2-Alkenyl and 2-Alkyl Derivatives of Adenosine and Adenosine-5′-*N*-Ethyluronamide: Different Affinity and Selectivity of *E*- and *Z*-Diastereomers at A_{2A} Adenosine Receptors

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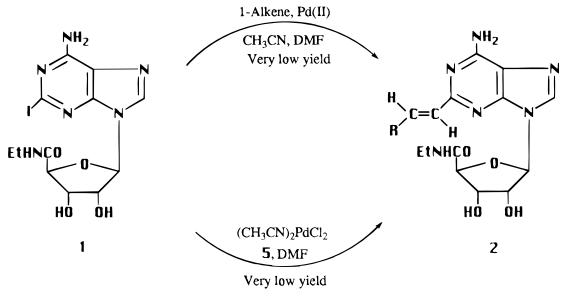
A series of new 2-(ar)alkenyl, both *Z*- and *E*-diastereomers, and 2-alkyl derivatives of adenosine-5′-*N*-ethyluronamide (NECA) and adenosine were synthesized and evaluated for their interaction with the A_1 and A_{2A} adenosine receptors, to better understand the conformational requirements of the receptor area interacting with the substituents in the 2- and 5′-positions. Partial reduction of the triple bond in 2-alkynyl derivatives of NECA led to compounds whose activity at the A_{2A} receptor subtype was related to *Z*−*E*-isomerism, the *E*-diastereomers being more potent and selective than the *Z*-ones. Saturation of the side chain markedly reduced compound affinity at adenosine receptors. Specifically, compounds bearing an (*E*)-alkenyl chain, while maintaining the same affinity at A_{2A} receptors as the corresponding alkynyl derivatives, showed an increase in A_{2A} vs A_1 selectivity. Hence, the new nucleosides (*E*)-2-hexenylNECA (**12a**) and (*E*)-2-(phenylpentenyl)NECA (**12b**) exhibited both high A_{2A} receptor affinity (K_i = 1.6 and 3.5 nM, respectively) and A_{2A} vs A_1 selectivity (157- and 290-fold, respectively). Moreover, **12a** displayed potent antiaggregatory activity, similar to that of the reference compound NECA. Comparison between NECA and adenosine derivatives further demonstrated that the 5′-ethylcarboxamido group is critical for the A_{2A} affinity. These studies indicated that the orientation of the substituent in the 2-position and the nature of the 5′-group in adenosine derivatives are critical to achieve high affinity and selectivity at the A_{2A} adenosine receptor subtype.

Adenosine elicits a number of biological responses through the interaction with at least four cell membrane receptors recently classified as A_1, A_{2A}, A_{2B}, and A_3.[1−3] These receptor subtypes have been cloned and characterized as belonging to the superfamily of receptors with seven transmembrane helices that couple to G proteins.[4] The physiological effects mediated by A_1 and A_{2A} receptors have been extensively investigated. Activation of the A_{2A} receptor subtype is associated with a variety of effects including vasodilation[5] and inhibition of platelet aggregation.[6] These pharmacological properties have indicated that A_{2A} agonists can be useful for the treatment of cardiovascular diseases such as hypertension, ischemic cardiomyopathy, and atherosclerosis.[7] As for the central nervous system, it has been reported that A_{2A} and D_2 dopamine receptors are coexpressed in the same regions in the brain[8] and within the same neuronal subpopulation within the striatum.[9] The interaction between the two receptor populations indicates that selective agonists and antagonists for A_{2A} receptors may have potential for the treatment of diseases associated with defects in D_2 signaling.[10] For example, it has been reported that A_{2A} agonists are effective in animal models of psychosis, whereas A_{2A} antagonists can be useful for treatment of Parkinson's desease.[11]

Over the last few years a considerable effort has been directed toward characterization of the A_1 and A_{2A} receptors and discovery of potent and selective agonists and antagonists.[12] At A_1 receptors the most active analogues are N^6-substituted adenosines,[13] whereas at A_{2A} receptors the most active compounds are C-2-substituted adenosine analogues.[14−16] Considering that adenosine-5′-*N*-ethyluronamide (NECA; Scheme 1) has affinity in the low nanomolar range for both A_{2A} and A_1 receptors[17] and is potent in several pharmacological models, a variety of substitutions at the C-2 have been introduced in the NECA structure. One of these modifications led to 2-[[4-(2-carboxyethyl)phenethyl]amino]-adenosine-5′-*N*-ethyluronamide (CGS 21680), which has become the reference A_{2A} receptor agonist in various pharmacological studies, having an A_{2A} vs A_1 ratio of about 50−140.[18,19]

Scheme 1



More recently, the introduction of the 2-hexynyl group in the NECA structure led to N-ethyl-1′-deoxy-1′-(6-amino-2-hexynyl-9*H*-purin-9-yl)-β-D-ribofuranuronamide (**15a**) (HENECA; Table 1), which has been characterized in many *in vitro* and *in vivo* models, showing interesting pharmacological properties.[20,21,7] HENECA has an A_{2A} selectivity similar to that of CGS 21680 but shows higher inhibitory activity on platelet aggregation,[22] a property of pharmacological interest

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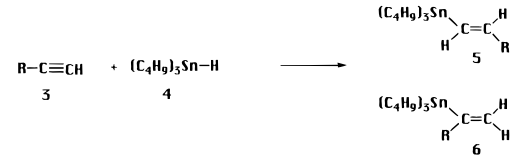
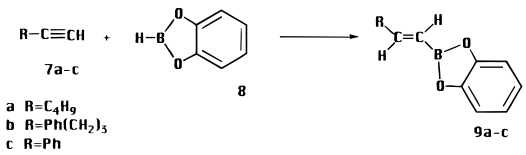
Scheme 2



Scheme 3



considering the potential of these drugs for treatment of cardiovascular diseases.[23,24] These data prompted us to perform the synthesis of a series of compounds bearing aliphatic or aromatic alkynes, in which the carbon−carbon triple bond was attached directly to the C-2 position of the adenine base. The pharmacological results, described in previous reports,[25,26] indicate that the 2-alkynylNECA derivatives are selective A_{2A} agonists possessing an interesting profile ranging from marked antiaggregatory activity to potent vasodilating properties. In an attempt to better understand the conformational requirements of the area of the A_{2A} receptor subtype interacting with the substituents in the 2- and 5′-positions of agonists, a series of 2-(ar)-alkenyl, both *Z* (*cis*)- and *E* (*trans*)-stereomers, and 2-alkyl derivatives of NECA and adenosine (Table 1) were synthesized and tested in the biochemical and functional assays as previously reported.[25]

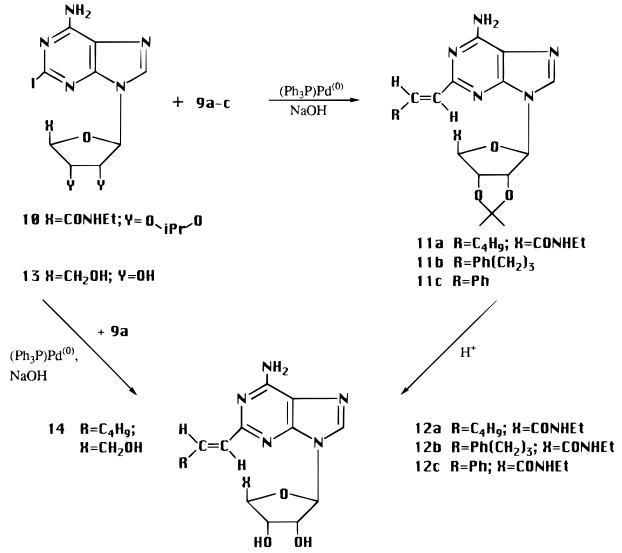
Chemistry

The first approach to the synthesis of 2-alkenyl and 2-aralkenyl derivatives of adenosine-5′-*N*-ethyluronamide was to react terminal alkenes with the 2-iodoNECA (**1**)[20] in the classical cross-coupling reaction conditions, using a mixture of acetonitrile and DMF as solvent and cuprous iodide and palladium(II) as catalysts (Scheme 1). Many different attempts, changing temperature, source of palladium(II), and ratios between the components of the reaction mixture gave the desired *E* derivatives only in very low yield.

Reaction between terminal alkynes **3** and tri-*n*-butyltin hydride (**4**) gave the (*E*)-tributyltin alkenyl derivatives **5**, together with their geminal isomers **6** (Scheme 2). Compounds of general structure **5** were in turn reacted with the same nucleoside 2-iodoNECA (**1**)[20] (Scheme 1) following a reported method.[27] The desired products, characterized mainly by NMR, were in that way obtained but once again with a very low yield.

The most useful synthetic route to obtain the (*E*)-2-alkenylNECA derivatives resulted to be that shown in Scheme 3: Reaction of catecholborane (**8**) with the appropriate terminal alkyne (**7a**−**c**) gave the (*E*)-alkenylcathecholborane derivatives **9a**−**c** in yields ranging between 47 and 75%.[28] These (*E*)-alkenes were in turn coupled to the *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-2′,3′-*O*-isopropylidene-*β*-D-ribofuranuronamide (**10**)[26] (Scheme 4) in dry acetonitrile and DMF with tetrakis(triphenylphosphine)palladium and NaOH as catalysts to provide compounds **11a**−**c**, which were then treated with trifluoroacetic acid at room temperature to give products **12a**−**c** in fair to good overall yields.

Scheme 4



The stability of isomerism of the double bond in the deblocking conditions was checked by NMR analysis; in fact, the double-bond protons bearing (*E*)-alkenes showed a very typical large coupling constant (for example, 15.4 Hz in the case of compound **12a**), whereas the corresponding *Z*-analogues showed a narrower constant (12.1 Hz for compound **16a**). Furthermore, the δ value for the double-bond proton β to the purine ring is shifted about 1 ppm downfield in the *E*-diastereomer, compared to the *Z*-one; in fact these protons in compounds **12a** and **16a** showed a δ value of 6.92 and 5.90, respectively.

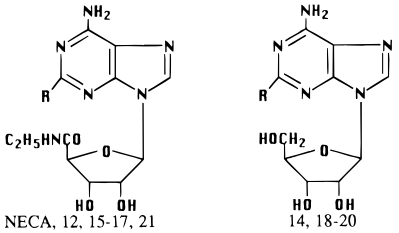
A similar synthetic way was performed for the preparation of the (*E*)-2-(1-hexenyl)adenosine (**14**) (Scheme 4). The unprotected 2-iodoadenosine (**13**)[15,29] and the borocatechol derivative **9a** were coupled in a mixture of 1:1 dry DMF and dry acetonitrile, using tetrakis(triphenylphosphine)palladium(0) and potassium carbonate as catalysts to give compound **14** in 35% yield.

The *Z*-derivatives **16a,b** were obtained starting from the corresponding alkynes **15a,b**[20,26] by partial reduction using the Lindlar catalyst in an atmosphere of hydrogen followed by very careful purification (Scheme 5). The separation of the desired compounds from the reaction mixture was very difficult owing to the fact that the reaction was not complete and, in addition to the desired compounds, contaminating amounts of the structurally similar *E*-diastereomers and saturated derivatives were found. The alkyl analogues **17a,b** (Scheme 5) were prepared by complete reduction of the triple bond using 10% palladium on carbon as catalyst to give the desired compounds in yields higher than 60%.

The same synthetic routes were followed to obtain (*Z*)-2-(1-hexenyl)adenosine (**19**) and 2-*n*-hexyladenosine (**20**) (Scheme 5). Compound **19** was obtained starting from the corresponding alkyne **18**[20] by partial reduction using the Lindlar catalyst in an atmosphere of hydrogen followed by preparative TLC purification. 2-*n*-Hexyladenosine (**20**) was prepared by complete reduction of the triple bond of compound **18**[20] using 10% palladium on carbon as catalyst to give the desired compound.

Receptor Binding Studies. Affinity of the new 2-alkenyl and 2-alkyl derivatives of adenosine and

Table 1. *In Vitro* Pharmacological Activity of 2-Substituted Derivatives of NECA and Adenosine



NECA, 12, 15-17, 21 14, 18-20

		binding assay[a] K_i (nM)			functional activity[b] EC_{50} (nM)		antiaggregation activity[c] potency ratio vs NECA
compd	R	rat brain A_1	rat striatum A_{2A}	selectivity A_1/A_{2A}	rat atria A_1	rat aorta A_{2A}	rabbit platelet A_{2A}
NECA	H	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
15a (HENECA)	C≡C$(CH_2)_3CH_3$	130 (116−145)	2.2 (1.9−2.6)	59	>1 μM	596 (244−1460)	3.00
12a	(*E*)-CH=CH$(CH_2)_3CH_3$	251 (137−460)	1.6 (1.1−2.5)	157	>1 μM	594 (188−1870)	1.20
16a	(*Z*)-CH=CH$(CH_2)_3CH_3$	951 (885−1022)	70 (51−96)	14	>1 μM	676 (211−2170)	0.05
17a	$(CH_2)_5CH_3$	>1 μM	136 (124−150)	>7	>1 μM	>1 μM	0.01
18	C≡C$(CH_2)_3CH_3$	98 (81−119)	2.2 (1.2−3.1)	49	>1 μM	79.2 (36.9−170)	0.58
14	(*E*)-CH=CH$(CH_2)_3CH_3$	332 (206−534)	14 (8.1−23)	24	nd	nd	0.15
19	(*Z*)-CH=CH$(CH_2)_3CH_3$	618 (578−660)	757 (603−952)	0.8	>1 μM	nd	<0.01
20	$(CH_2)_5CH_3$	>1 μM	≥1 μM		>1 μM	nd	<0.01
15b	C≡C$(CH_2)_3C_6H_5$	209 (194−226)	1.2 (1.1−1.4)	174	>1 μM	497 (265−931)	0.35
12b	(*E*)-CH=CH$(CH_2)_3C_6H_5$	1017 (804−1286)	3.5 (2.2−5.6)	291	>1 μM	576 (227−1460)	0.14
16b	(*Z*)-CH=CH$(CH_2)_3C_6H_5$	>1 μM	51 (32−80)	>20	>1 μM	>1 μM	0.01
21	C≡CC_6H_5	698 (611−798)	120 (112−128)	5.8	>1 μM	>1 μM	0.01
12c	(*E*)-CH=CHC_6H_5	117 (97−141)	234 (111−490)	0.5	420 (317−557)	194 (73.6−512)	nd

[a] Receptor binding affinity at A_1 and A_{2A} receptors was determined using [^{3}H]CHA and [^{3}H]CGS 21680 as radioligands, respectively. Data are geometrical means from at least three separate experiments; 95% confidence limits are in parentheses. [b] Data are means from at least three separate experiments; 95% confidence limits are in parentheses. [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.

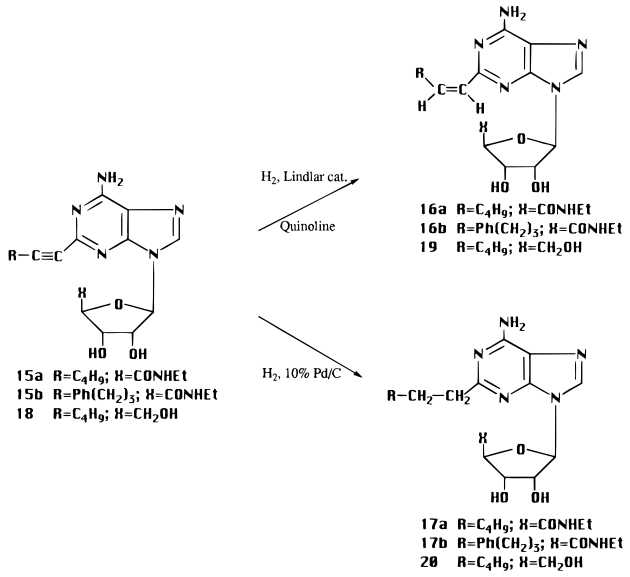
NECA at A_1 and A_{2A} adenosine receptors was evaluated in binding studies in rat brain membranes, using [^{3}H]-CHA[30] and [^{3}H]CGS 21680[18b] as radioligands, respectively. NECA and HENECA[25] were tested as reference compounds. The results are reported in Table 1.

In comparison with HENECA, (*E*)-2-hexenylNECA (**12a**) showed similar A_{2A} receptor affinity (K_i = 1.6 nM) but a 3-fold higher selectivity for this receptor subtype. Conversely, affinity and selectivity of the *Z*-derivative **16a** for the same receptor subtype was markedly reduced (K_i A_{2A} = 70 nM), and saturation of the side chain induced a further decrease in affinity (**17a**, K_i A_{2A} = 140 nM).

The adenosine derivatives followed the same pattern. In fact the *E*-diastereoisomer resulted to be more potent than the *Z*-one at A_{2A} receptors (**14**, K_i A_{2A} = 14 nM; **19**, K_i A_{2A} = 760 nM, respectively). However, the decrease in affinity of both alkenyl derivatives of adenosine **14** and **19** as compared with 2-hexynyladenosine (**18**) was more marked than that of the corresponding NECA derivatives **12a** and **16a** vs 2-hexynyl-NECA (**15a**). Thus, the presence of the 5′-*N*-ethylcarboxamido group become more critical for A_{2A} receptor affinity when the triple bond was reduced.

The 3-fold increase in selectivity, obtained by substitution of the hexynyl with a (*E*)-hexenyl chain in the HENECA structure, prompted us to synthesize the (*E*)- and (*Z*)-alkenyl derivatives of 2-(phenylpentenyl)NECA (**15b**), which has been reported to have A_{2A} vs A_1 selectivity of about 170-fold.[26] The (*E*)-2-(phenylpen-

Scheme 5



tenyl)NECA (**12b**) retained a good affinity at A_{2A} receptors (K_i = 3.5 nM) and showed a 5-fold decrease in A_1 binding affinity (K_i = 1017 nM). Thus, compound **12b** resulted as the most selective A_{2A} agonist of the series, displaying 290-fold A_{2A} vs A_1 selectivity. Consistent with previous results, the *Z*-isomer **16b** showed an affinity at the A_{2A} receptor 15- and 40-fold lower than that of **12b** and **15b**, respectively.

In our previous studies we demonstrated that the introduction of an unsubstituted phenyl ring conjugated to the triple bond produces a decrease in the affinity at both A_1 and A_{2A} receptors as compared to 2-hexynyl-NECA.[26] In the present study, we found that the substitution of the phenylethynyl group of compound **21** (Table 1) with a (*E*)-styryl substituent led to a compound with moderate A_1 selectivity (**12c**, K_i A_1 = 117 nM, K_i A_{2A} = 234 nM).

Functional Studies on Isolated Preparations. According to methods described elsewhere,[19,25] the A_1-mediated negative chronotropic activity was tested in spontaneously beating rat atria, whereas functional activity at A_{2A} receptors was assessed in rat aorta and rabbit platelets by measuring vasorelaxation and antiaggregatory activity. Results are summarized in Table 1.

In general, all the new alkenyl and alkyl derivatives of NECA showed a similar vasorelaxant activity as compared with that of the corresponding alkynyl derivatives and NECA itself, with slight effect on heart rate. However, compound **12c**, which has an aromatic ring conjugated with the *E*-double bond, appeared to have a higher vasodilating activity and more marked negative chronotropic effect on heart rate than the alkynyl derivative 2-(phenylethynyl)NECA (**21**). In addition, **12c** showed a vasorelaxant activity similar to that of HENECA (EC_{50} = 194 and 596 nM, respectively), although its A_{2A} binding affinity was 100-fold lower than that of HENECA itself.

The antiaggregatory effect of the new alkenyl and alkyl derivatives of adenosine and NECA on rabbit platelet aggregation induced by ADP is reported in Table 1 as potency ratio vs NECA. All the new derivatives showed an antiaggregatory activity weaker than that of the corresponding alkynyl nucleosides. All of them are also less potent than NECA itself, except for (*E*)-2-hexenylNECA (**12a**), which showed a comparable antiaggregatory activity with a potency ratio vs NECA of 1.2. In agreement with previous results, the adenosine derivatives **18**, **14**, **19**, and **20** were shown to be less potent than their corresponding NECA derivatives **15a**, **12a**, **16a**, and **17a**. In general, the (*E*)-alkenyl diastereomers are more potent than the *Z*-ones and the corresponding saturated derivatives.

Retention of nucleosides on a reverse-phase HPLC column has been measured according to a method reported in the literature.[25,31] As expected, the *E*-diastereomers were found to be more lipophilic than the corresponding *Z*-diastereomers and alkynyl derivatives. Although the hydrophobicity index of the new nucleosides barely correlated with the data obtained in both binding and functional studies, it is worthwhile to note that compounds **12b** and **15b**, which showed the highest A_1/A_{2A} selectivity, are the most liphophilic molecules in the series.

Conclusions

The major finding of this study is that partial reduction of the triple bond in 2-alkynyl derivatives of NECA and adenosine leads to compounds whose activity is related to *Z*−*E*-isomerism, the *E*-diastereomers being more active and selective than the *Z*-ones. Moreover, saturation of the side chain markedly reduces adenosine receptor affinity.

Specifically, compounds bearing an (*E*)-alkenyl chain, while maintaining the same affinity at A_{2A} receptors as the corresponding alkynyl derivatives, showed a reduced affinity for the A_1 receptor subtype. Hence, the new nucleosides **12a**,**b** exhibited both high affinity and selectivity at the A_{2A} receptor subtype. However, in the case of compound **21**, bearing an unsubstituted phenyl ring conjugated to the triple bond, substitution of this phenylethynyl group with a (*E*)-styryl one (**12c**) produced at the A_1 receptor an increase in both affinity and selectivity. Partial or total reduction of the alkynyl side chain of NECA and adenosine derivatives appears to be detrimental for the antiaggregatory activity.

These studies indicate that A_{2A} adenosine receptors strongly differentiate between geometric isomers in term of affinity and selectivity. Specifically, the orientation of the substituent in the 2-position and the nature of the 5′-group in adenosine derivatives are critical for both affinity and selectivity at A_{2A} receptors. Hence, the presence of an (*E*)-alkenyl chain and a 5′-*N*-ethylcarboxamido group results in highly selective A_{2A} adenosine receptor agonists (compounds **12a**,**b**).

Experimental Section

Chemistry. Melting points were determined with a Buchi apparatus and are uncorrected. ^{1}H NMR spectra were obtained with a Varian VX 200 MHz spectrometer. Analytical TLC was carried out on precoated TLC plates with silica gel 60 F-254 (Merck; 200 μm thickness). The same plates were also used for prepatative TLC (200 × 200 mm, 1000 μm thickness). For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are within ±0.4% of theoretical values.

Preparation of (*E*)-1-Alkenyl-1-catecholborane Derivatives 9a−c. A mixture of 1 g of catecholborane (8.34 mmol) and 8.34 mmol of the appropriate alkyne was heated at 70 °C for 3 h. The resulting oil was chromatographed on a flash silica gel column using the suitable solvent mixture to give the desired (*E*)-alkenylcatecholborane derivatives in good yield.

9a: elution mixture, cyclohexane−ethyl acetate, 96−4 → 90−10 (gradient); colorless thick oil; yield 75%; ^{1}H NMR (Me_2-SO-d_6) δ 0.93 (t, 3H, J = 6.9 Hz, $(CH_2)_3CH_3$), 1.25−1.58 (m, 4H, $CH_2CH_2CH_3$), 2.30 (m, 2H, $CHCH_2$), 5.79 (d, 1H, J = 18.0 Hz, B*CH*=CH), 7.05 (m, 3H, 2H-Ph, BCH=*CH*), 7.21 (m, 2H, Ph). Anal. ($C_{12}H_{15}BO_2$) C, H.

9b: elution mixture, cyclohexane−ethyl acetate, 98−2 → 90−10 (gradient); colorless thick oil; yield 68%; ^{1}H NMR (Me_2-SO-d_6) δ 1.88 (m, 2H, $CH_2CH_2CH_2$), 2.34 (m, 2H, $CHCH_2$), 2.69 (m, 2H, CH_2Ph), 5.83 (d, 1H, J = 18.0 Hz, B*CH*=CH), 6.80−7.30 (m, 10H, 9H-Ar, BCH=*CH*). Anal. ($C_{17}H_{17}BO_2$) C, H.

9c: elution mixture, petroleum ether−methanol, 97−3; white solid, mp 107−110 °C dec; yield 47.5%; ^{1}H NMR (Me_2-SO-d_6) δ 6.36 (d, 1H, J = 18.0 Hz, B*CH*=CH), 6.80−7.30 (m, 10H, 9H-Ar, BCH=*CH*). Anal. ($C_{14}H_{11}BO_2$) C, H.

(*E*)-*N*-Ethyl-1′-deoxy-1′-[6-amino-2-(1-hexen-1-yl)-9*H*-purin-9-yl]-2′,3′-*O*-isopropylidene-β-D-ribofuranuronamide (11a). To a solution of 237 mg (0.5 mmol) of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-2′,3′-isopropylidene-β-D-ribofuranuronamide[26] (**10**) in 20 mL of acetonitrile was added 30 mg of tetrakis(triphenylphosphine)palladium(0), and the mixture was stirred at room temperature for 15 min; then

0.5 mL of NaOH solution (6 N) and 1.5 mmol of (*E*)-1-(borocatechol)-1-hexene (**7a**) were added, and the suspension was refluxed for 16 h. The mixture was then added to 1.0 mmol of **7a** and further refluxed for 8 h.

The mixture was purified on a silica gel flash chromatography column eluting with chloroform−acetonitrile−methanol−ammonia solution (32% in water), 98.5−0.5−0.5−0.5, to give compound **11a** (157 mg, 73%) as a chromatographically pure thick oil: ^{1}H NMR (Me_2SO-d_6) δ 0.56 (t, 3H, J = 7.1 Hz, NCH_2CH_3), 0.92 (t, 3H, J = 6.8 Hz, $CH_2CH_2CH_3$), 1.36 and 1.55 (s, 3H each, $C(CH_3)_2$), 1.41 (m, 2H, $CH_2CH_2CH_2$), 2.24 (m, 2H, $CHCH_2$), 2.78 (m, 2H, $NHCH_2$), 4.54 (d, 1H, J = 1.9 Hz, H-4′), 5.38 (d, 1H, J = 6.0 Hz, H-3′), 5.53 (dd, 1H, J = 2.0, 6.2 Hz, H-2′), 6.24 (d, 1H, J = 15.5 Hz, Ar*CH*=CH), 6.36 (s, 1H, H-1′), 6.89 (m, 1H, ArCH=*CH*), 7.14 (br s, 2H, NH_2), 7.31 (t, 1H, NH), 8.20 (s, 1H, H-8). Anal. ($C_{21}H_{30}N_6O_4$) C, H, N.

(*E*)-*N*-Ethyl-1′-deoxy-1′-[6-amino-2-(5-phenyl-1-penten-1-yl)-9*H*-purin-9-yl]-2′,3′-*O*-isopropylidene-β-D-ribofuranuronamide (11b). To a solution of 237 mg (0.5 mmol) of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-2′,3′-isopropylidene-β-D-ribofuranuronamide[26] (**10**) in 20 mL of acetonitrile were added 75 mg each of triphenylphosphine and palladium(II) chloride, and the mixture was stirred at room temperature for 15 min; then 0.5 mL of NaOH solution (6 N) and 1.5 mmol of (*E*)-1-(borocatechol)-1-(5-phenyl)pentene (**7b**) were added, and the suspension was refluxed for 16 h. The mixture was then added to 1.0 mmol more of **7b** and further refluxed for 8 h.

The mixture was evaporated and then purified on a silica gel flash chromatography column eluting with chloroform−cyclohexane−acetonitrile−methanol, 86−10−2−2, to give compound **11b** (157 mg, 63%) as a chromatographically pure thick oil: ^{1}H NMR (Me_2SO-d_6) δ 0.52 (t, 3H, J = 7.2 Hz, NCH_2CH_3), 1.35 and 1.54 (s, 3H each, $C(CH_3)_2$), 1.77 (m, 2H, $CH_2CH_2CH_2$), 2.70 (m, 4H, $CHCH_2$, $NHCH_2$), 4.54 (d, 1H, J = 2.1 Hz, H-4′), 5.37 (d, 1H, J = 6.6 Hz, H-3′), 5.57 (dd, 1H, J = 2.2, 4.0 Hz, H-2′), 6.26 (d, 1H, J = 15.5 Hz, Ar*CH*=CH), 6.38 (s, 1H, H-1′), 6.66 (m, 2H, Ph), 6.90 (m, 1H, ArCH=*CH*), 7.27 (m, 5H, 3H, Ph, NH_2), 8.21 (s, 1H, H-8), 8.85 (br s, 1H, NH). Anal. ($C_{26}H_{32}N_6O_4$) C, H, N.

(*E*)-*N*-Ethyl-1′-deoxy-1′-[6-amino-2-(2-phenyl-1-ethen-1-yl)-9*H*-purin-9-yl]-2′,3′-*O*-isopropylidene-β-D-ribofuranuronamide (11c). To a solution of 237 mg (0.5 mmol) of *N*-ethyl-1′-deoxy-1′-(6-amino-2-iodo-9*H*-purin-9-yl)-2′,3′-isopropylidene-β-D-ribofuranuronamide[26] (**10**) in 20 mL of acetonitrile was added 5 mg of tetrakis(triphenylphosphine)palladium(0), and the mixture was stirred at room temperature for 15 min; then 0.5 mL of NaOH solution (6 N) and 2 mmol of (*E*)-1-(borocatechol)-2-phenyl-1-ethene (**7c**) were added, and the suspension was refluxed for 2.5 h. The mixture was evaporated and then purified on a silica gel chromatography column eluting with chloroform−cyclohexane−methanol, 70−25−5, to give compound **11c** (196 mg, 87.2%) as a chromatographically pure thick oil: ^{1}H NMR (Me_2SO-d_6) δ 0.53 (t, 3H, J = 7.1 Hz, NCH_2CH_3), 1.38 and 1.57 (s, 3H each, $C(CH_3)_2$), 2.75 (m, 2H, $NHCH_2$), 4.59 (d, 1H, J = 2.0 Hz, H-4′), 5.42 (d, 1H, J = 5.9 Hz, H-3′), 5.65 (dd, 1H, J = 2.2, 6.4 Hz, H-2′), 6.43 (s, 1H, H-1′), 7.01 (d, 1H, J = 16.2 Hz, Ar*CH*=CH), 7.15−7.50 (m, 7H, Ph, NH_2), 7.72 (m, 2H, ArCH=*CH*, NH), 8.27 (s, 1H, H-8). Anal. ($C_{23}H_{26}N_6O_4$) C, H, N.

General Procedure for the Synthesis of (*E*)-2-(Ar)-alkenyladenosine-5′-*N*-ethyluronamides 12a−c. Isopropylidene derivative (0.4 mmol) was dissolved in 10 mL of trifluoroacetic acid, and the solution was stirred at room temperature for 6 h. The mixture was then evaporated and coevaporated three times with 10 mL of distilled water and twice with 10 mL of absolute ethanol; the residue was purified on a thin layer chromatography plate eluting with the suitable mixture of solvents, to give compounds **12a**−**c**, which were then crystallized from acetonitrile.

(*E*)-*N*-Ethyl-1′-deoxy-1′-[6-amino-2-(1-hexen-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (12a). (a) General Method: elution solvent, chloroform−*n*-hexane−methanol, 90−5−5; 0.156 mmol, yield 39%; mp 180−183 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.92 (t, 3H, J = 7.2 Hz, $CH_2CH_2CH_3$), 1.03 (t, 3H, J = 7.0 Hz, NCH_2CH_3), 1.42 (m, 4H, $CH_2CH_2CH_3$), 2.25 (m, 2H, $CHCH_2$), 3.24 (m, 2H, $NHCH_2$), 4.21 (m, 1H, H-3′), 4.31 (s, 1H, H-4′), 4.73 (m, 1H, H-2′), 5.57 (d, 1H, J = 6.2 Hz, OH), 5.71 (d, 1H, J = 4.4 Hz, OH), 5.98 (d, 1H, J = 7.3 Hz, H-1′), 6.29 (d, 1H, J = 15.4 Hz, Ar*CH*=CH), 6.92 (m, 1H, ArCH=*CH*), 7.27 (br s, 2H, NH_2), 8.31 (t, 1H, NH), 8.39 (s, 1H, H-8). Anal. ($C_{18}H_{26}N_6O_4$) C, H, N.

(b) From 1-hexene: A mixture of 2-iodoNECA (**1**)[20] (45 mg, 0.1 mmol), 1-hexene (67 mg, 0.8 mmol), palladium(II) acetate (2.5 mg), tri-*o*-tolylphosphine (7 mg), dry piridine (1 mL), and dry DMF (2 mL) was heated at 80 °C for 15 h; the solvents were then removed, and the residue was purified by TLC, eluting with chloroform−acetonitrile−methanol−aqueous ammonia, 69.5−20−10−0.5, to obtain 4 mg (0.09 mmol, 9%) of the title compound. All the analytical data were identical with the same product obtained following the general method.

(c) From 1-(tributylstannyl)-1-hexene: To a solution of 110 mg (0.25 mmol) of 2-iodoNECA (**1**)[20] in 5 mL of dry DMF were added 6 mg (0.002 mmol) of bis(acetonitrile)palladium(II) chloride and 132 mg (0.35 mmol) of (*E*)-1-(tributylstannyl)-1-hexene[32] (**5**), and the mixture was stirred under reflux for 20 h. The solution was then evaporated and purified on a thin layer chromatography plate eluting with chloroform−methanol−benzene−aqueous ammonia, 79.5−10−10−0.5, to give 10 mg (0.026 mmol, 10.4%) of the title compound. All the analytical data were identical with the same product obtained following the general method.

(*E*)-*N*-Ethyl-1′-deoxy-1′-[6-amino-2-(5-phenyl-1-penten-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (12b): elution solvent, chloroform−*n*-hexane−acetonitrile, 90−5−5; 0.272 mmol, yield 68%; mp 116−119 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.02 (t, 3H, J = 7.2 Hz, NCH_2CH_3), 1.79 (m, 2H, $CH_2CH_2CH_2$), 2.28 (m, 2H, $CHCH_2$), 2.65 (t, 2H, J = 7.4 Hz, $PhCH_2$), 3.23 (m, 2H, $NHCH_2$), 4.22 (m, 1H, H-3′), 4.31 (d, 1H, J = 1.7 Hz, H-4′), 4.74 (m, 1H, H-2′), 5.60 (d, 1H, J = 6.0 Hz, OH), 5.74 (d, 1H, J = 4.8 Hz, OH), 5.97 (d, 1H, J = 7.5 Hz, H-1′), 6.32 (d, 1H, J = 15.6 Hz, Ar*CH*=CH), 6.95 (m, 1H, ArCH=*CH*), 7.28 (m, 7H, Ph, NH_2), 8.32 (t, 1H, NH), 8.40 (s, 1H, H-8). Anal. ($C_{23}H_{28}N_6O_4$) C, H, N.

(*E*)-*N*-Ethyl-1′-deoxy-1′-[6-amino-2-(5-phenyl-1-ethen-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (12c): elution solvent, chloroform−*n*-hexane−methanol, 75−13−12; 0.252 mmol, yield 63%; mp 150−153 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.03 (t, 3H, J = 7.3 Hz, NCH_2CH_3), 3.20 (m, 2H, $NHCH_2$), 4.27 (m, 1H, H-3′), 4.34 (d, 1H, J = 1.7 Hz, H-4′), 4.77 (dd, 1H, J = 4.7, 6.8 Hz, H-2′), 5.90 (m, 2H, 2OH), 6.04 (d, 1H, J = 7.3 Hz, H-1′), 7.07 (d, 1H, J = 15.9 Hz, $PhCH$=CH), 7.20−7.68 (m, 7H, Ph, NH_2) 7.77 (d, 1H, J = 15.9 Hz, PhCH=*CH*), 8.31 (t, 1H, NH), 8.46 (s, 1H, H-8). Anal. ($C_{20}H_{22}N_6O_4$) C, H, N.

(*E*)-2-(1-Hexen-1-yl)adenosine (14). To a solution of 322 mg (0.82 mmol) of 2-iodoadenosine[15] (**13**) in 30 mL of a mixture of 1:1 acetonitrile:DMF was added 50 mg of tetrakis(triphenylphosphine)palladium(0), and the mixture was stirred at room temperature for 15 min; then 500 mg each of K_2CO_3 and (*E*)-1-(borocatechol)-1-hexene (**9a**) (2.46 mmol) were added, and the suspension was refluxed for 4 h. The mixture was filtered, evaporated, and then purified on a silica gel flash chromatography column eluting with dichloromethane−methanol, 95−5, to give 101 mg (0.29 mmol, 35%) of **14** as a chromatographically pure solid. A sample was crystallized from acetonitrile: mp 119−121 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 0.92 (t, 3H, J = 6.7 Hz, CH_2CH_3), 1.43 (m, 4H, $CH_2CH_2CH_3$), 2.24 (m, 2H, =$CHCH_2$), 3.61 (m, 2H, CH_2-5′), 3.98 (d, 1H, J = 2.5 Hz, H-4′), 4.16 (m, 1H, H-3′), 4.66 (m, 1H, H-2′), 5.20 (d, 1H, J = 4.0 Hz, OH), 5.44 (d, 1H, J = 6.1 Hz, OH), 5.52 (m, 1H, OH), 5.87 (d, 1H, J = 6.6 Hz, H-1′), 6.28 (d, 1H, J = 15.4 Hz, Ar*CH*=CH), 6.92 (m, 1H, ArCH=*CH*), 7.25 (br s, 2H, NH_2), 8.29 (s, 1H, H-8). Anal. ($C_{20}H_{22}N_5O_4$) C, H, N.

(*Z*)-*N*-Ethyl-1′-deoxy-1′-[6-amino-2-(1-hexen-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (16a). A mixture of 60 mg (0.154 mmol) of **15a**,[20] 15 mL of acetone, 100 mg of palladium on calcium carbonate (Lindlar catalyst), and 0.5 mL of quinoline was hydrogenated on a Parr apparatus at 18 psi for 9 h. The mixture was then filtered, evaporated, and purified on a thin layer chromatography plate eluting with chloroform−acetonitrile−methanol−aqueous ammonia, 69.5−

20−10−0.5, to give 45 mg (0.115 mmol, 75%) of the title compound: mp 164−167 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.90 (t, 3H, J = 6.3 Hz, NCH_2CH_3), 1.02 (t, 3H, J = 7.4 Hz, $CH_2CH_2CH_3$), 1.38 (m, 4H, $CH_2CH_2CH_3$), 2.82 (m, 2H, =CHCH_2), 3.23 (m, 2H, NHCH_2), 4.19 (m, 1H, H-3′), 4.32 (s, 1H, H-4′), 4.79 (m, 1H, H-2′), 5.65 (br m, 2H, 2OH), 5.90 (m, 1H, ArCH=CH), 5.98 (d, 1H, J = 7.1 Hz, H-1′), 6.27 (d, 1H, J = 12.1 Hz, ArCH=CH), 7.24 (s, 2H, NH_2), 8.23 (t, 1H, NH), 8.43 (s, 1H, H-8). Anal. ($C_{18}H_{26}N_6O_4$) C, H, N.

(*Z*)-*N*-Ethyl-1′-deoxy-1′-[6-amino-2-(5-phenyl-1-penten-1-yl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (16b). A mixture of 100 mg (0.222 mmol) of **15b**,[26] 20 mL of acetone, 160 mg of palladium on calcium carbonate (Lindlar catalyst), and 0.6 mL of quinoline was hydrogenated on a Parr apparatus at 18 psi for 5 h. The mixture was then filtered, evaporated, and purified on a thin layer chromatography plate eluting with chloroform−acetonitrile−methanol−aqueous ammonia, 74.5−17−8−0.5, to give 60 mg (0.133 mmol, 60%) of the title compound as a thick oil: ^{1}H NMR (Me_2SO-d_6) δ 1.02 (t, 3H, J = 6.4 Hz, NCH_2CH_3), 1.79 (m, 2H, $CH_2CH_2CH_2$), 2.64 (m, 2H, $CHCH_2$), 2.83 (t, 2H, J = 7.2 Hz, PhCH_2), 3.16 (m, 2H, NHCH_2), 4.22 (m, 1H, H-3′), 4.33 (s, 1H, H-4′), 4.78 (m, 1H, H-2′), 5.68 (br m, 2H, OH), 5.99 (m, 2H, H-1′, ArCH=CH), 6.29 (d, 1H, J = 11.4 Hz, ArCH=CH), 7.24 (m, 7H, Ph, NH_2), 8.27 (t, 1H, NH), 8.46 (s, 1H, H-8). Anal. ($C_{23}H_{28}N_6O_4$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(1-hexyl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (17a).** A mixture of 50 mg (0.129 mmol) of **15a**,[20] 30 mL of absolute ethanol, and 30 mg of palladium on carbon (10%) was hydrogenated on a Parr apparatus at 40 psi for 4 h. The mixture was filtered, evaporated, and purified on a thin layer chromatography plate eluting with chloroform−acetonitrile−methanol−aqueous ammonia, 69.5−20−10−0.5, to give 31 mg (0.079 mmol, 61%) of the title compound: mp 140−143 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.87 (t, 3H, J = 6.2 Hz, $NHCH_2CH_3$), 1.06 (t, 3H, J = 7.1 Hz, $CH_2CH_2CH_3$), 1.31 (m, 6H, $CH_2CH_2CH_2CH_3$), 1.74 (m, 2H, $ArCH_2CH_2$), 2.65 (t, 2H, J = 7.9 Hz, ArCH_2CH_2), 3.24 (m, 2H, NHCH_2), 4.17 (dd, 1H, J = 1.5, 4.6 Hz, H-3′), 4.31 (d, 1H, J = 1.7 Hz, H-4′), 4.64 (dd, 1H, J = 4.8, 7.4 Hz, H-2′), 5.69 (br m, 2H, 2OH), 5.96 (d, 1H, J = 7.5 Hz, H-1′), 7.25 (s, 2H, NH_2), 8.35 (s, 1H, H-8), 8.48 (t, 1H, NH). Anal. ($C_{18}H_{28}N_6O_4$) C, H, N.

***N*-Ethyl-1′-deoxy-1′-[6-amino-2-(5-phenyl-1-pentyl)-9*H*-purin-9-yl]-β-D-ribofuranuronamide (17b).** A mixture of 48 mg (0.106 mmol) of **15b**,[26] 20 mL of absolute ethanol, and 20 mg of palladium on carbon (10%) was hydrogenated on a Parr apparatus at 40 psi for 6 h. The mixture was filtered, evaporated, and purified on a thin layer chromatography plate eluting with chloroform−acetonitrile−methanol−aqueous ammonia, 69.5−20−10−0.5, to give 30 mg (0.066 mmol, 62%) of the title compound: mp 108−110 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.03 (t, 3H, J = 6.8 Hz, $NHCH_2CH_3$), 1.23−1.85 (br m, 6H, $CH_2CH_2CH_2CH_3$), 2.60 (m, 4H, ArCH_2, PhCH_2), 3.24 (m, 2H, NHCH_2), 4.18 (m, 1H, H-3′), 4.31 (d, 1H, J = 1.6 Hz, H-4′), 4.65 (m, 1H, H-2′), 5.55 (d, 1H, J = 6.4 Hz, OH), 5.72 (d, 1H, J = 4.2 Hz, OH), 5.96 (d, 1H, J = 7.0 Hz, H-1′), 7.21 (br m, 7H, Ph, NH_2), 8.34 (s, 1H, H-8), 8.45 (t, 1H, NH). Anal. ($C_{23}H_{30}N_6O_4$) C, H, N.

(*Z*)-2-(1-Hexenyl)adenosine (19). A mixture of 80 mg (0.230 mmol) of 2-(1-hexyn-1-yl)adenosine **(18)**,[20] 10 mL of acetone, 100 mg of palladium on calcium carbonate (Lindlar catalyst), and 0.5 mL of quinoline was hydrogenated on a Parr apparatus at 20 psi for 3 days. The mixture was filtered, evaporated, and purified on a thin layer chromatography plate eluting with chloroform−acetonitrile−methanol, 70−20−10, to give 32 mg (0.092 mmol, 40%) of the title compound: mp 106−109 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.90 (t, 3H, J = 6.3 Hz, CH_2CH_3), 1.39 (m, 4H, $CH_2CH_2CH_3$), 2.86 (m, 2H, $CHCH_2CH_2$), 3.61 (m, 2H, CH_2-5′), 3.96 (d, 1H, J = 2.9 Hz, H-4′), 4.15 (m, 1H, H-3′), 4.66 (m, 1H, H-2′), 5.20−5.50 (br m, 3H, 3OH), 5.87 (m, 2H, H-1′, =$CH$$CH_2$), 6.23 (d, 1H, J = 11.9 Hz, ArCH=), 7.22 (m, 2H, NH_2), 8.34 (s, 1H, H-8). Anal. ($C_{20}H_{22}N_5O_4$) C, H, N.

2-(1-Hexyl)adenosine (20). A mixture of 60 mg (0.173 mmol) of 2-(1-hexyn-1-yl)adenosine **(18)**,[20] 20 mL of absolute ethanol, and 20 mg of palladium on carbon (10%) was hydrogenated on a Parr apparatus at 45 psi for 18 h. The mixture was then filtered, evaporated, and purified on a thin layer chromatography plate eluting with chloroform−acetonitrile−methanol−aqueous ammonia, 69.5−20−10−0.5, to give 36 mg (0.102 mmol, 58.2%) of the title compound: mp 108−110 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.87 (t, 3H, J = 6.6 Hz, CH_2CH_3), 1.30 (m, 6H, $CH_2CH_2CH_2CH_3$), 1.71 (m, 2H, $ArCH_2CH_2$), 2.64 (t, 2H, J = 7.0 Hz, ArCH_2CH_2), 3.61 (m, 2H, CH_2-5′), 3.99 (d, 1H, J = 2.2 Hz, H-4′), 4.15 (dd, 1H, J = 2.0, 4.8 Hz, H-3′), 4.67 (dd, 1H, J = 5.1, 6.8 Hz, H-2′), 5.42 (br m, 2H, 2OH), 5.72 (m, 1H, OH), 5.85 (d, 1H, J = 6.6 Hz, H-1′), 7.26 (s, 2H, NH_2), 8.25 (s, 1H, H-8). Anal. ($C_{16}H_{25}N_5O_4$) C, H, N.

Biological Studies. Receptor Binding Assay. Cerebral membranes were obtained from male Sprague−Dawley rats (Charles River, Calco, Italy) weighing 150−200 g. Tissue preparation was carried out according to Jarvis et al.[18b] Adenosine A_1 and A_{2A} receptor binding assays were performed according to Bruns et al.[30] and Jarvis et al.[18b] using [^{3}H]-N^6-cyclohexyladenosine ([^{3}H]CHA) and [^{3}H]-2-[[*p*-(2-carboxyethyl)-phenethyl]amino]-5′-(*N*-ethylcarbamoyl)adenosine ([^{3}H]CGS 21680). The IC_{50} values were estimated by probit models.[33] K_i values were calculated from the Cheng−Prusoff equation[34] 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_{2A} binding studies, respectively.

Isolated Tissue Preparations. 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.[6] Briefly, isolated spontaneously beating rat atria were used to measure drug interaction with A_1 receptors. The decrease in beating rate evoked by cumulative addition of agonist was measured. Vascular tissue is specific to measure the interaction of adenosine analogues with A_{2A} receptors. Specimens of vessel were cleaned of connective tissue, cut into rings, and allowed to equilibrate in an organ bath. Submaximal contractions of vascular rings were obtained by PGF2α (3 μM). The compounds were then added cumulatively and the evoked relaxation was measured isometrically. The relationship between the contractile response (y) and the log dose was modeled by a straight line after arcsin transformation of the dependent variable in order to obtain least-squares estimates of EC_{50} values for each preparation.[35] The average dose−response function was computed as a mean 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 PROC GLM.[36]

Platelet Aggregation Assay. Platelet aggregation assay was performed according to the Born turbidimetric technique,[37] as previously described.[22] Compounds were dissolved in saline containing 10% 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 5 μM ADP, 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 the reference adenosine analogue NECA, after logit−log transformation, and fitted by weighted least-squares method.[33] The antiaggregatory activity was evaluated using a concentration of the test compound close to the IC_{50} value. The resulting single-dose potency ratio is only a rough estimate because the dose−response relationship and the deviation from parallelism could not be evaluated.

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