N^6-Alkyl-2-alkynyl Derivatives of Adenosine as Potent and Selective Agonists at the Human Adenosine A_3 Receptor and a Starting Point for Searching A_{2B} Ligands

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A series of N^6-alkyl-2-alkynyl derivatives of adenosine (Ado) have been synthesized and evaluated for their affinity at human A_1, A_{2A}, and A_3 receptors and for their potency at A_{2B} adenosine receptor subtypes. The corresponding 2-(1-alkynyl) derivatives of 5′-*N*-ethylcarboxamidoadenosine (NECA) and Ado are used as reference compounds. Binding studies demonstrated that the activities of 2-alkynylAdos were slightly increased for the adenosine A_1 receptor and slightly decreased for both A_3 and A_{2B} subtypes compared to those of their corresponding NECA derivatives, whereas the A_{2A} receptor affinities of the two series of nucleosides were similar. The presence of a methyl group on N^6 of the 2-alkynyladenosines, inducing an increase in affinity at the human A_3 receptor and a decrease at the other subtypes, resulted in an increase in A_3 selectivity. In particular, 2-phenylethynyl-N^6-methylAdo (**8b**) showed an A_3 affinity in the low nanomolar range ($K_i(A_3) = 3.4$ nM), with a A_1/A_3 and A_{2A}/A_3 selectivity of about 500 and 2500, respectively. These findings motivated us to search for the preparation of new selective radioligands for the A_3 subtype; hence, a procedure to introduce a tritiated alkylamino group in these molecules was carried out. As far as the potency at the A_{2B} receptor, the type of 2-alkynyl chain and the presence of the ethylcarboxamido group on the sugar seem to be very important; in fact, the (*S*)-2-phenylhydroxypropynylNECA [(*S*)-PHPNECA, **1e**, $EC_{50}(A_{2B}) = 0.22\ \mu$M] proved to be one of the most potent A_{2B} agonist reported so far. On the other hand, the (*S*)-2-phenylhydroxypropynyl-N^6-ethylAdo (**9e**, $EC_{50}(A_{2B}) = 0.73\ \mu$M) showed a significantly increase of potency at the A_{2B} subtype in comparison with the N^6-methyl, N^6-isopropyl, and the unsubstituted adenosine derivatives, although it resulted in being less potent than (*S*)-PHPNECA (**1e**, $EC_{50}(A_{2B}) = 0.22\ \mu$M). These observations suggest that the introduction of an ethyl group in the N^6-position and an ethylcarboxamido substituent in the 4′-position of (*S*)-2-phenylhydroxypropynyladenosine could lead to a compound endowed with high potency at the A_{2B} receptor.

Introduction

Adenosine and natural adenosine derivatives are involved in the regulation of many aspects of cellular metabolism, and purinergic mechanism is an expanding therapeutic target for the discovery of novel drugs.[1,2] However, to date only adenosine itself is approved for human use, although over the past years much effort has been directed toward the discovery of potent and selective adenosine agonists.

Most actions of adenosine are mediated by four extracellular receptors termed A_1, A_{2A}, A_{2B}, and A_3.[3,4] In particular, the physiological role of the A_3 receptor subtype is not clearly understood owing to its recent characterization and a lack of truly selective ligands for in vivo studies. There are reports that agonists acting via the adenosine A_3 receptor have cardioprotective effects,[5−7] although it was proven that the A_3 receptor serves a different cardioprotective function compared to the adenosine A_1 receptor.[8] Chronic administration of adenosine A_3 receptor agonists has also been shown to have protective actions in the brain, in contrast to acute high-dose administration, which can cause toxicity.[9,10] More recently, it has been suggested that adenosine exerts a cytostatic effect on the proliferation of the Nb2-11C rat lymphoma cell line mainly through binding to its A_3 receptor.[11]

In a search for selective adenosine receptor ligands, it has been found that 2-hexynyladenosine-5′-*N*-ethyluronamide (HENECA, **1a**) displays high affinity at rat A_{2A} receptors combined with a good A_{2A} vs A_1 selectivity (Figure 1).[12−15] Moreover, HENECA was also found to possess good affinity for the rat A_3 receptor subtype.[16] Furthermore, several 2-alkynyl derivatives of NECA were tested in membranes of CHO cells that express the human A_3, and in particular, HENECA, 2-phenylethynylNECA (PENECA, **1b**), and 2-phenylhydroxypropynylNECA (PHPNECA, **1c**) showed high affinity and different degrees of selectivity for the human adenosine A_3 receptor.[17] The ethyl substituent of the carboxamido group in the 5′-position of HENECA (**1a**) has been replaced with a cyclopentyl or benzyl group. The introduction of a cyclopentyl ring led to a compound

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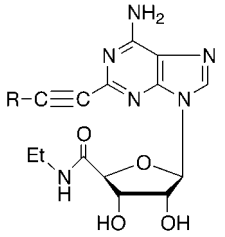


1a (HENECA); R = -$(CH_2)_3$-CH_3

1b (PENECA); R = -C_6H_5

1c [(*R,S*) PHPNECA]; R = (*R,S*) -CH(OH)-C_6H_5

1d [(*R*) PHPNECA]; R = (*R*) -CH(OH)-C_6H_5

1e [(*S*) PHPNECA]; R = (*S*) -CH(OH)-C_6H_5

Figure 1. Some 2-alkynyl-N^6-ethylcarboxamidoadenosines that resulted in potent ligands for A_3 adenosine receptors.

that showed an 8-fold decrease of affinity at all human receptor subtypes compared with HENECA; when a benzyl group was introduced in the same position, the corresponding derivative showed a more pronounced loss in affinity, demonstrating that a bulky substituent is less tolerated at the 5′-position.[18] Furthermore, NECA derivatives bearing arylcarbamoyl substituents on N^6 were found to possess high affinity and moderate selectivity for the rat A_3 receptor.[19]

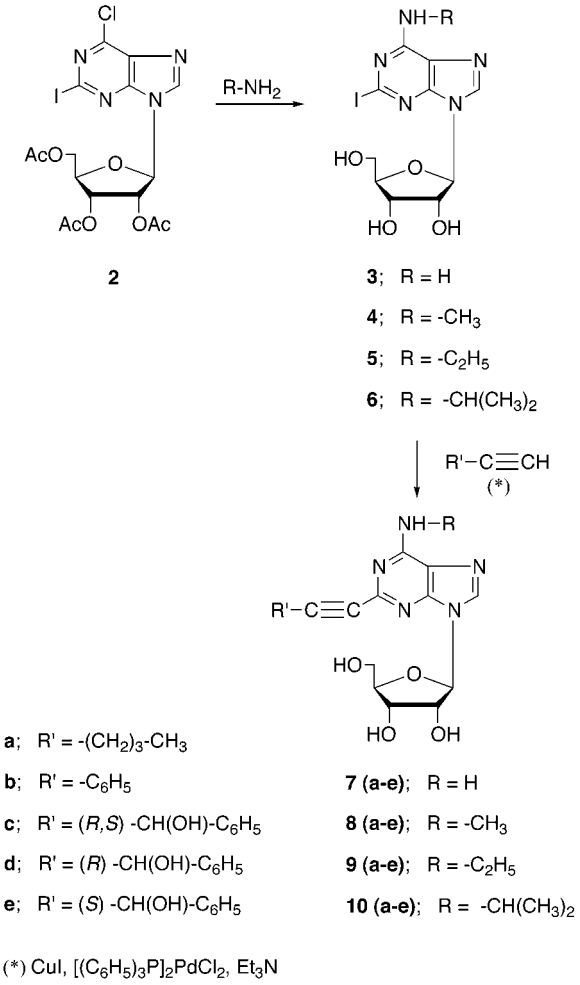
In an attempt to potentiate the affinity and selectivity at A_3 receptors through a trisubstitution, we synthesized NECA derivatives bearing arylcarbamoyl substituents on N^6 and (ar)alkynyl chains in the 2-position. However, these trisubstituted derivatives showed a reduced affinity for both the A_1 and A_3 subtypes (K_i in the high nanomolar range), together with loss of A_2 activity, resulting in compounds with good A_{2A}/A_1 and A_{2A}/A_3 selectivity.[20]

These observations prompted us to investigate the effect of small substituents in the N^6-position of such molecules.

Taking into account both the differences in synthesis (2-alkynylAdo derivatives can be obtained from commercially available guanosine through five steps, whereas for the synthesis of the corresponding NECA derivatives, 10 steps are needed) and the fact that 2-alkynyl-NECAs have affinity for adenosine receptors similar to that of the corresponding adenosine derivatives,[12] we synthesized 2-alkynyl- (hexynyl-, phenylethynyl-, (*R,S*)-phenylhydroxypropynyl-) adenosines bearing on N^6 a methyl (**8a**−**c**), an ethyl (**9a**−**c**), and an isopropyl (**10a**−**c**). As reference compounds, the corresponding 2-alkynyladenosines [HEAdo (**7a**),[12,21] PEAdo (**7b**), and PHPAdo (**7c**)] were prepared.[22] In the case of PHPAdo, both the racemic mixture of nucleosides and the (*R*) and (*S*) diastereomers were synthesized, leading to **7c**, **7d**, and **7e**.[23] (During the preparation of this manuscript, the synthesis of **7d** and **7e** was reported by IJzerman et al.,[24] with some differences in the method, yields, and ^{1}H NMR spectrum attributions). The (*R*) (**8d**−**10d**) and (*S*) (**8e**−**10e**) diastereomers of N^6-methyl-, N^6-ethyl-, and N^6-isopropylPHPAdo (**8c**−**10c**) were also prepared (Scheme 1).

Finally, a procedure to study the possibilities for introduction of a tritiated alkylamino group in these

Scheme 1



molecules was carried out to obtain a new selective radioligand for the A_3 subtype (Schemes 2 and 3).

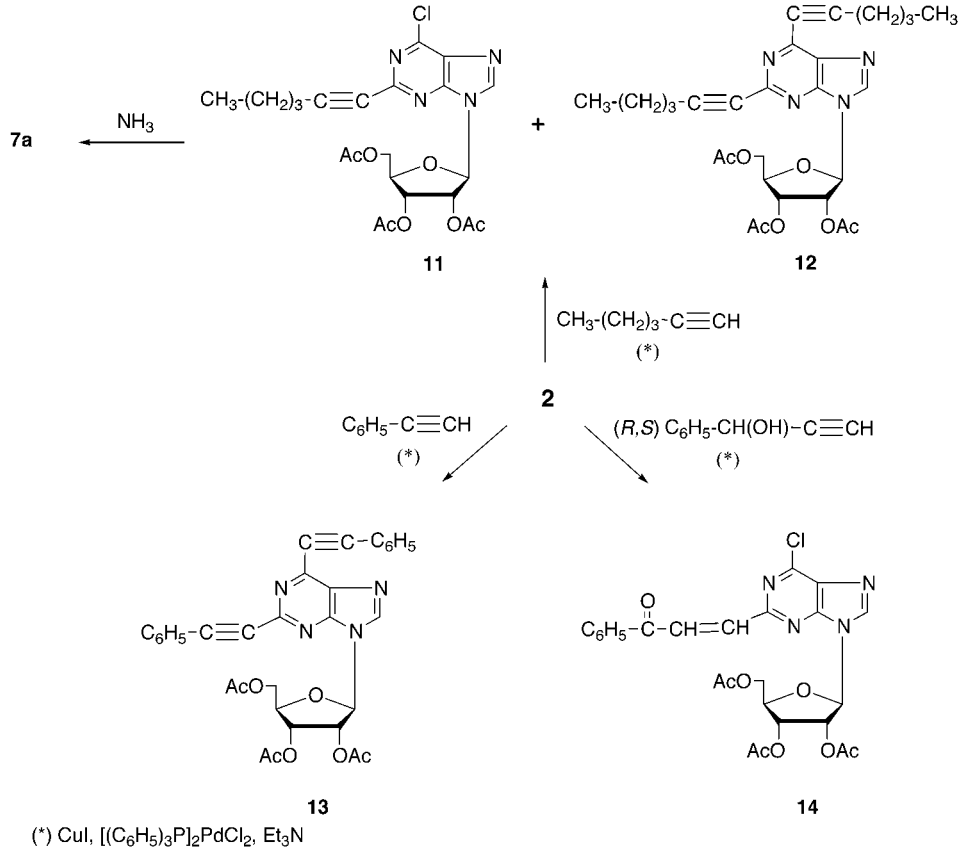
Results and Discussion

Chemistry. The synthesis of 2-alkynyladenosines (**7a**−**e**) and 2-alkynyl-N^6-alkyladenosines (**8a**−**e**, **9a**−**e**, and **10a**−**e**) was carried out starting from 9-(2,3,5,-tri-*O*-acetyl-β-D-ribofuranosyl)-6-chloro-2-iodopurine (**2**).[25] Reaction of **2** with liquid ammonia at room temperature or with the suitable alkylamine at −20 °C for 30 min and then at room temperature for 1 h gave compounds **3**[21] and **4**−**6**, respectively, obtained by selective substitution of the chlorine atom in the 6-position and contemporary deprotection of the sugar (Scheme 1).

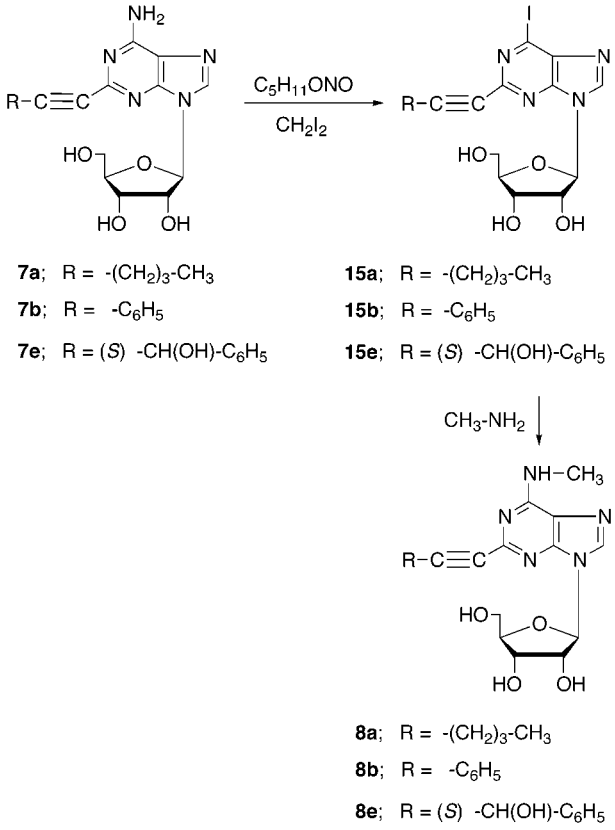
Substitution of the iodine in the 2-position of 2-iodoadenosine (**3**)[25] or of 2-iodo-N^6-methyladenosine (**4**−**6**) with five different alkynyl chains [commercially available 1-hexyne, ethynylbenzene, (*R,S*)-1-phenyl-2-propyn-1-ol, (*R*)-1-phenyl-2-propyn-1-ol, and (*S*)-1-phenyl-2-propyn-1-ol] was carried out by a modification of the palladium-catalyzed cross-coupling reaction[12] to give compounds **7**−**10a**−**e**.

Furthermore, to find a convenient synthetic procedure for the preparation of radiolabeled N^6-methyl-2-alkynyladenosines, we carried out an attempt to couple **2** selectively with the suitable alkyne, using the cross-coupling reaction conditions to obtain monosubstituted 2-alkynyl-6-chloro-9-(2,3,5,-tri-*O*-acetyl-β-D-ribofuranosyl)purines. The 6-chlorine atom of these intermediates

Scheme 2



Scheme 3

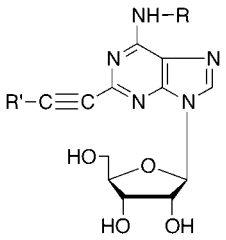


could have been easily substituted by tritiated methylamine to give the corresponding radioligands of **8a**−**e**.

Unfortunately, reaction of **2** with the desired alkynes in general failed to give the monosubstituted nucleosides in satisfactory yield. In particular, the results of such reactions are reported in Scheme 2. Coupling of **2** with 1-hexyne afforded a mixture of the monosubstituted compound 6-chloro-2-hexynyl-9-(2,3,5,-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**11**) and the disubstituted compound **12** in 31% and 45% yields, respectively. The alkynylation site of **11** was confirmed by a chemical proof. In fact, reaction of **11** with liquid ammonia at room temperature for 24 h gave 2-hexynyladenosine (HEAdo, **7a**) in 50% yield. In the case of phenylethyne, the coupling reaction gave only the disubstituted nucleoside **13** in 51% yield. Coupling of **2** with (*R*,*S*)-1-phenyl-2-propyn-1-ol resulted in a monosubstituted derivative in 60% yield, which was identified as the phenylketopropenyl analogue **14** due to a tautomeric rearrangement on the side chain. The structure of (*E*)-6-chloro-2-(3-keto-3-phenyl-1-propen-1-yl)-9-(2,3,5,-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**14**) was confirmed by ^{1}H NMR. The ^{1}H NMR spectrum of **14** displayed two doublets of the (E) double bond protons at δ 7.75 and 8.35 with a coupling constant of 15.8 Hz, while signals corresponding to the protons of the CHOH group were missing. On the other hand, the CHOH protons were clearly identified in the ^{1}H NMR spectra of the phenylhydroxypropynyl derivatives **7c**−**e**, **8c**−**e**, **9c**, **10c**, and **15e**, where conversely the double bond protons are missing.

An alternative synthetic route, depicted in Scheme 3, was designed to avoid the undesired disubstitution. Hence, the 2-alkynyladenosines **7a**, **7b**, and **7e** were used as the starting material and reaction of these

Table 1. Affinities of the Adenosine Derivatives **7a**−**e**, **8a**−**e**, **9a**−**e**, and **10a**−**e** in Radioligand Binding Assays at Human A_1, A_{2A}, and A_3 Adenosine Receptors



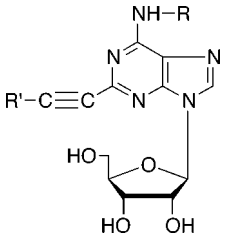
compd	R	R′	$K_i(A_1)$,[a] nM	$K_i(A_{2A})$,[b] nM	$K_i(A_3)$,[c] nM	A_1/A_3	A_{2A}/A_3
1a, HENECA			60 (50−72)	6.4 (3.8−11)	2.4 (2.0−2.9)	25	3
7a, HEAdo	H	$CH_3(CH_2)_3$	18 (9.4−36)	5.7 (5.2−6.2)	4.7 (4.4−5.2)	4	1
8a	CH_3	$CH_3(CH_2)_3$	327 (255−419)	1,230 (453−3310)	1.1 (0.71−1.80)	297	1118
9a	C_2H_5	$CH_3(CH_2)_3$	138 (119−158)	267 (201−355)	2.3 (1.0−5.2)	60	116
10a	$CH(CH_3)_2$	$CH_3(CH_2)_3$	43 (34−56)	196 (132−293)	9.7 (9.4−10)	4	20
1b, PENECA			560 (480−650)	620 (300−1300)	6.2 (5.1−7.5)	90	100
7b, PEAdo	H	C_6H_5	391 (284−556)	363 (285−462)	16 (13−19)	24	23
8b	CH_3	C_6H_5	1,690 (1020−2790)	8,530 (7400−9850)	3.4 (2.0−5.8)	497	2509
9b	C_2H_5	C_6H_5	1,176 (849−1630)	4,450 (3460−5720)	4.9 (3.1−7.9)	240	908
10b	$CH(CH_3)_2$	C_6H_5	429 (386−478)	1,100 (783−1550)	17 (8.0−34)	25	65
1c, (*R*,*S*)-PHPNECA			2.7 (1.7−4.1)	3.1 (2.4−3.9)	0.42 (0.17−1.0)	6	7
7c, (*R*,*S*)-PHPAdo	H	(*R*,*S*)-$C_6H_5CH(OH)$	0.67 (0.55−0.80)	7.0 (3.7−13)	3.3 (2.3−4.8)	0.2	2
8c	CH_3	(*R*,*S*)-$C_6H_5CH(OH)$	8.4 (6.8−10)	273 (216−346)	0.76 (0.60−0.96)	11	359
9c	C_2H_5	(*R*,*S*)-$C_6H_5CH(OH)$	2.7 (2.4−2.9)	94 (72−123)	0.97 (0.58−1.6)	3	97
10c	$CH(CH_3)_2$	(*R*,*S*)-$C_6H_5CH(OH)$	1.5 (0.96−2.2)	96 (65−142)	2.3 (1.6−3.4)	0.7	42
1d, (*R*)-PHPNECA			1.9 (1.8−2.1)	39 (25−59)	5.5 (3.6−8.5)	0.3	7
7d, (*R*)-PHPAdo	H	(*R*)-$C_6H_5CH(OH)$	0.44 (0.38−0.52)	29 (19−45)	5.0 (3.2−7.7)	0.1	6
8d	CH_3	(*R*)-$C_6H_5CH(OH)$	7.1 (5.4−9.5)	1,560 (989−2460)	1.2 (0.71−2.1)	6	1300
9d	C_2H_5	(*R*)-$C_6H_5CH(OH)$	2.2 (2.0−2.4)	1,181 (864−1614)	1.4 (0.70−2.9)	2	843
10d	$CH(CH_3)_2$	(*R*)-$C_6H_5CH(OH)$	1.1 (0.79−1.4)	324 (165−636)	4.9 (2.3−10.4)	0.2	66
1e, (*S*)-PHPNECA			2.1 (1.2−3.7)	2.0 (1.2−3.5)	0.75 (0.52−1.1)	3	3
7e, (*S*)-PHPAdo	H	(*S*)-$C_6H_5CH(OH)$	0.67 (0.47−0.96)	1.8 (1.1−3.0)	1.4 (0.78−2.4)	0.5	1
8e	CH_3	(*S*)-$C_6H_5CH(OH)$	9.6 (7.6−12)	163 (154−173)	0.53 (0.46−0.61)	18	307
9e	C_2H_5	(*S*)-$C_6H_5CH(OH)$	3.4 (2.7−4.2)	64 (34−120)	0.20 (0.14−0.28)	17	320
10e	$CH(CH_3)_2$	(*S*)-$C_6H_5CH(OH)$	1.7 (1.4−2.0)	43 (24−79)	1.5 (1.0−2.0)	1	29

[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] Displacement of specific [^{3}H]NECA binding in CHO cells stably transfected with human recombinant A_3 adenosine receptor, expressed as K_i (nM).

compounds with diiodomethane and isopentylnitrite gave the versatile synthons 2-alkynyl-6-iodoadenosine **15a**, **15b**, and **15e**. Treatment of them with methylamine at 0 °C for 5 h afforded the desired compounds **8a**, **8b**, and **8e**. This procedure allows the introduction in the final step at low temperature and with high yield of a tritiated methylamine.

Pharmacology. The 2-alkynyl derivatives of adenosine and of N^6-alkyladenosines **7a**−**e**, **8a**−**e**, **9a**−**e**, and **10a**−**e** 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 adenylate cyclase activity assays (A_{2B}),[26] and the results are reported in Tables 1 and 2.

Table 2. Effects of the Adenosine Derivatives **7a**−**e**, **8a**−**e**, **9a**−**e**, and **10a**−**e** on Adenylyl Cyclase Activity at Human A_{2B} Adenosine Receptor



compd	R	R′	EC_{50},[a] μM
1a			6.1 (4.0−9.3)
7a	H	$CH_3(CH_2)_3$	100
8a	CH_3	$CH_3(CH_2)_3$	100
9a	C_2H_5	$CH_3(CH_2)_3$	100
10a	$CH(CH_3)_2$	$CH_3(CH_2)_3$	100
1b			>100
7b	H	C_6H_5	>100
8b	CH_3	C_6H_5	100
9b	C_2H_5	C_6H_5	>100
10b	$CH(CH_3)_2$	C_6H_5	>100
1c			1.1 (0.47−2.6)
7c	H	(*R,S*)-$C_6H_5CH(OH)$	2.4 (1.5−3.7)
8c	CH_3	(*R,S*)-$C_6H_5CH(OH)$	2.7 (1.3−5.6)
9c	C_2H_5	(*R,S*)-$C_6H_5CH(OH)$	1.7 (0.97−3.0)
10c	$CH(CH_3)_2$	(*R,S*)-$C_6H_5CH(OH)$	6.1 (4.3−8.5)
1d			2.4 (1.5−3.8)
7d	H	(*R*)-$C_6H_5CH(OH)$	6.2 (2.9−13)
8d	CH_3	(*R*)-$C_6H_5CH(OH)$	100
9d	C_2H_5	(*R*)-$C_6H_5CH(OH)$	9.1 (6.9−12)
10d	$CH(CH_3)_2$	(R)-$C_6H_5CH(OH)$	8.9 (4.5−18)
1e			0.22 (0.22−0.23)
7e	H	(*S*)-$C_6H_5CH(OH)$	0.92 (0.71−1.2)
8e	CH_3	(*S*)-$C_6H_5CH(OH)$	2.6 (1.7−4.0)
9e	C_2H_5	(*S*)-$C_6H_5CH(OH)$	0.73 (0.61−0.89)
10e	$CH(CH_3)_2$	(*S*)-$C_6H_5CH(OH)$	0.96 (0.50−1.9)

[a] Measurement of receptor-stimulated adenylyl cyclase activity in CHO cells stably transfected with human recombinant A_{2B} adenosine receptor, expressed as EC_{50} (μM).

HENECA (**1a**),[12] PENECA (**1b**),[15] (*R,S*)-PHPNECA (**1c**),[14] (*R*)-PHPNECA (**1d**),[27] and (*S*)-PHPNECA (**1e**)[27] have been used as reference compounds.

The results of binding and cyclase studies showed that removing the *N*-ethylcarboxamido group of HENECA (**1a**) and of all other NECA derivatives **1b**−**e**, reported in Figure 1, led to adenosine derivatives that maintain an affinity comparable to that of the parent compounds for the A_1, A_{2A}, and A_3 human adenosine receptor subtypes. In particular, 2-hexynyladenosine (HEAdo, **7a**) showed slightly higher affinity at the A_1 receptor than the corresponding NECA derivative **1a** (K_i = 18 vs 60 nM), very similar affinity at the A_{2A} subtype (K_i = 5.7 vs 6.4 nM), and slightly lower affinity at the A_3 receptor (K_i = 4.7 vs 2.4 nM). The potency at the A_{2B} subtype is diminished (EC_{50} = 100 vs 6.1 μM) as shown in Table 2. A similar profile is obtained by comparing PENECA (**1b**), PHPNECAs **1c**−**e**, and the corresponding adenosine derivatives PEAdo (**7b**), (*R,S*)-PHPAdo (**7c**), (*R*)-PHPAdo (**7d**), and (*S*)-PHPAdo (**7e**).

The presence of a methyl group on N^6 of 2-alkynyladenosine derivatives always decreased the affinity for the A_1 and A_{2A} receptors and correspondingly increased A_3 affinity, leading to a significant improvement of A_3 selectivity in comparison with all the other subtypes.

In particular, the N^6-methyl derivative of PEAdo (**8b**) showed low affinity for A_1 and A_{2A} subtypes ($K_i(A_1)$ = 1690 nM and $K_i(A_{2A})$ = 8530 nM) and much higher affinity at the A_3 receptor ($K_i(A_3)$ = 3.4 nM), resulting in an A_1/A_3 and A_{2A}/A_3 selectivity ratio of about 500 and 2500, respectively.

The compounds obtained when the N^6-methyl group is replaced by an ethyl (**9a**−**c**) or an isopropyl group (**10a**−**c)** maintained high affinity for the A_3 receptor but showed in most cases decreased A_1/A_3 and A_{2A}/A_3 selectivity.

The potency of the synthesized compounds at the A_{2B} receptor has been evaluated, and the results are shown in Table 2. All compounds resulted in full agonists in stimulating adenylate cyclase activity in comparison with NECA.

In addition to the consideration that the NECA derivatives **1a**−**e** are always more potent than the corresponding adenosine nucleosides, it is clear that the influence of the alkynyl chain in the 2-position is determinant for the activity of these molecules at the A_{2B} receptor subtype. In fact, most of the 2-hydroxyphenylpropynyl derivatives showed EC_{50} in the low micromolar range (see all **c**−**e** derivatives), the most potent being the (*S*)-PHPNECA (**1e**)[27] with an EC_{50} in the nanomolar range (EC_{50} = 0.22 μM).

On the other hand, the contemporary presence of a hydroxyphenylpropynyl group in the 2-position and of an ethyl substituent on N^6 increased the potency at the A_{2B} adenosine receptor subtype (Table 2). In fact, the EC_{50} of the N^6-ethyl derivative of (*S*)-PHPAdo (**9e**) was 0.73 μM, and this compound resulted in being slightly more potent than the corresponding N^6-methyl and N^6-isopropyl derivatives (**8e**, EC_{50} = 2.6 μM; **10e**, EC_{50} = 0.96 μM) and more potent than the unsubstituted (*S*)-PHPAdo (**7e**, EC_{50} = 0.92 μM).

Conclusions

From this study, it is possible to conclude that removing the carboxamido group of the 2-alkynyl derivatives of NECA **1a**−**e** led to the adenosine derivatives **7a**−**e**, which in general maintain high affinity at all adenosine receptor subtypes. In particular, the activities of 2-alkynylAdos were slightly higher at A_1 and lower at A_3 and A_{2B} subtypes than those of the corresponding NECA derivatives. The affinities at the A_{2A} subtype are similar for the two series of nucleosides.

Introduction of a methyl group on N^6 of the 2-alkynyladenosines brought about an increase in affinity at the human A_3 receptor and a corresponding decrease at the other subtypes, resulting in a relevant increase in A_3 selectivity (Figure 2). In particular, 2-phenylethynyl-N^6-methylAdo (**8b**) showed an A_3 affinity in the low

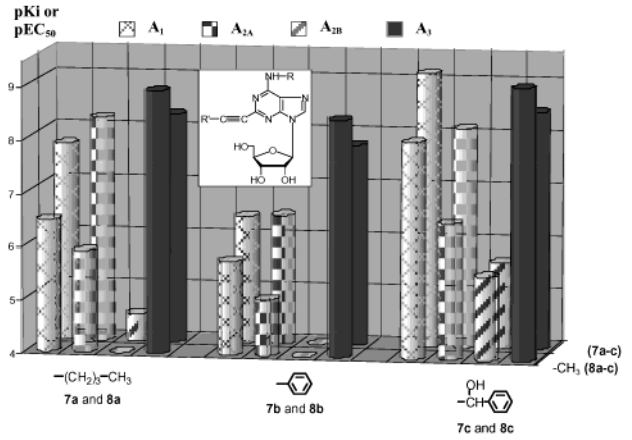


Figure 2. 2-Phenylhydroxypropynyladenosine derivatives. In all cases the (*S*) diastereomers are more active than the corresponding (*R*) diastereomers at A_{2A}, A_{2B}, and A_3 receptors; however, they are slightly less active at A_1 receptors.

nanomolar range and an A_1/A_3 and A_{2A}/A_3 selectivity ratio of about 500 and 2500, respectively.

On the other hand, the presence of an ethyl group in N^6 has significantly increased the potency at the A_{2B} subtype in comparison with the N^6-methyl, N^6-isopropyl, and the unsustituted adenosine derivatives. However, for the potency at the A_{2B} receptor, it seems to be more important than the type of alkynyl chain in the 2-position and the presence of the ribose carboxyamido group, the (*S*)-2-phenylhydroxypropynylNECA[27] [(*S*)-PHPNECA, **1c**, $EC_{50}(A_{2B}) = 0.22\ \mu M$] being one of the most potent A_{2B} agonist reported so far.

The combination of an ethyl group in N^6-position and an ethylcarboxamido substituent in the 4′-position of (*S*)-2-hydroxypropynyladenosine could lead to a compound endowed with high potency at the A_{2B} receptor.

Finally, it is worthwhile to note that the four human adenosine receptors showed a different behavior toward the diastereomers of all the phenylhydroxypropynyl derivatives **1d**, **1e**, **7d**, **7e**, **8d**, and **8e**. In fact, the A_1 subtype had a slight preference for the (*R*) diastereomer whereas the other subtypes showed a significant preference for the compounds in the (*S*) configuration, as clearly illustrated by Figure 3.

Experimental Section

Chemistry. Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian VXR 300 MHz spectrometer; δ is in ppm, J is in Hz. All exchangeable protons were confirmed by addition of D_2O. TLC experiments were carried out on precoated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Elemental analyses were determined on a Fisons model EA 1108 analyzer and are within ±0.4% of theoretical values.

General Procedure for the Synthesis of 2-Iodoadenosine (3) and N^6-Alkyl-2-iodoadenosines (4−6). To 1.86 mmol (1.003 g) of 9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-6-chloro-2-iodopurine (**2**)[25] 8 mL of the suitable amine was added. The mixture was set aside at room temperature or at −20 °C for the time reported below. The residual amine was evaporated, and the residue was chromatographed on a silica gel column, eluting with a suitable mixture of solvents to give compounds **3**−**6**.

2-Iodo-N^6-methyladenosine (4). Reaction of **2** with methylamine at −20 °C for 30 min and then at room temperature for 1 h, followed by silica gel chromatography with elution with $CHCl_3-CH_3OH$ (93:7), gave **4** as a white solid in 84% yield: mp 180−182 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.92 (d, 3H, J = 4.1 Hz, NH*CH*$_3$), 3.61 (m, 2H, CH_2-5′), 3.95 (m, 1H, H-4′), 4.14 (m, 1H, H-3′), 4.53 (m, 1H, H-2′), 5.82 (d, 1H, J = 6.1 Hz, H-1′), 8.14 (m, 1H, NH), 8.31 (s, 1H, H-8). Anal. ($C_{11}H_{14}IN_5O_4$) C, H, N.

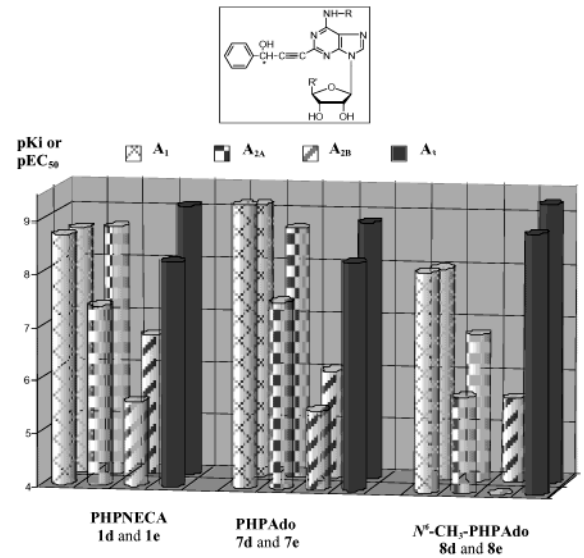


Figure 3. 2-Alkynyladenosines and the corresponding N^6-methyl derivatives. Comparison of the activities at A_1, A_{2A}, A_{2B}, and A_3 receptors reveals that A_3 receptors have a preference for the N^6-methyl derivatives. These compounds, which show reduced activities at the other receptor subtypes in comparison with the N^6-unsubstituted derivatives, are endowed with high A_3 selectivity.

N^6-Ethyl-2-iodoadenosine (5). Reaction of **2** with ethylamine at −20 °C for 30 min and then at room temperature for 1 h, followed by silica gel flash chromatography elution with $CHCl_3-CH_3OH$ (97:3), gave **5** as a white solid in 76% yield: mp 95−97 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3H, J = 7.0 Hz, NHCH$_2$*CH*$_3$), 3.57 (m, 4H, NH*CH*$_2$CH$_3$ and CH_2-5′), 3.95 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.52 (m, 1H, H-2′), 5.82 (d, 1H, J = 6.3 Hz, H-1′), 8.22 (m, 1H, NH), 8.31 (s, 1H, H-8′). Anal. ($C_{12}H_{16}IN_5O_4$) C, H, N.

2-Iodo-N^6-isopropyladenosine (6). Reaction of **2** with ethylamine at −20 °C for 30 min and then at room temperature for 16 h, followed by silica gel flash chromatography with elution with $CHCl_3-CH_3OH$ (98:2), gave **6** as a white solid in 72% yield: mp 100−102 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.21 (d, 6H, J = 6.6 Hz, NCH(*CH*$_3$)$_2$), 3.61 (m, 2H, CH_2-5′), 3.95 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.33 (m, 1H, N*CH*(CH$_3$)$_2$), 4.53 (m, 1H, H-2′), 5.83 (d, 1H, J = 6.2 Hz, H-1′), 8.10 (m, 1H, NH), 8.32 (s, 1H, H-8). Anal. ($C_{13}H_{18}IN_5O_4$) C, H, N.

General Procedure for the Synthesis of 2-Alkynyladenosines (7a−e) and 2-Alkynyl-N^6-alkyladenosines (8a−e, 9a−e, and 10a−e). To a solution of 200 mg (0.51 mmol) of 2-iodoadenosine (**3**)[25] or 2-iodo-N^6-alkyladenosine (**4**−**6**) in 8 mL of dry DMF and 2.3 mL of triethylamine under an atmosphere of N_2 8 mg (0.011 mmol) of bis(triphenylphosphine)palladium dichloride and 0.51 mg (0.003 mmol) of CuI were added. The appropriate terminal alkyne (3.1 mmol) was added, and the reaction mixture was stirred at room temperature for the time reported below. After evaporation in vacuo, the residual oil was purified by silica gel column chromatography with elution with a suitable mixture of solvents.

2-Phenylethynyladenosine (7b). Reaction of **3** with ethynylbenzene for 3 h, followed by silica gel column chromatography with elution with $CHCl_3-CH_3OH$ (90:10), gave **7b** as a white solid in 78% yield ($CHCl_3-CH_3CN$): mp 215−216 °C; ^{1}H NMR (DMSO-d_6) δ 3.65 (m, 2H, CH_2-5′), 3.99 (m, 1H, H-4′), 4.15 (m, 1H, H-3′), 4.57 (m, 1H, H-2′), 5.92 (d, 1H, J = 6.2 Hz, H-1′), 7.48 (m, 3H, H−Ph), 7.62 (m, 4H, H−Ph and NH_2), 8.47 (s, 1H, H-8). Anal. ($C_{18}H_{17}N_5O_4$) C, H, N.

(*R*,*S*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)adenosine (7c). Reaction of **3** with (*R*,*S*)-1-phenyl-2-propyn-1-ol for

6 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (85:15), gave **7c** as a white solid in 65% yield (EtOH): mp 183−185 °C; ^{1}H NMR (DMSO-d_6) δ 3.63 (m, 2H, CH_2-5′), 3.96 (m, 1H, H-4′), 4.14 (m, 1H, H-3′), 4.52 (m, 1H, H-2′), 5.61 (d, 1H, J = 6.2 Hz, *CH*OH), 5.88 (d, 1H, J = 5.8 Hz, H-1′), 6.28 (d, 1H, J = 6.0 Hz, CH*OH*), 7.46 (m, 7H, H−Ph and NH_2), 8.45 (s, 1H, H-8). Anal. ($C_{19}H_{19}N_5O_5$) C, H, N.

(*R*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)adenosine (7d). Reaction of **3** with (*S*)-1-phenyl-2-propyn-1-ol for 6 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH−*c*C_6H_{12} (77:13:10), gave **7d** as a white solid in 55% yield (EtOH): mp 183−185 °C; ^{1}H NMR (DMSO-d_6) δ 3.64 (m, 2H, CH_2-5′), 3.96 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.51 (m, 1H, H-2′), 5.61 (d, 1H, J = 6.0 Hz, *CH*OH), 5.89 (d, 1H, J = 5.9 Hz, H-1′), 6.28 (d, 1H, J = 6.0 Hz, CH*OH*), 7.46 (m, 7H, H−Ph and NH_2), 8.45 (s, 1H, H-8). Anal. ($C_{19}H_{19}N_5O_5$) C, H, N.

(*S*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)adenosine (7e). Reaction of **3** with (*R*)-1-phenyl-2-propyn-1-ol for 6 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (88:12), gave **7e** as a white solid in 67% yield (MeOH−EtOH): mp 143−145 °C (dec); ^{1}H NMR (DMSO-d_6) δ 3.64 (m, 2H, CH_2-5′), 3.96 (m, 1H, H-4′), 4.15 (m, 1H, H-3′), 4.53 (m, 1H, H-2′), 5.62 (d, 1H, J = 6.1 Hz, *CH*OH), 5.88 (d, 1H, J = 5.9 Hz, H-1′), 6.28 (d, 1H, J = 5.9 Hz, CH*OH*), 7.46 (m, 7H, H−Ph and NH_2), 8.45 (s, 1H, H-8). Anal. ($C_{19}H_{19}N_5O_5$) C, H, N.

2-(1-Hexyn-1-yl)-*N*^{6}-methyladenosine (8a). Reaction of **4** with 1-hexyne for 16 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (92:8), gave **8a** as a white solid in 78% yield (EtOH): mp 152−155 °C; ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 6.9 Hz, CH_3), 1.50 (m, 4H, $(CH_2)_2CH_3$), 2.44 (t, 2H, J = 6.6 Hz, $CH_2C{\equiv}C$), 2.94 (br s, 3H, NH*CH*$_3$), 3.63 (m, 2H, CH_2-5′), 3.96 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.54 (m, 1H, H-2′), 5.88 (d, 1H, J = 5.9 Hz, H-1′), 7.89 (m, 1H, NH), 8.40 (s, 1H, H-8). Anal. ($C_{17}H_{23}N_5O_4$) C, H, N.

***N*^{6}-Methyl-2-phenylethynyladenosine (8b).** Reaction of **4** with ethynylbenzene for 16 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (92:8), gave **8b** as a white solid in 84% yield (EtOH): mp 229−231 °C; ^{1}H NMR (DMSO-d_6) δ 2.99 (br s, 3H, NH*CH*$_3$), 3.64 (m, 2H, CH_2-5′), 3.98 (m, 1H, H-4′), 4.15 (m, 1H, H-3′), 4.57 (m, 1H, H-2′), 5.93 (d, 1H, J = 6.2 Hz, H-1′), 7.47 (m, 3H, H−Ph), 7.65 (m, 2H, H−Ph), 8.07 (m, 1H, NH), 8.47 (s, 1H, H-8). Anal. ($C_{19}H_{19}N_5O_4$) C, H, N.

(*R*,*S*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-*N*^{6}-methyladenosine (8c). Reaction of **4** with (*R*,*S*)-1-phenyl-2-propyn-1-ol for 6 h, followed by silica gel column chromatography with elution with $CHCl_3$−*c*C_6H_{12}−CH_3OH (75:15:10), gave **8c** as a white solid in 73% yield (EtOH): mp 115 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.94 (br s, 3H, NH*CH*$_3$), 3.63 (m, 2H, CH_2-5′), 3.95 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.50 (m, 1H, H-2′), 5.64 (d, 1H, J = 5.8 Hz, *CH*OH), 5.88 (d, 1H, J = 5.7 Hz, H-1′), 6.31 (d, 1H, J = 5.9 Hz, CH*OH*), 7.40 (m, 3H, H−Ph), 7.57 (m, 2H, H−Ph), 7.95 (m, 1H, NH), 8.44 (s, 1H, H-8). Anal. ($C_{20}H_{21}N_5O_5$) C, H, N.

(*R*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-*N*^{6}-methyladenosine (8d). Reaction of **4** with (*S*)-1-phenyl-2-propyn-1-ol for 6 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (90:10), gave **8d** as a white solid in 62% yield (EtOEt): mp 127 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.94 (br s, 3H, NH*CH*$_3$), 3.63 (m, 2H, CH_2-5′), 3.95 (m, 1H, H-4′), 4.13 (br s, 1H, H-3′), 4.50 (m, 1H, H-2′), 5.64 (d, 1H, J = 5.9 Hz, *CH*OH), 5.89 (d, 1H, J = 5.9 Hz, H-1′), 6.31 (d, 1H, J = 6.0 Hz, CH*OH*), 7.40 (m, 3H, H−Ph), 7.57 (d, 2H, J = 6.7 Hz, H−Ph), 7.97 (m, 1H, NH), 8.44 (s, 1H, H-8). Anal. ($C_{20}H_{21}N_5O_5$) C, H, N.

(*S*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-*N*^{6}-methyladenosine (8e). Reaction of **4** with (*R*)-1-phenyl-2-propyn-1-ol for 6 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (90:10), gave **8e** as a white solid in 60% yield (EtOEt): mp 126−128 °C (dec); ^{1}H NMR (DMSO-d_6) δ 2.94 (m, 3H, NH*CH*$_3$), 3.64 (m, 2H, CH_2-5′), 3.96 (m, 1H, H-4′), 4.14 (m, 1H, H-3′), 4.51 (m, 1H, H-2′), 5.64 (d, 1H, J = 5.9 Hz, *CH*OH), 5.88 (d, 1H, J = 5.9 Hz, H-1′), 6.31 (d, 1H, J = 5.9 Hz, CH*OH*), 7.40 (m, 3H, H−Ph), 7.57 (m, 2H, H−Ph), 7.96 (m, 1H, NH), 8.44 (s, 1H, H-8). Anal. ($C_{20}H_{21}N_5O_5$) C, H, N.

***N*^{6}-Ethyl-2-(1-hexyn-1-yl)adenosine (9a).** Reaction of **5** with 1-hexyne for 24 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (92:8), gave **9a** as a white solid in 72% yield (EtOH): mp 187−189 °C; ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7.0 Hz, $(CH_2)_2CH_3$), 1.18 (t, 3H, J = 14 Hz NCH_2CH_3), 1.51 (m, 4H, $(CH_2)_2CH_3$), 2.46 (m, 2H, $CH_2C{\equiv}C$), 3.61 (m, 4H, CH_2-5′ and NCH_2CH_3), 3.96 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.54 (m, 1H, H-2′), 5.87 (d, 1H, J = 6.1 Hz, H-1′), 7.94 (m, 1H, NH), 8.40 (s, 1H, H-8). Anal. ($C_{18}H_{25}N_5O_4$) C, H, N.

***N*^{6}-Ethyl-2-phenylethynyladenosine (9b).** Reaction of **5** with ethynylbenzene for 4 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (92:8), gave **9b** as a white solid in 83% yield (EtOH): mp 195−199 °C; ^{1}H NMR (DMSO-d_6) δ 1.21 (t, 3H, J = 14.2 Hz, $NHCH_2CH_3$), 3.59 (m, 4H, NH*CH*$_2$ and CH_2-5′), 3.98 (m, 1H, H-4′), 4.15 (m, 1H, H-3′), 4.57 (m, 1H, H-2′), 5.92 (d, 1H, J = 6.2 Hz, H-1′), 7.49 (m, 3H, H−Ph), 7.64 (m, 2H, H−Ph), 8.06 (m, 1H, NH), 8.46 (s, 1H, H-8). Anal. ($C_{20}H_{21}N_5O_4$) C, H, N.

(*R*,*S*)-*N*^{6}-Ethyl-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)-adenosine (9c). Reaction of **5** with (*R*,*S*)-1-phenyl-2-propyn-1-ol for 6 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (92:8), gave **9c** as a white solid in 69% yield (EtOH): mp 95−98 °C; ^{1}H NMR (DMSO-d_6) δ 1.18 (t, 3H, J = 7.0 Hz, $NHCH_2CH_3$), 3.57 (m, 4H, NH*CH*$_2$-CH_3 and CH_2-5′), 3.97 (m, 1H, H-4′), 4.14 (m, 1H, H-3′), 4.52 (m, 1H, H-2′), 5.64 (d, J = 5.9 Hz, *CH*OH), 5.89 (d, 1H, J = 6.0 Hz, H-1′), 6.31 (d, 1H, J = 5.9 Hz, CH*OH*), 7.40 (m, 3H, H−Ph), 7.58 (m, 2H, H−Ph), 8.02 (m, 1H, NH), 8.45 (s, 1H, H-8). Anal. ($C_{21}H_{23}N_5O_5$) C, H, N.

(*R*)-*N*^{6}-Ethyl-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)adenosine (9d). Reaction of **5** with (*S*)-1-phenyl-2-propyn-1-ol for 16 h, followed by silica gel column chromatography with elution with $CHCl_3$−*c*C_6H_{12}−CH_3OH (60:35:5), gave **9d** as a white solid in 47% yield (MeOH): mp 122−125 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (t, 3H, J = 7.0 Hz, $NHCH_2CH_3$), 3.57 (m, 4H, $NHCH_2CH_3$ and CH_2-5′), 3.95 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.50 (m, 1H, H-2′), 5.64 (d, 1H, J = 5.6 Hz, *CH*OH), 5.87 (d, 1H, J = 5.8 Hz, H-1′), 6.31 (d, 1H, J = 5.8 Hz, CH*OH*), 7.42 (m, 3H, H−Ph), 7.57 (m, 2H, H−Ph), 8.00 (m, 1H, NH), 8.44 (s, 1H, H-8). Anal. ($C_{21}H_{23}N_5O_5$) C, H, N.

(*S*)-*N*^{6}-Ethyl-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)adenosine (9e). Reaction of **5** with (*R*)-1-phenyl-2-propyn-1-ol for 7 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (93:7), gave **9e** as a white solid in 25% yield (MeOH): mp 102−106 °C; ^{1}H NMR (DMSO-d_6) δ 1.17 (t, 3H, J = 7.0 Hz, $NHCH_2CH_3$), 3.56 (m, 4H, NH*CH*$_2$-CH_3 and CH_2-5′), 3.95 (m, 1H, H-4′), 4.12 (m, 1H, H-3′), 4.49 (m, 1H, H-2′), 5.63 (d, 1H, J = 6.0 Hz, *CH*OH), 5.87 (d, 1H, J = 5.8 Hz, H-1′), 6.31 (d, 1H, J = 5.8 Hz, CH*OH*), 7.41 (m, 3H, H−Ph), 7.56 (m, 2H, H−Ph), 7.98 (m, 1H, NH), 8.43 (s, 1H, H-8). Anal. ($C_{21}H_{23}N_5O_5$) C, H, N.

2-(1-Hexyn-1-yl)-*N*^{6}-isopropyladenosine (10a). Reaction of **6** with 1-hexyne for 12 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (92:8), gave **10a** as a white solid in 74% yield (EtOH): mp 193−195 °C; ^{1}H NMR (DMSO-d_6) δ 0.93 (t, 3H, J = 7.0 Hz, $(CH_2)_2CH_3$), 1.21 (d, 6H, J = 6.6 Hz, $CH(CH_3)_2$), 1.49 (m, 4H, $(CH_2)_2CH_3$), 2.44 (t, 2H, J = 13.6 Hz, $CH_2C{\equiv}C$), 3.61 (m, 4H, CH_2-5′), 3.96 (m, 1H, H-4′), 4.12 (t, 1H, J = 7.9 Hz, H-3′), 4.53 (m, 2H, H-2′ and $CH(CH_3)_2$), 5.87 (d, 1H, J = 6.1 Hz, H-1′), 7.74 (d, 1H, J = 8.8 Hz, NH), 8.39 (s, 1H, H-8). Anal. ($C_{19}H_{27}N_5O_4$) C, H, N.

2-Phenylethynyl-*N*^{6}-isopropyladenosine (10b). Reaction of **6** with ethynylbenzene for 4 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (92:8), gave **10b** as a white solid in 65% yield (EtOH): mp 192−195 °C; ^{1}H NMR (DMSO-d_6) δ 1.24 (d, 6H, J = 6.9, $CH(CH_3)_2$), 3.63 (m, 2H, CH_2-5′), 3.98 (m, 1H, H-4′), 4.14 (m, 1H, H-3′),

4.55 (m, 2H, H-2′ and *CH*(CH_3)$_2$), 5.91 (d, 1H, J = 5.9 Hz, H-1′), 7.49 (m, 3H, H−Ph), 7.63 (m, 2H, H−Ph), 7.92 (m, 1H, NH), 8.47 (s, 1H, H-8). Anal. ($C_{21}H_{23}N_5O_4$) C, H, N.

(*R,S*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-*N*^{6}-isopropyladenosine (10c). Reaction of **6** with (*R,S*)-1-phenyl-2-propyn-1-ol for 6 h, followed by silica gel column chromatography with elution with $CHCl_3$−CH_3OH (92:8), gave **10c** as a white solid in 74% yield (EtOH): mp 115−120 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.21 (d, 6H, J = 6.6 Hz, NHCH(*CH*$_3$)$_2$), 3.57 (m, 2H, CH_2-5′), 3.95 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.38 (m, 1H, NH*CH*(CH_3)$_2$), 4.50 (m, 1H, H-2′), 5.63 (d, J = 5.9 Hz, *CH*OH), 5.88 (d, 1H, J = 5.9 Hz, H-1′), 6.29 (d, 1H, J = 6.2 Hz, CH*OH*), 7.40 (m, 3H, H−Ph), 7.56 (m, 2H, H−Ph), 7.80 (d, 1H, J = 8.4 Hz, NH), 8.44 (s, 1H, H-8). Anal. ($C_{22}H_{25}N_5O_5$) C, H, N.

(*R*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-*N*^{6}-isopropyladenosine (10d). Reaction of **6** with (*S*)-1-phenyl-2-propyn-1-ol for 16 h, followed by silica gel column chromatography with elution with $CHCl_3$−*c*C_6H_{12}−CH_3OH (75:15:10), gave **10d** as a white solid in 61% yield (MeOH): mp 205 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.19 (d, 6H, J = 6.6 Hz, NHCH(*CH*$_3$)$_2$), 3.60 (m, 2H, CH_2-5′), 3.94 (m, 1H, H-4′), 4.11 (m, 1H, H-3′), 4.47 (m, 2H, H-2′ and NH*CH*(CH_3)$_2$), 5.61 (d, 1H, J = 6.2 Hz, *CH*OH), 5.87 (d, 1H, J = 6.0 Hz, H-1′), 6.29 (d, 1H, J = 6.0 Hz, CH*OH*), 7.38 (m, 3H, H−Ph), 7.55 (m, 2H, H−Ph), 7.81 (d, 1H, J = 8.8 Hz, NH), 8.42 (s, 1H, H-8). Anal. ($C_{22}H_{25}N_5O_5$) C, H, N.

(*S*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-*N*^{6}-isopropyladenosine (10e). Reaction of **6** with (*R*)-1-phenyl-2-propyn-1-ol for 16 h, followed by silica gel column chromatography with elution with $CHCl_3$−*c*C_6H_{12}−CH_3OH (75:15:10), gave **10e** as a white solid in 41% yield (MeOH): mp 96−98 °C (dec); ^{1}H NMR (DMSO-d_6) δ 1.20 (d, 6H, J = 6.6 Hz, NHCH(*CH*$_3$)$_2$), 3.60 (m, 2H, CH_2-5′), 3.95 (m, 1H, H-4′), 4.12 (m, 1H, H-3′), 4.49 (m, 2H, H-2′ and NH*CH*(CH_3)$_2$), 5.63 (d, 1H, J = 6.0 Hz, *CH*OH), 5.87 (d, 1H, J = 5.8 Hz, H-1′), 6.30 (d, 1H, J = 6.0 Hz, CH*OH*), 7.39 (m, 3H, H−Ph), 7.56 (m, 2H, H−Ph), 7.83 (d, 1H, J = 8.8 Hz, NH), 8.44 (s, 1H, H-8). Anal. ($C_{22}H_{25}N_5O_5$) C, H, N.

General Procedure for the Preparation of 2-Alkynyl-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purines. To a solution of **2** (0.51 mmol) in dry DMF (15 mL) and Et_3N (2.3 mL) under an atmosphere of N_2 were added bis-(triphenylphosphine)palladium dichloride (8.1 mg, 0.012 mmol) and CuI (0.51 mg, 0.003 mmol). The suitable alkyne (3.1 mmol) was added, and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for the time reported for each compound. The solvent was removed in vacuo, and the residue was chromatographed on a silica gel column, eluting with a suitable mixture of solvents to give compounds **11**−**14** as amorphous solids.

6-Chloro-2-(hexyn-1-yl)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (11) and 2,6-(Dihexyn-1-yl)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (12). Reaction of **2** with 1-hexyne for 16 h, followed by chromatography on a silica gel column with elution with $CHCl_3$−*c*C_6H_{12}−MeCN (50:45:5), gave **11** (31%) and **12** (45%) as amorphous solids.

11: ^{1}H NMR (DMSO-d_6) δ 0.94 (t, 3H, J = 7.1 Hz, CH_3), 1.55 (m, 4H, (*CH*$_2$)$_2$$CH_3$), 2.04 (s, 3H, $COCH_3$), 2.06 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 2.52 (t, 2H, J = 6.6 Hz, CH_2C≡C), 4.39 (m, 3H, CH_2-5′ and H-4′), 5.63 (m, 1H, H-3′), 6.00 (m, 1H, H-2′), 6.34 (d, 1H, J = 5.4 Hz, H-1′), 8.94 (s, 1H, H-8). Anal. ($C_{22}H_{25}ClN_4O_7$) C, H, N.

12: ^{1}H NMR (DMSO-d_6) δ 0.94 (m, 6H, J = 7.1 Hz, 2CH_3), 1.53 (m, 8H, 2(*CH*$_2$)$_2$$CH_3$), 2.04 (s, 3H, $COCH_3$), 2.05 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 2.53 and 2.64 (m, 2H each, CH_2C≡C), 4.35 (m, 3H, CH_2-5′ and H-4′), 5.63 (m, 1H, H-3′), 5.94 (m, 1H, H-2′), 6.31 (d, 1H, J = 5.43 Hz, H-1′), 8.96 (s, 1H, H-8). Anal. ($C_{28}H_{34}N_4O_7$) C, H, N.

2,6-(Diphenylethyn-1-yl)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (13). Reaction of **2** with ethynylbenzene for 16 h, followed by chromatography on a silica gel column with elution with $CHCl_3$−*c*C_6H_{12}−MeCN (52:40:8), gave **13** (51%) as an amorphous solid: ^{1}H NMR (DMSO-d_6) δ 2.04 (s, 3H, $COCH_3$), 2.08 (s, 3H, $COCH_3$), 2.15 (s, 3H, $COCH_3$), 4.39 (m, 3H, CH_2-5′ and H-4′), 5.74 (m, 1H, H-3′), 6.00 (m, 1H, H-2′), 6.40 (d, 1H, J = 5.3 Hz, H-1′), 7.57 (m, 6H, H−Ph), 7.74 (m, 6H, H−Ph), 8.99 (s, 1H, H-8). Anal. ($C_{32}H_{26}N_4O_7$) C, H, N.

(*E*)-6-Chloro-2-(3-keto-3-phenyl-1-propen-1-yl)-9-(2,3,5,-tri-*O*-acetyl-β-D-ribofuranosyl)purine (14). Reaction of **2** with (*R,S*)-1-phenyl-2-propyn-1-ol for 48 h, followed by chromatography on a silica gel column with elution with *c*C_6H_{12}−EtOAc (75:25), gave **14** (60%) as an amorphous solid: ^{1}H NMR (DMSO-d_6) δ 1.89 (s, 3H, $COCH_3$), 2.12 (s, 3H, $COCH_3$), 2.14 (s, 3H, $COCH_3$), 4.39 (m, 3H, CH_2-5′ and H-4′), 5.92 (m, 1H, H-3′), 6.15 (m, 1H, H-2′), 6.43 (d, J = 3.0 Hz, 1H, H-1′), 7.75 (m, 4H, H−Ph and =CH), 8.14 (d, J = 7.5, 2-H, H−Ph), 8.35 (d, J = 15.8 Hz, 1H, =CH), 8.95 (s, 1H, H-8). Anal. ($C_{25}H_{23}ClN_4O_8$) C, H, N.

General Procedure for the Synthesis of 6-Iodo-2-alkynylpurines 15a, 15b, and 15e. To a solution of the suitable 2-alkynyladenosine (0.27 mmol) in dry DMF (2 mL) diiodomethane (3.61 mL) and isopentylnitrite (1.12 mL) were added, and the mixture was heated at 60 °C for 30 min. The solvent was evaporated, and the residue was chromatographed on a silica gel column with elution with a suitable mixture of solvents to give compounds **15a**, **15b**, and **15e**.

2-(Hexyn-1-yl)-6-iodo-9-(β-D-ribofuranosyl)purine (15a). Reaction of **7a** according to the general procedure and purification with elution with $CHCl_3$−*c*C_6H_{12}−MeOH (83:10:7) gave **15a** as an amorphous solid in 38% yield: ^{1}H NMR (DMSO-d_6) δ 0.95 (t, 3H, J = 7.1 Hz, $(CH_2)_2CH_3$), 1.54 (m, 4H, $(CH_2)_2CH_3$), 2.52 (m, 2H, CH_2C≡C), 3.68 (m, 2H, CH_2-5′), 3.99 (m, 2H, H-4′), 4.18 (m, 1H, H-3′), 4.52 (m, 1H, H-2′), 5.97 (d, 1H, J = 5.1 Hz, H-1′), 8.94 (s, 1H, H-8). Anal. ($C_{16}H_{19}IN_4O_4$) C, H, N.

6-Iodo-2-(phenylethyn-1-yl)-9-(β-D-ribofuranosyl)purine (15b). Reaction of **7b** according to the general procedure and purification with elution with $CHCl_3$−*c*C_6H_{12}−MeOH (83:10:7) gave **15b** as an amorphous solid in 37% yield: ^{1}H NMR (DMSO-d_6) δ 3.68 (m, 2H, CH_2-5′), 4.01 (m, 2H, H-4′), 4.20 (m, 1H, H-3′), 4.55 (m, 1H, H-2′), 6.03 (d, 1H, J = 5.2 Hz, H-1′), 7.53 (m, 3H, H−Ph), 7.73 (m, 2H, H−Ph), 9.00 (s, 1H, H-8). Anal. ($C_{18}H_{15}IN_4O_4$) C, H, N.

(*S*)-2-(3-Hydroxy-3-phenyl-1-propyn-1-yl)-6-iodo-9-(β-D-ribofuranosyl)purine (15e). Reaction of **7e** according to the general procedure and purification with elution with $CHCl_3$−MeOH−*c*C_6H_{12} (82:10:8) gave **15e** as an amorphous solid in 28% yield: ^{1}H NMR (DMSO-d_6) δ 3.68 (m, 2H, CH_2-5′), 4.00 (m, 1H, H-4′), 4.18 (m, 1H, H-3′), 4.51 (m, 1H, H-2′), 5.71 (d, 1H, J = 6.0 Hz, *CH*OH), 5.98 (d, 1H, J = 4.5 Hz, H-1′), 6.28 (d, 1H, J = 6.0 Hz, CH*OH*), 7.42 (m, 3H, H−Ph), 7.58 (m, 2H, H−Ph), 8.98 (s, 1H, H-8). Anal. ($C_{19}H_{17}IN_4O_5$) C, H, N.

Biological Studies. Cloning of the human adenosine receptors, stable transfection of cells, cell culture, membrane preparation, radioligand binding, and determination of adenylyl cyclase activity have been fully described elsewhere.[26] 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. Because of the lack of a suitable radioligand, the relative potency of agonists at A_{2B} adenosine receptors was determined in adenylyl cyclase experiments.

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