2-Alkynyl Derivatives of Adenosine-5′-*N*-ethyluronamide: Selective A_2 Adenosine Receptor Agonists with Potent Inhibitory Activity on Platelet Aggregation

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A series of new 2-alkynyl and 2-cycloalkynyl derivatives of adenosine-5′-*N*-ethyluronamide (NECA) and of *N*-ethyl-1′-deoxy-1′-(6-amino-2-hexynyl-9*H*-purin-9-yl)-β-D-ribofuranuronamide (1, HE-NECA), bearing hydroxy, amino, chloro, and cyano groups in the side chain, were synthesized. The compounds were studied in binding and functional assays to assess their potency for the A_2 compared to A_1 adenosine receptor. The presence of an α-hydroxyl group in the alkynyl chain of NECA derivatives accounts for the A_2 agonist potency, leading to compounds endowed with sub-nanomolar affinity in binding studies. However, these analogues also possess good A_1 receptor affinity resulting in low A_2 selectivity. From functional experiments the 4-hydroxy-1-butynyl (**6**) and the 4-(2-tetrahydro-2H-pyranyloxy)-1-butynyl (**16**) derivatives appear to be very potent in inducing vasorelaxation without appreciable effect on heart rate. The new compounds were also tested as inhibitors of platelet aggregation induced by ADP. Introduction of an α-hydroxyl group in the alkynyl side chain caused a greater increase in antiaggregatory activity than either NECA or HE-NECA, resulting in the most potent inhibitors of platelet aggregation so far known in the nucleoside series. The presence of an α-quaternary carbon such as the 3-hydroxy-3,5-dimethyl-1-hexynyl (**12**) and the 3-hydroxy-3-phenyl-1-butynyl (**15**) derivatives markedly reduced the antiaggregatory potency without affecting the A_2 affinity. The hydrophobicity index (k') of the new nucleosides barely correlated with the binding data, whereas high k' values were associated with increased A_2 vs A_1 selectivity but with reduced activity in all functional assays. Some of the compounds synthesized possess interesting pharmacological properties. Compounds having an appropriate balance between vasorelaxation and antiplatelet activity, if confirmed *in vivo*, deserve further development for the treatments of cardiovascular disorders.

Adenosine is known to modulate a number of physiological functions,[1] and a variety of studies have demonstrated that most adenosine actions are mediated by at least two extracellular receptors divided into two major subtypes, designated A_1 and A_2.[2]

Both receptors are widespread in the different systems of the organism. In some tissues, however, only one subtype is present. For example, the A_1 receptor prevails in the heart, whereas the A_2 receptor is present mainly in vessels and platelets. In turn, the A_2 receptor has been further subdivided into A_{2a} (high affinity, in brain striatum) and A_{2b} (low affinity, in fibroblasts).[3] Compounds which interact selectively with the A_2 receptors could have an interesting pharmacological profile. For example, the combination of vasodilating activity and antiaggregatory property can lead to useful therapeutical application in the treatment of severe cardiovascular pathologies, such as ischemic cardiomyopathy, hypertension, and atherosclerosis.

At A_1 receptors the most active analogs are N^6-substituted adenosines,[4] whereas at A_2 receptors the most active compounds are C-2 substituted adenosine analogs.[5-7] Considering that adenosine-5′-*N*-ethyluronamide (NECA) is potent in several pharmacological models and that it has high affinity in the low nanomolar range for both A_2 and A_1 receptors,[8] a variety of substitution at C-2 has been introduced in NECA structure.[9] One of these, the 2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5′-*N*-ethyluronamide (CGS 21680), has become the reference A_2 receptor agonist in various pharmacological studies, being about 50–140-fold selective for A_2 vs A_1 receptors.[9a,10] Recently, we have reported the synthesis of *N*-ethyl-1′-deoxy-1′-(6-amino-2-hexynyl-9*H*-purin-9-yl)-β-D-ribofuranuronamide (1, HE-NECA), which possesses high affinity at A_2 receptors combined with a good A_2 vs A_1 selectivity.[11] In addition, HE-NECA was found to be the most potent inhibitor of platelet aggregation so far known in the nucleoside series.[10,11]

The therapeutic potential of compound **1** for the treatment of cardiovascular diseases prompted us to synthesize a number of new 2-alkynyl and 2-cycloalkynyl derivatives of NECA **4-25**, bearing hydroxyl, amino, chloro, and cyano groups on the side chain. The new compounds were evaluated in the most reliable biological assays, which provide information as to the potency and selectivity of A_2 receptor agonists.[10,12]

Results and Discussion

Chemistry. The synthesis of 2-alkynyl derivatives of adenosine-5′-*N*-ethyluronamide **5–25** was accomplished by the reactions described in Scheme 1. The synthesis of compounds **3** and **5–25** was carried out by a modification of the palladium-catalyzed cross-coupling reaction.[13]

Treatment of a solution of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**2**)[11] in dry acetonitrile, DMF, and triethylamine with cuprous iodide, $PdCl_2$, triphenylphosphine, and the appropriate terminal alkyne, at room temperature for several hours in an atmosphere of N2, allowed complete conversion of the iodonucleoside to the alkynyl derivatives (Table 1).

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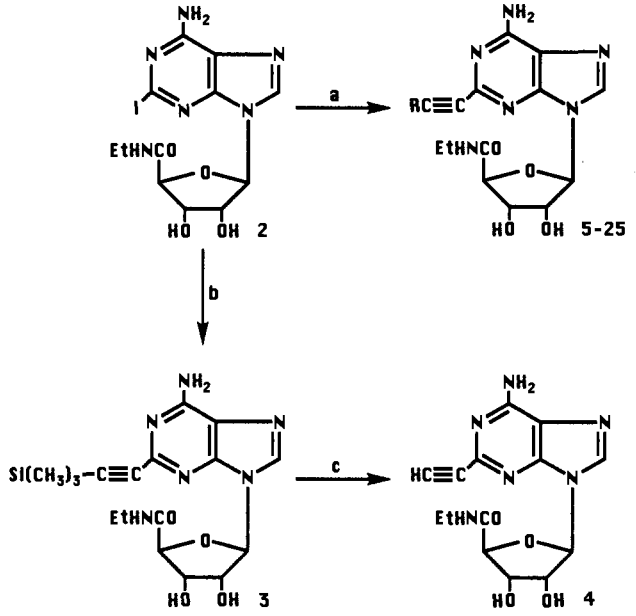
Scheme 1[a]



[a] (a) CuI, $(Ph)_3P$-$PdCl_2$, RC≡CH; (b) CuI, $(Ph)_3P$-$PdCl_2$, $(CH_3)_3SiC{\equiv}CH$; (c) KOH.

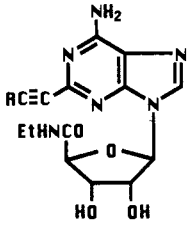
Synthesis of *N*-ethyl-1′-deoxy-1′-[6-amino-2-(3,3-diethoxy-1-propyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (**17**) required reaction at 40 °C for 48 h.

The synthesis of the ethynyl derivative **4** was accomplished by reaction of **2** with (trimethylsilyl)acetylene to give **3**, followed by deprotection in basic medium.

Adenosine Receptor Binding Affinity. The interaction of the new 2-alkynyl derivatives of NECA **4–25** with adenosine receptors was evaluated using both radioligand binding technique and functional assays. Affinity for A_2 receptors was determined in competition assays in rat striatum using [^{3}H]CGS 21680 as radioligand.[14] Affinity for A_1 receptors was determined in compeittion assays in rat brain using [^{3}H]CHA.[15] NECA, HE-NECA,[11] and CCPA[4] were included as reference compounds. The results are reported in Table 1.

Compounds **5–15** have a hydroxyl group in a different position with respect to the triple bond. In comparison with HE-NECA, the derivatives **5–8**, bearing a primary alcohol, showed weaker A_2 binding affinity and a slight increase in A_1 affinity, which resulted in a pronounced loss of A_2 vs A_1 selectivity. Lengthening of the alkynylic chain did not influence the compounds' A_2 affinity, whereas it produced a decrease in A_1 binding affinity. Introduction

Table 1. *In Vitro* Pharmacological Activity of 2-Alkynyl Derivatives of NECA



compd	R	binding assay[a] K_i (nM) rat brain A_1	rat striatum A_2	selectivity A_1/A_2	functional activity[b] EC_{50} (nM) rat atria A_1	rat aorta A_2	antiaggr activity[c] potency ratio vs NECA: rabbit platelet A_2	k'[d]
HE-NECA	$(CH_2)_3CH_3$	130 (116–145)	2.2 (1.9–2.6)	59	>10 μM	596 (244–1460)	3.00	2.57
NECA		10.4 (9.4–11.6)	7.8 (6.6–9.1)	1.3	54.8 (34.8–78.2)	394 (209–742)	1.00	0.19
CCPA		1.3 (1.1–1.4)	650 (555–762)	0.002	8.2 (4.4–15.3)	>10 μM	0.003	2.76
4	H	35.4 (21.9–57.2)	57.8 (48.9–68.3)	0.6	167 (116–240)	440 (242–797)	0.30	0.25
5	CH_2OH	14.1 (7.8–256)	9.1 (6.0–13.7)	1.5	84.2 (19.0–347)	367 (237–568)	2.30	0.17
6	$(CH_2)_2OH$	47.3 (42.8–52.4)	10.8 (9.8–12.0)	4.4	>10 μM	59.4 (11.3–314)	1.10	0.20
7	$(CH_2)_3OH$	99.9 (89.6–111)	11.3 (10.1–12.5)	8.8	>10 μM	300 (113–794)	2.20	0.22
8	$(CH_2)_4OH$	42.1 (39.9–44.5)	6.8 (6.0–7.7)	6.2	>10 μM	391 (180–850)	4.80	0.35
9	$CH(OH)CH_3$	11.1 (10.1–12.2)	7.6 (6.6–8.7)	1.5	657 (493–876)	828 (232–2950)	4.70	0.23
10	$CH(OH)CH_2CH_3$	20.4 (18.5–22.6)	12.4 (10.8–14.2)	1.6	153 (67.6–348)	69.3 (28.3–170)	14.10	0.42
11	$CH_2CH(OH)CH_3$	69.6 (64.7–74.9)	56.4 (52.3–60.8)	1.2	6050 (1260–29 000)	1650 (544–4990)	3.20	0.29
12	$C(OH,CH_3)CH_2$-$CH(CH_3)CH_3$	3.0 (2.5–3.6)	0.5 (0.4–0.6)	6.0	>10 μM	338 (145–787)	0.54	1.84
13	1-hydroxycyclopentyl	4.0 (3.5–4.5)	0.6 (0.5–0.7)	6.7	475 (229–984)	159 (54.5–466)	5.30	0.77
14	CH(OH)Ph	2.5 (2.2–2.9)	0.9 (0.7–1.3)	2.8	110 (35.4–345)	123 (50.4–301)	15.70	0.82
15	$C(OH,CH_3)Ph$	32.7 (29.7–35.9)	1.7 (1.6–1.8)	19	2430 (990–5960)	456 (316–657)	0.26	1.32
16	$(CH_2)_2OPyr$[e]	267 (191–375)	5.0 (4.8–5.2)	53	>10 μM	115 (71.1–186)	0.30	1.32
17	$CH(OCH_2CH_3)_2$	656 (515–835)	441 (336–578)	1.5	>10 μM	382 (157–930)	0.02	1.63
18	CH_2NH_2	48.6 (31.8–74.4)	38.3 (25.4–57.7)	1.3	730 (548–1120)	564 (317–1000)	0.40	0.15
19	$CH_2N(CH_3)_2$	27.9 (25.0–31.1)	2.3 (2.2–2.4)	12	332 (194–570)	140 (36.0–546)	2.30	0.37
20	1-aminocyclohexyl	398 (363–436)	39.4 (25.2–61.7)	10	>10 μM	779 (414–1470)	0.60	0.60
21	$(CH_2)_3Cl$	37.9 (34.1–42.0)	1.0 (0.8–1.2)	38	2520 (1570–4060)	715 (463–1100)	2.30	1.35
22	$(CH_2)_3CN$	184 (167–204)	4.7 (4.1–5.5)	39	2370 (1770–3170)	111 (79.2–155)	2.10	0.32
23	1-methylvinyl	167 (111–252)	27.3 (22.1–33.7)	6.1	>10 μM	1200 (591–2420)	0.66	1.27
24	1-cyclohexenyl	3295 (3068–3538)	61.3 (55.5–67.6)	54	>10 μM	3030 (682–13 400)	0.03	3.90
25	cyclohexyl	236 (203–274)	9.2 (7.4–11.4)	26	>10 μM	329 (62.3–1730)	0.66	4.54

[a] Receptor binding affinity at A_1 and A_2 receptors were determined using [^{3}H]CHA and [^{3}H]CGS21680 as radioligands, respectively. Data are geometrical means from at least three separate experiments; 95% confidence limits in parentheses. [b] Data are means from at least three separate experiments; 95% confidence limits in parenthesis. [c] The potency ratio was calculated using the concentration of the test compound close to the IC_{50} value. In our experimental conditions the IC_{50} value for NECA was 0.2 μM. [d] Hydrophobicity index. Data are means from three separate experiments. [e] Pyr = 2-tetrahydro-2*H*-pyran.

of an α-hydroxyl group in a more lipophilic side chain as for compounds 12–15 produced an increase in the A_2 affinity which led to the most potent A_2 agonists made thus far (12: K_i = 0.5 nM). However, a similar increase in potency was observed for affinity at the A_1 receptor; consequently, compounds 12–15 retained little A_2 vs A_1 selectivity. Introduction of an α,α-diethoxy group markedly decreased affinity for both A_2 and A_1 receptors (17: $K_i(A_1)$ = 656 and $K_i(A_2)$ = 441 nM vs 5: $K_i(A_1)$ = 14.1 and $K_i(A_2)$ = 9.1 nM). The presence of a β-oxy-2-tetrahydro-2*H*-pyranyl group (16) increased A_2 selectivity (53-fold) by reducing A_1 affinity.

Introduction of an α-amino group (18 and 20) produced a decrease in the affinity for both receptors with respect to the corresponding hydroxyl derivatives. However, the *N*,*N*-dimethylamino substitution (19) showed higher binding affinity at the A_2 site than the corresponding α-amino (18) and α-hydroxy (5) substituted compounds.

The presence of a conjugated double bond (23 and 24) decreased binding affinity at both sites, but as for the unsaturated cycloalkyl substituent, the affinity for the A_1 receptor was markedly reduced, resulting in a 54-fold A_2-selective compound (24).

Replacing of the distal methyl in the HE-NECA side chain by a chlorine atom (21) or a cyano group (22) did not produce any improvement. It is worth noting that the unsubstituted ethynyl group present in the compound 4 reduced the A_2 activity, leaving a moderate A_1 selectivity.

Effect in Isolated Tissues. Negative chronotropic activity (A_1) was tested in spontaneously beating rat atria and vasodilation (A_2) in rat aorta according to a method described elsewhere.[12] Results are summarized in Table 1.

Most of the compounds showed vasorelaxant activity higher than that of NECA. Of the compounds 5–8, bearing primarny hydroxyl groups, the derivative 6 was the most interesting. In fact, it was very potent in inducing vasorelaxation without any appreciable effect on heart rate. As regards compounds whose hydroxyl group is introduced in a more lipophilic chain, there was a reduction of vasorelaxant activity as well as decrease of A_2 vs A_1 selectivity. Moreover, the substitution of the pirmary hydroxyl group by chlorine atom (21) or a cyano (22) as well as an amino group (18 and 19) reduced the separation between responses mediated by A_2 vs A_1 receptors.

The presence of cycloalkyl groups (13, 20, 24, and 25) and a conjugate double bond (23 and 24) decreased heart rate in comparison with the unsubstituted compound 4. The introduction of a β-oxytetrahydro-2*H*-pyran group (16) led to a compound with a clear vasodilating activity and no influence on heart rate.

Platelet Aggregation Studies. The antiaggregatory effect of the new alkynyl derivatives of NECA on rabbit platelet aggregation induced by ADP is reported in Table 1 as the potency ratio calculated versus NECA.

Two major findings emerge from this study. First, some of the alkynyl derivatives of NECA showed an antiaggegatory activity higher than that of either NECA or HE-NECA itself, resulting in the most potent agonists which inhibit platelet aggregation. The presence of a hydroxyl group improved the activity, in particular an α-hydroxyl substituent conferred a potency 16-fold that of NECA (10 and 14). However, contemporary α-methylation markedly decreased the antiaggregatory activity (12 and 15). When an ω-hydroxyl group is present, the highest activity is

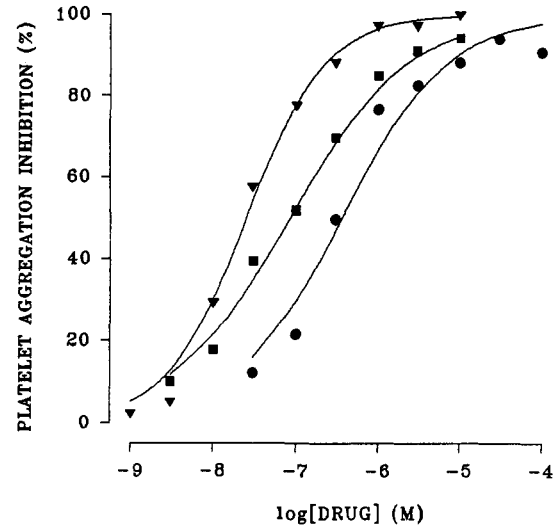


Figure 1. Dose–response curves of the inhibitory effects of NECA (●), *N*-ethyl-1′-deoxy-1′-[6-amino-2-(6-hydroxy-1-hexyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (8) (■), and *N*-ethyl-1′-deoxy-1′-[6-amino-2-(3-hydroxy-1-pentyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (10) (▼) on ADP-induced aggregation in rabbit platelets. Data are means of at least four separate experiments.

obtained with a six-carbon side chain (8). The presence of an α-amino group resulted in compounds less potent than NECA (18 and 20), whereas introduction of a *N*,*N*-dimethylamino substituent enhanced the effect (19). The dose–response curves of the platelet aggregation inhibitory effect of NECA, *N*-ethyl-1′-deoxy-1′-[6-amino-2-(6-hydroxy-1-hexyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (8) and *N*-ethyl-1′-deoxy-1′-[6-amino-2-(3-hydroxy-1-pentyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (10) are reported in Figure 1.

Again, replacing of the distal methyl in the HE-NECA side chain with a chlorine atom (21) or a cyano group (22) did not improve the antiaggregatory potency. Introduction of an α,α-diethoxy group (17) or a cyclohexenyl substituent (24) and, to a lesser extent, a β-oxy-2-tetrahydro-2*H*-pyranyl (16) and a cyclohexyl (25) group worsened the effect.

Secondly, some alkynyl derivatives displayed a good activity in the platelt aggregation assay which did not correspond to the affinity for the A_2 receptors as measured in rat striatum. Thus, the compound 12, which was 16-fold more active than NECA at the A_2 receptor, showed a weak antiaggregatory potency. Conversely, compound 10 presented binding affinity at the A_2 receptor comparable to that of NECA, with a marked activity as inhibitor of platelet aggregation. These findings lend further support to the hypothesis that the A_2 receptor on the platelets is not a typical A_{2a} recognition site as we have suggested in a recent study conducted in a series of 2-(ar)alkoxyadenosine derivatives.[16]

Conclusions

We have identified a number of 2-alkynyl derivatives of NECA possessing a potent activity at A_2 receptors in both binding assays and functional *in vitro* models. Specifically, compounds having an α-hydroxyl group in the alkynyl chain (e.g., 12–14) are the most potent agonists at A_2 receptors so far synthesized. These substituents are, however, detrimental to A_2/A_1 selectivity since the compounds have also high affinity for A_1 receptors. With

regard to the antiaggregatory property, derivatives **10** and **14** are the most potent of the series, being about 15 times more active than NECA and 5 times more potent than HE-NECA. Moreover, it is worth noting that the presence of an α-quaternary carbon such as in compounds **12** and **15** markedly reduced the antiaggregatory potency, the heart rate, and the vasorelaxant activity without affecting the A_2 binding affinity.

The α-hydroxyl-2-alkynyl derivatives of NECA are also effective vasorelaxant compounds having a potency equal to or higher than that of NECA. Interestingly, compounds **6**, **12**, **16**, and **17** induce vasorelaxation without interacting with heart rate, a physiological response mediated by A_1 receptors.

Retention of nucleosides on a reverse-phase HPLC column is reported as a measure of the relative hydrophobicity (k', Table 1).[22] The hydrophobicity index of the new nucleosides barely correlated with the binding data, whereas high k' values were associated with increased A_2 vs A_1 selectivity but with reduced activity in all functional assays.

A few selected compounds need to be examined further to assess their potency and duration of action in *in vivo* models. Compounds possessing the proper balance between vasorelaxant properties and antiplatelet activity, in the absence of effects on the central nervous system, deserve further evaluation for their potential in the treatments of cardiovascular disorders.

Experimental Section

Chemistry. Melting points were determined with a Büchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian VX 300 MHz spectrometer. TLC were carried out on precoated TLC plates with silica gel 60 F-254 (Merck). For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are within ±0.4% of theoretical values.

General Procedure for the Preparation of 2-Alkynyladenosine-5'-*N*-ethyluronamides. To a solution of 250 g (0.58 mmol) of *N*-ethyl-1'-deoxy-1'-(6-amino-2-iodo-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**2**)[9] in 10 mL of dry acetonitrile, 5 mL of DMF, and 2.5 mL of triethylamine under an atmosphere of N_2 was added 8.1 mg (0.0115 mmol) of bis(triphenylphosphine)-palladium dichloride and 0.58 mg (0.003 mmol) of cuprous iodide. To the mixture was added the appropriate terminal alkyne (2.9 mmol), and the reaction mixture was stirred under an atmosphere of N_2 at room temperature for the time reported below. 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 **3**–**25** as chromatographically pure solids.

***N*-Ethyl-1'-deoxy-1'-[6-amino-2-[2-(trimethylsilyl)ethynyl]-9*H*-purin-9-yl]-β-D-ribofuranuronamide (3).** The reaction of **2** with (trimethylsilyl)acetylene for 16 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (96:4), gave compound **3** (95%): mp 208–211 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.22 (m, 9H, CH_3 silyl), 1.05 (t, 3H, CH_2CH_3), 3.29 (m, 2H, CH_2CH_3), 4.10 (m, 1H, H-3'), 4.29 (s, 1H, H-4'), 4.56 (m, 1H, H-2'), 5.93 (d, 1H, J = 7.8 Hz, H-1'), 7.64 (s, 2H, NH_2), 8.46 (s, 1H, H-8), 8.63 (t, 1H, NH). Anal. ($C_{17}H_{24}N_6O_4Si\cdot H_2O$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-(6-amino-2-ethynyl-9*H*-purin-9-yl)-β-D-ribofuranuronamide (4).** To 1.6 g (3.96 mmol) of **3** in 30 mL of methanol was added 0.32 g of KOH in 10 mL of methanol. The reaction mixture was stirred at room temperature for 1 h, and then the solvent was removed under vacuum. The residue was partitioned between ethyl acetate and water, and the combined organic layers were dried (Na_2SO_4) and evaporated to give 1.0 g (816) of **4** as a chromatographically pure solid: mp 245–248 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.04 (t, 3H, CH_2CH_3), 3.26 (m, 2H, CH_2CH_3), 4.10 (s, 1H, C≡C*H*), 4.12 (m, 1H, H-3'), 4.29 (d, J = 1.5, 1H, H-4'), 4.55 (m, 1H, H-2'), 5.92 (d, 1H, J = 7.5 Hz, H-1'), 7.62 (s, 2H, NH_2), 8.46 (s, 1H, H-8), 8.63 (t, 1H, NH). Anal. ($C_{14}H_{16}N_6O_4\cdot H_2O$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-[6-amino-2-(3-hydroxy-1-propyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (5).** The reaction of **2** with 2-propyn-1-ol for 3 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (85:15), gave compound **5** (52%, crystallized from ethanol): mp 173–175 °C dec; ^{1}H NMR (Me_2SO-d_6) 1.04 (t, 3H, CH_2CH_3), 3.27 (m, 2H, CH_2CH_3), 4.10 (m, 1H, H-3'), 4.26 (m, 3H, H-4' and CH_2OH), 4.54 (m, 1H, H-2'), 5.39 (t, 1H, CH_2OH), 5.91 (d, 1 H, J = 7.5 Hz, H-1'), 7.54 (s, 2H, NH_2), 8.43 (s, 1H, H-8), 8.67 (t, 1H, NH). Anal. ($C_{15}H_{18}N_6O_5\cdot H_2O$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-[6-amino-2-(4-hydroxy-1-butyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (6).** The reaction of **2** with 3-butyn-1-ol for 20 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol–benzene (80:12:8), gave compound **6** (70%, crystallized from ethanol): mp 245–248 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.06 (t, 3H, CH_2CH_3), 2.50 (t, 2H, CH_2CH_2OH), 3.28 (m, 2H, CH_2CH_3), 3.57 (m, 2H, CH_2OH), 4.10 (m, 1H, H-3'), 4.28 (d, 1H, J = 1.5 Hz, H-4'), 4.55 (m, 1H, H-2''), 4.93 (t, 1H, CH_2OH), 5.91 (d, 1H, J = 7.8 Hz, H-1'), 7.53 (s, 2H, NH_2), 8.41 (s, 1H, H-8), 8.76 (t, 1H, NH). Anal. ($C_{16}H_{20}N_6O_5\cdot H_2O$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-[6-amino-2-(5-hydroxy-1-pentyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (7).** The reaction of **2** with 4-pentyn-1-ol for 20 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol–benzene (70:20:10), gave compound **7** (57%, crystallized from methanol: mp 230–232 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.06 (t, 3H, CH_2CH_3), 1.66 (m, 2H, CH_2CH_2C≡C), 2.43 (t, 2H, CH_2C≡C), 3.28 (m, 2H, CH_2CH_3), 3.48 (m, 2H, CH_2OH), 4.10 (m, 1H, H-3'), 4.28 (d, 1H, J = 1.5 Hz, H-4'), 4.55 (m, 2H, H-2' and CH_2OH), 5.91 (d, 1H, J = 7.8 Hz, H-1'), 7.53 (s, 2H, NH_2), 8.40 (s, 1H, H-8), 8.77 (t, 1H, NH). Anal. ($C_{17}H_{22}N_6O_5\cdot H_2O$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-[6-amino-2-(6-hydroxy-1-hexyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (8).** The reaction of **2** with 5-hexyn-1-ol for 24 h at 40 °C, followed by chromatography on a silica gel column, eluting with chloroform–methanol–benzene (70:20:10), gave compound **8** (69%, crystallized from methanol): mp 245–247 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.06 (t, 3H, CH_2CH_3), 1.54 (m, 4H, CH_2CH_2), 2.42 (m, 2H, CH_2), 3.30 (m, 2H, CH_2CH_3), 3.41 (m, 2H, CH_2OH), 4.09 (m, 1H, H-3'), 4.28 (s, 1H, H-4'), 4.44 (t, 1H, CH_2OH), 4.55 (m, 1H, H-2'), 5.90 (d, 1H, J = 7.8 Hz, H-1'), 7.54 (s, 2H, NH_2), 8.40 (s, 1H, H-8), 8.77 (t, 1H, NH). Anal. ($C_{18}H_{24}N_6O_5\cdot H_2O$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-[6-amino-2-(3-hydroxy-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (9).** The reaction of **2** with 3-butyn-2-ol for 20 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol–benzene (70:20:10), gave compound **9** (52%): mp 211–214 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.05 (t, 3H, CH_2CH_3), 1.36 (d, 3H, J = 6.6 Hz, CH_3HOH), 3.30 (m, 2H, CH_2CH_3), 4.12 (m, 1H, H-3'), 4.29 (d, 1H, J = 1.5 Hz, H-4'), 4.55 (m, 2H, H-2' and CHOH), 5.55 (d, 1H, J = 5.4 Hz, CHO*H*), 5.92 (d,1H, J = 7.8 Hz, H-1'), 7.57 (s, 2H, NH_2), 8.44 (s, 1H, H-8), 8.70 (t, 1H, NH). Anal. ($C_{16}H_{20}N_6O_5\cdot H_2O$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-[6-amino-2-(3-hydroxy-1-pentyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (10).** The reaction of **2** with 1-pentyn-3-ol for 5 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (85:15), gave compound **10** (37%, crystallized from ethanol): mp 148–150 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.95 (t, 3H, CH_2CH_3), 1.04 (t, 3H, NCH_2CH_3), 1.64 (m, 2H, CH_2CHOH), 3.28 (m, 2H, CH_2CH_3), 4.10 (m, 1H, H-3'), 4.28 (d, 1H, J = 1.8 Hz, H-4'), 4.33 (m, 1H, CHOH), 4.55 (m, 2H, H-2'), 5.58 (d, 1H, CHO*H*), 5.92 (d, 1H, J = 7.8 Hz, H-1'), 7.56 (s, 2H, NH_2), 8.44 (s, 1H, H-8), 8.62 (t, 1H, NH). Anal. ($C_{20}H_{28}N_6O_5\cdot H_2O$) C, H, N.

***N*-Ethyl-1'-deoxy-1'-[6-amino-2-(4-hydroxy-1-pentyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (11).** The reaction of **2** with 4-pentyn-2-ol for 5 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (85:15), gave compound **11** (39%, crystallized from ethanol): mp 232–234 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.05 (t, 3H, CH_2CH_3), 1.17 (d, 3H, J = 6.0 Hz, CH_3CHOH), 2.45 (m, 2H, CH_2C≡C), 3.28 (m, 2H, CH_2CH_3), 3.83 (m, 2H, CHOH), 4.09 (m, 1H, H-3'), 4.28 (d, 1H, J = 1.5 Hz, H-4'), 4.55 (m, 1H, H-2'), 4.88 (d, 1H, J = 4.8

Hz, CHO*H*), 5.91 (d, 1H, *J* = 7.5 Hz, H-1′), 7.53 (s, 2H, NH_2), 8.40 (s, 1H, H-8), 8.74 (t, 1H, NH). Anal. ($C_{17}H_{22}N_6O_5 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(3-hydroxy-3,5-dimethyl-1-hexyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (12).** The reaction of **2** with 3,5-dimethyl-1-hexyn-3-ol for 5 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (87:13), gave compound **12** (41%, crystallized from ethanol): mp 135–127 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.95 (d, 6H, CH(CH_3)$_2$), 1.03 (t, 3H, CH_2CH_3), 1.42 (s, 3H, CH_3COH), 1.53 (d, 2H, *J* = 6.0 Hz, CH_2COH), 1.92 (m, 1H, CH(CH$_3$)$_2$), 3.26 (m, 2H, CH_3CH_3), 4.10 (m, 1H, H-3′), 4.28 (s, 1H, H-4′), 4.57 (m, 2H, H-2′), 5.39 (s, 1H, CO*H*), 5.92 (d, 1H, *J* = 7.5 Hz, H-1′), 7.54 (s, 2H, NH_2), 8.45 (s, 1H, H-8), 8.51 (t, 1H, NH). Anal. ($C_{20}H_{28}N_6O_5 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-[2-(1-hydroxycyclopentyl)-ethynyl]-9*H*-purin-9-yl]-β-D-ribofuranuronamide (13).** The reaction of **2** with 1-ethynyl-1-cyclopentanol for 5 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (85:15), gave compound **13** (30%, crystallized from ethanol): mp 153–155 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.04 (t, 3H, CH_2CH_3), 1.67 (m, 4H, H-cyclopentyl), 1.87 (m, 3H, H-cyclopentyl), 3.29 (m, 2H, CH_2CH_3), 4.10 (m, 1H, H-3′), 4.28 (d, *J* = 1.5 Hz, 1H, H-4′), 4.56 (m, 1H, H-2′), 5.92 (d, 1H, *J* = 7.8 Hz, H-1′), 7.58 (s, 2H, NH_2), 8.45 (s, 1H, H-8), 8.59 (t, 1H, NH). Anal. ($C_{20}H_{26}N_6O_4 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(3-hydroxy-3-phenyl-1-propyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (14).** The reaction of **2** with 1-phenyl-2-propyn-1-ol for 20 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (85:15), gave compound **14** (67%, crystallized from methanol): mp 150–152 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.95 (m, 3H, CH_2CH_3), 3.18 (m, 2H, CH_2CH_3), 4.10 (m, 1H, H-3′), 4.28 (s, 1H, H-4′), 4.55 (m, 1H, H-2′), 5.58 (d, 1H, *J* = 8.1 Hz, CHOH), 5.92 (d, 1H, *J* = 7.5 Hz, H-1′), 6.26 (d, 1H, *J* = 8.1 Hz, CHO*H*), 7.36 (m, 3H, H-Ph), 7.50 (d, *J* = 6.9 Hz, 2H, H-Ph), 7.58 (s, 2H, NH_2), 8.45 (s, 1H, H-8), 8.58 (t, 1H, NH). Anal. ($C_{20}H_{20}N_6O_5 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(3-hydroxy-3-phenyl-1-butyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (15).** The reaction of **2** with 2-phenyl-3-butyn-2-ol for 20 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol–benzene (75:15:10), gave compound **15** (75%): mp 203–205 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.97 (t, 3H, CH_2CH_3), 1.71 (s, 3H, CH_3C), 3.21 (m, 2H, CH_2CH_3), 4.11 (m, 1H, H-3′), 4.28 (s, 1H, H-4′), 4.57 (m, 1H, H-2′), 5.93 (d, 1H, *J* = 7.2 Hz, H-1′), 7.28 (t, 1H, H-Ph), 7.37 (t, 2H, H-Ph), 7.60 (m, 4H, H-Ph and NH_2), 8.49 (m, 2H, H-8 and NH). Anal. ($C_{22}H_{24}N_6O_5 \cdot H_2O$), C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-[4-(2-tetrahydro-2*H*-pyranyloxy)-1-butyn-1-yl]-9*H*-purin-9-yl]-β-D-ribofuranuronamide (16).** The reaction of **2** with tetrahydro-2-(2-propynyloxy)-2*H*-pyran for 48 h, followed by chromatography on a silica gel column, eluting with chloroform–benzene–methanol (80:10:10), gave compound **16** (70%, crystallized from ethanol): mp 125–127 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.06 (m, 3H, CH_2CH_3), 1.40–1.87 (m, 6H, CH_2 pyran), 2.71 (t, 2H, CH_2C≡C), 3.31 (m, 2H, NCH_2CH_3), 3.48 (m, 2H, OCH_2CH_2), 3.78 (m, 2H, OCH_2 pyrane), 4.12 (m, 1H, H-3′), 4.33 (s, 1H, H-4′), 4.59 (m, 1H, OCH), 4.65 (m, 1H, H-2′), 5.93 (d, 1H, *J* = 7.6 Hz, H-1′), 7.58 (s, 2H, NH_2), 8.45 (s, 1H, H-8), 8.79 (t, 1H, NH). Anal. ($C_{21}H_{28}N_6O_6 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(3,3-diethoxy-1-propyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (17).** The reaction of **2** with 3,3-diethoxy-1-propyne for 48 h at 40 °C, followed by chromatography on a silica gel column, eluting with chloroform–benzene–methanol (70:18:12), gave compound **17** (56%, crystallized from ethanol): mp 184–187 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.07 (m, 3H, NCH_2CH_3), 1.17 (t, 6H, OCH_2CH_3), 3.25 (m, 2H, NCH_2CH_3), 3.34–3.75 (m, 4H, OCH_2CH_3), 4.15 (m, 1H, H-3′), 4.12 (m, 1H, H-3′), 4.34 (s, 1H, H-4′), 4.61 (m, 1H, H-2′), 5.55 (s, 1H, OC*H*), 5.96 (d, 1H, *J* = 7.6 Hz, H-1′), 7.70 (s, 2H, NH_2), 8.51 (s, 1H, H-8), 8.63 (t, 1H, NH). Anal. ($C_{19}H_{26}N_6O_6 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′deoxy-1′-[6-amino-2-(3-amino-1-propyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (18).** The reaction of **2** with 2-propynylamine for 36 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol–ammonia (90:9.5:0.5), gave compound **18** (52%): mp >220 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.09 (t, 3H, CH_2CH_3), 1.96 (br, s, 2H, CH_2NH_2), 3.30 (m, 2H, CH_2CH_3), 3.50 (s, 1H, CH_2NH_2), 4.16 (m, 1H, H-3′), 4.32 (d, 1H, *J* = 1.5 Hz, H-4′), 4.58 (m, 1H, H-2′), 5.95 (d, 1H, *J* = 7.7 Hz, H-1′), 7.57 (s, 2H, NH_2), 8.46 (s, 1H, H-8), 8.77 (t, 1H, NH). Anal. ($C_{15}H_{19}N_7O_4 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-[3-(*N*,*N*-dimethylamino)-1-propyn-1-yl]-9*H*-purin-9-yl]-β-D-ribofuranuronamide (19).** The reaction of **2** with *N*,*N*-dimethyl-2-propynylamine for 20 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol–benzene (65:25:10), gave compound **19** (61%, crystallized from methanol): mp 227–230 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.06 (t, 3H, CH_2CH_3), 2.23 and 2.24 (s, 3H each, NCH_3), 3.26 (m, 2H, CH_2CH_3), 3.33 (s, 2H, CH_2C≡C), 4.12 (m, 1H, H-3′), 4.29 (d, 1H, *J* = 1.2 Hz, H-4′), 4.56 (m, 1H, H-2′), 5.92 (d, 1H, *J* = 7.5 Hz, H-1′), 7.58 (s, 2H, NH_2), 8.45 (s, 1H, H-8), 8.65 (t, 1H, NH). Anal. ($C_{17}H_{23}N_7O_4 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′deoxy-1′-[6-amino-2-[2-(1-aminocyclohexyl)-ethynyl]-9*H*-purin-9-yl]-β-D-ribofuranuronamide (20).** The reaction of **2** with 1-ethynyl-1-cyclohexylamine for 30 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol–benzene (80:10:10), gave compound **20** (76%, crystallized from methanol–chloroform): mp 211–213 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.03 (t, 3H, CH_2CH_3), 1.19, 1.22, 1.47, 1.63, 1.83 (m, 10H, H-cyclohexyl), 3.24 (m, 2H, CH_2CH_3), 4.12 (m, 1H, H-3′), 4.29 (d, *J* = 1.5 Hz, 1H, H-4′), 4.57 (m, 1H, H-2′), 5.94 (d, 1H, *J* = 7.2 Hz, H-1′), 7.53 (s, 2H, NH_2), 8.43 (t, 1H, NH), 8.49 (s, 1H, H-8). Anal. ($C_{20}H_{27}N_7O_4 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(5-chloro-1-pentyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (21).** The reaction of **2** with 5-chloro-1-pentyne for 20 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (90:10), gave compound **21** (52%, crystallized from methanol): mp 156–158 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.06 (t, 3H, CH_2CH_3), 1.98 (m, 2H, CH_2CH_2C≡C), 2.56 (t, 2H, CH_2C≡C), 3.30 (m, 2H, CH_2CH_3), 3.74 (t, 2H, CH_2Cl), 4.11 (m, 1H, H-3′), 4.28 (d, 1H, *J* = 1.2 Hz, H-4′), 4.54 (m, 1H, H-2′), 5.91 (d, 1H, *J* = 7.8 Hz, H-1′), 7.56 (s, 2H, NH_2), 8.42 (s, 1H, H-8), 8.74 (t, 1H, NH). Anal. ($C_{17}H_{21}ClN_6O_4 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′deoxy-1′-[6-amino-2-(5-cyano-1-pentyn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (22).** The reaction of **2** with 5-hexynenitrile for 48 h, followed by chromatography on a silica gel column, eluting with chloroform–acetonitrile–benzene–methanol (70:10:10:10), gave compound **22** (65%, crystallized from ethanol): mp 131–134 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.10 (t, 3H, CH_2CH_3), 1.87 (m, 2H, CH_2CH_2C≡C), 2.55 (t, 2H, CH_2C≡C), 2.64 (t, 2H, CH_2CN), 3.30 (m, 2H, CH_2CH_3), 4.14 (m, 1H, H-3′), 4.28 (d, 1H, *J* = 1.3 Hz, H-4′), 4.59 (m, 1H, H-2′), 5.95 (d, 1H, *J* = 7.5 Hz, H-1′), 7.60 (s, 2H, NH_2), 8.46 (s, 1H, H-8), 8.77 (t, 1H, NH). Anal. ($C_{18}H_{21}N_7O_4 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(3-methylbut-3-en-1-yn-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (23).** The reaction of **2** with 2-methyl-1-buten-3-yne for 20 h, followed by chromatography on a silica gel column, eluting with chloroform–benzene–methanol (82:10:8), gave compound **23** (65%, crystallized from methanol–ethyl acetate): mp 175–177 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.05 (t, 3H, CH_2CH_3), 1.94 (s, 3H, CH_3CH), 3.28 (m, 2H, CH_2CH_3), 4.12 (m, 1H, H-3′), 4.30 (s, 1H, H-4′), 4.58 (m, 1H, H-2′), 5.48 and 5.51 (s, 1H each, CH_2=), 5.94 (d, 1H, *J* = 7.5 Hz, H-1′), 7.61 (s, 2H, NH_2), 8.46 (s, 1H, H-8), 8.63 (t, 1H, NH). Anal. ($C_{17}H_{20}N_6O_4 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(1-cyclohexenylethynyl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (24).** The reaction of **2** with 1-ethynylcyclohexene for 3 h, followed by chromatography on a silica gel column, eluting with chloroform–methanol (91:9), gave compound **24** (75%): mp 210–213 °C dec; ^{H} NMR (Me_2SO-d_6) δ 1.03 (t, 3H, CH_2C_{H3}), 1.57 (m, 4H, H-cyclohexenyl), 2.12 (m, 4H, H-cyclohexenyl), 3.28 (m, 2H, CH_2CH_3), 4.10 (m, 1H, H-3′), 4.28 (d, *J* = 1.5 Hz, 1H, H-4′), 4.56 (m, 1H, H-2′), 5.92 (d, 1H, *J* = 7.8 Hz, H-1′), 6.27 (m, 1H, H-2 cyclohexenyl), 7.55 (s, 2H, NH_2), 8.42 (s, 1H, H-8), 8.62 (t, 1H, NH). Anal. ($C_{20}H_{24}N_6O_4 \cdot H_2O$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(cyclohexylethynyl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (25).** The reaction of **2**

with cyclohexylacetylene for 6 h, followed by chromatography on a silica gel column, eluting with chloroform–benzene–methanol (82:10:8), gave compound **25** (68%): mp 200–203 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.04 (t, 3H, CH_2CH_3), 1.30, 1.45, 1.67, 1.83 (m, 10H, H-cyclohexyl), 2.59 (m, 1H, H-1 cyclohexyl), 3.28 (m, 2H, CH_2CH_3), 4.11 (m, 1H, H-3′), 4.27 (d, J = 1.5 Hz, 1H, H-4′), 4.55 (m, 1H, H-2′), 5.90 (d, 1H, J = 7.8 Hz, H-1′), 7.53 (s, 2H, NH_2), 8.40 (s, 1H, H-8), 8.68 (t, 1H, NH). Anal. ($C_{20}H_{26}N_6O_4 \cdot H_2O$) C, H, N.

Biological Studies. Receptor Binding Assays. Cerebral membranes were obtained from male Sprague–Dawley rats (Charles River, Calco, Italy) weighing 150–200 g. Adenosine A_1 and A_2 receptor binding assays were performed according to Bruns et al.[15] and Jarvis et al.[14] using [^{3}H]-N^6-cyclohexyladenosine ([^{3}H]-CHA) and [^{3}H]-2-[p-(2-carboxyethyl)phenethyl]aminoadenosine 5′-N-ethylcarboxamide ([^{3}H]CGS 21680), respectively. The IC_{50} values were estimated by probit models.[17] K_i values were calculated from the Cheng-Prusoff equation[18] using 1 nM as the K_d for [^{3}H]CHA and 18.5 nM for [^{3}H]CGS 21680 in A_1 and A_2 binding studies, respectively.

Isolated Tissues. Rats were sacrificed by decapitation, and both heart and thoracic aorta were removed and placed in Krebs Henseleit's solution according to a method described elsewhere.[12] Briefly, spontaneously beating rat atria were used to measure drug interaction with A_1 receptors. The decrease in heart rate evoked by cumulative addition of agonist was measured. Vascular tissue is specifically used to measure the interaction of adenosine analogues with A_2 receptors. Specimens of rat aorta were cleaned of connective tissue, cut into rings, and allowed to equilibrate in an organ bath. Submaximal contractions of vascular rings were obtained by PGF2a (3 mM). The compounds were then added cumulatively, and relaxation was measured isometrically. The relationship between the contractile response (y) and the log dose was modeled with a straight line after arcsin transformation of the dependent variable in order to obtain least-square estimates of EC_{50} values for each preparation.[19] The average dose–response function was computed as a means constant curve (i.e., a curve whose constants are the mean of those estimated from each preparation). The effective dose of each compound was expressed as mean EC_{50} with 95% confidence limits. The analysis was carried out by SAS PROC GLM.[20]

Platelet Aggregation Assay. Platelet aggregation assay was performed according to the Born turbidimetric technique,[21] as previously described.[10] Compounds were dissolved in saline containing 10% of dimethyl sulfoxide (DMSO), which was present in the platelet-rich plasma at a final concentration of 0.3%. The maximal amplitude of aggregation, recorded 5 min after the addition of ADP 5 μM, was used for quantitative evaluation of the aggregation process. Percentage of inhibition was calculated in relation to control values. The potency ratio was calculated versus NECA, the reference adenosine analogue, after logit-log transformation, and fitted by the weighted least-square method.[17] The antiaggregatory activity was evaluated using a concentration of test compound close to the IC_{50} value.

Hydrophobicity Index k'. Retention of nucleosides on a reverse-phase HPLC column is reported to be a useful measure of the relative hydrophobicity.[22] This hydrophobicity index, k', is calculated by the formula $k' = (t - t_0)/t_0$, where t_0 represents the transit time of the solvent and t the retention time of each compound. A column Supelcosil (150 × 4.6 mm) LC-8-DB 3u, eluted with a mixture of CH_3OH and 0.1 M $HCOONH_4$, pH 7.5 (56:44), was used.

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