4 Hz, H-1′), 6.29 (bs, 1H, OH), 7.31–7.60 (m, 7H, H–Ph and NH_2), 8.50 (s, 1H, H-8), 8.55 (m, 1*H*, N*H*–CH_2). Anal. calcd. for $C_{23}H_{24}N_6O_5$ (452.5): C 58.40; H 5.35; N 18.57. Found: C 58.20; H 5.69; N 18.76.

2-[(*S*)-3-Hydroxy-3-phenyl-1-propyn-1-yl)-9-(*N*-propyl-β-D-ribofuranuronamide)adenine (16). The reaction of **13** with (*R*)-1-phenyl-2-propyn-1-ol, followed by chromatography on a silica gel column eluting with $CHCl_3$-MeOH (90:10), gave **16** (57 mg; 31%), mp 157–160°C; ^{1}H NMR (DMSO-d_6): ∂ 0.78 (m, 3H, *J* = 7.3 Hz, $CONHCH_2CH_2CH_3$), 1.40 (m, 2H, $CONHCH_2CH_2CH_3$), 3.17 (m, 2H, $CONHCH_2$), 4.13 (m, 1H, H-3′), 4.33 (m, 1H, H-4′), 4.60 (m, 1H, H-2′), 5.62 (m, 2H, C*H*C≡C and O*H*), 5.78 (bs, 1H, OH), 5.96 (d, 1H, *J* = 7.4 Hz, H-1′), 6.30 (bs, 1H, OH), 7.31–7.60 (m, 7H, H–Ph and NH_2), 8.49 (s, 1H, H-8), 8.55 (m, 1*H*, N*H*–CH_2). Anal. calcd. for $C_{23}H_{24}N_6O_5$ (452.5): C 58.40; H 5.35; N 18.57. Found: C 58.12; H 5.61; N 18.78.

***N*^{6}-Ethyl-9-(*N*-ethyl-β-D-ribofuranuronamide)-2-[(*R,S*)-3-hydroxy-3-phenyl-1-propyn-1-yl)adenine (19).** The reaction of **18** with (*R,S*)-1-phenyl-2-propyn-1-ol, followed by chromatography on a silica gel column eluting with $CHCl_3$–cC_6H_{12}–CH_3OH (60:33:7), gave **19** as a white solid (50 mg; 30%), mp 135–137°C (dec); ^{1}H NMR (DMSO-d_6): ∂ 0.97 (m, 3H, $CONHCH_2CH_3$), 1.19 (m, 3H, $NHCH_2CH_3$), 3.18 (m, 2H, $CONHCH_2$), 3.48 (m, 2H, $NHCH_2CH_3$), 4.17 (m, 1H, H-3′), 4.30 (s, 1H, H-4′), 4.58 (m, 1H, H-2′), 5.61 (m, 2H, C*H*C≡C and O*H*), 5.76 (d, 1H, *J* = 4.3 Hz, OH), 5.95 (d, 1H, *J* = 7.6 Hz, H-1′), 6.32 (d, 1H, *J* = 6.0, OH), 7.40 (m, 3H, H–Ph), 7.54 (d, 2H, *J* = 6.6 Hz, H–Ph), 8.10 (ps t, 1H, N*H*CH_2), 8.48 (s, 1H, H-8), 8.60 (m, 1H, N*H*CO). Anal. calcd. for $C_{23}H_{26}N_6O_5$ (466.5): C 59.22; H 5.62; N 18.02. Found: C 58.93; H 5.33; N 18.34.

***N*^{6}-Ethyl-9-(*N*-ethyl-β-D-ribofuranuronamide)-2-[(*S*)-3-hydroxy-3-phenyl-1-propyn-1-yl)adenine (20).** The reaction of **18** with (*R*)-1-phenyl-2-propyn-1-ol, followed by chromatography on a silica gel column eluting with $CHCl_3$–cC_6H_{12}–CH_3OH (60:33:7), and then on a flash chromatography eluting with $CHCl_3$-CH_3OH (97:3), gave **20** as a white solid (87 mg; 53%), mp 137–140°C; ^{1}H NMR (DMSO-d_6): ∂ 0.95 (m, 3H, $CONHCH_2CH_3$), 1.19 (m, 3H, $NHCH_2CH_3$), 3.17 (m, 2H, $CONHCH_2$), 3.52 (m, 2H, $NHCH_2CH_3$), 4.13 (m, 1H, H-3′), 4.30 (s, 1H, H-4′), 4.58 (m, 1H, H-2′), 5.61 (m, 2H, C*H*C≡C and O*H*), 5.76 (d, 1H, *J* = 4.3 Hz, OH), 5.95 (d, 1H, *J* = 7.6 Hz, H-1′), 6.30 (d, 1H, *J* = 5.9 Hz, OH), 7.39 (m, 3H, H–Ph), 7.56 (m, 2H, H–Ph), 8.08 (ps t, 1H, N*H*CH_2), 8.48 (s, 1H, H-8), 8.55 (m, 1H, N*H*CO). Anal. calcd. for $C_{23}H_{26}N_6O_5$ (466.5): C 59.22; H 5.62; N 18.02. Found: C 58.98; H 5.39; N 18.31.

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BIOLOGICAL STUDIES

Cloning of the human adenosine receptors, stable transfection of cells, cell culture, membrane preparation, radioligand binding, and adenylyl cyclase activity have been fully described elsewhere.[5] Briefly, all human subtypes were stably transfected into Chinese hamster ovary (CHO) cells in order to be able to study their pharmacological profile in an identical cellular background utilizing radioligand binding studies (A_1, A_{2A}, A_3) or adenylyl cyclase activity assays (A_{2B}).

Receptor binding affinity was determined using [^{3}H]CCPA as radioligand at A_1 receptors, whereas [^{3}H]NECA was used at A_{2A} and A_3 subtypes. The procedure was performed as described previously.[10] Due to the lack of a suitable radioligand the relative potency of agonists at A_{2B} adenosine receptors was determined in adenylyl cyclase experiments. The procedure was carried out as described previously with minor modifications.[5]

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