2-(N'-Aralkylidenehydrazino)adenosines: Potent and Selective Coronary Vasodilators

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This study aimed at the development of 2-(N'-aralkylidenehydrazino)adenosines as coronary vasodilators. The reaction of aromatic aldehydes or ketones with 2-hydrazinoadenosine in refluxing methanol formed the target compounds **2–27** as crystalline products in good yields. Two kinds of receptors mediate the actions of adenosine on the heart. Retardation of impulse conduction through the atrioventricular node, the negative dromotropic action, is an example of adenosine's action at an A_1 receptor (A_1AR) and coronary vasodilation reflects adenosine's action at an A_2 receptor (A_2AR). Accordingly, bioassays employing guinea pig heart Langendorff preparations assessed the selectivity of **2–27** as coronary vasodilators. Analogues **2–27** were weak negative dromotropic agents; the EC_{50} of the most active analogue, 2-[N'-(1-naphthylmethylene)hydrazino]-adenosine, **23**, was 0.8 μM, several orders of magnitude less than many A_1AR agonists. Some of the analgoues were quite active coronary vasodilators; 2-(N'-benzylidenehydrazino)adenosine, **2**, and several of its para-substituted derivatives, namely, the fluoro (**7**), methyl (**13**), methoxy (**16**), and *tert*-butylcarbonylethyl, **31**, had EC_{50}s for coronary vasodilation in the range 1.7–3.2 nM. The selectivity ratios, EC_{50}(negative dromotropic)/EC_{50}(coronary vasodilatory), of these five analogues ranged between 5100 (analogue **31**) and 43 000 (analogue **2**). Phenyl ring substitutions of other kinds or at other positions, replacement of the phenyl ring by other aryl or heteroaryl groups, or the replacement of the benzylic H by a methyl group lowered coronary vasoactivity significantly. The unselective adenosine receptor antagonist 8-(*p*-sulfophenyl)theophylline raised the EC_{50} of the negative dromotropic activities of **2**, **16**, and 2-[N'-(2-naphthylmethylene)hydrazino]adenosine, **24**, by 3-, 18-, and 7-fold, and raised the EC_{50}s of coronary vasoactivity by 11-, 3-, and 30-fold, respectively evidence that vasoactivity was receptor-mediated.

A previous report[1] described the synthesis, properties, and cardiovascular activities of a series of 2-(alkylidenehydrazino)adenosines that were prepared by the condensation of aliphatic aldehydes or ketones with 2-hydrazinoadenosine. Like their oxygen and nitrogen isosteres the 2-alkoxyadenosines[2] and the 2-(alkylamino)adenosines,[3] the 2-(N'-alkylidenehydrazino)adenosines include some agonists that have very high activity as coronary vasodilators but only weakly retard conduction through the atrioventricular (AV) node, the negative dromotropic effect. An A_1AR coupled to a $K_{ACh.Ado}$ channel is thought to mediate the negative dromotropic action, and an A_2-AR, the coronary vasoactivity of adenosine.[3] Because aromatic groups in a C-2 substituent can increase the A_2-AR agonist potency and coronary vasoactivity of adenosine,[4-6] the study was extended to the 2-(N'-aralkylidenehydrazino)adenosines described below. Although in the present study the antagonism of both the negative dromotropic and coronary vasodilatory actions by dialkylxanthines is evidence that adenosine receptors mediate the cardiac actions of the 2-(N'-aralkylidenehydrazino)-adenosines, the study does not identify the type of receptor responsible for either of those actions.

Currently CGS 21,680, which is *N*-ethyl-2-[[2-(4-carboxyethylphenyl)ethyl]amino]adenosine-5'-uronamide,[7] is the prototypical A_2AR agonist.[8] However, the instability of the phenethylamine that serves as the C-2 substituent complicates the synthesis of CGS 21,680, and for this reason we explored the possibility that the hydrazino isostere, which is much easier to prepare, might be a suitable substitute.

Chemistry

The reaction of 2-hydrazinoadenosine, **1**, with an aromatic carboxaldehyde (analogues **2–24**, **31**, and **32**) or with an aryl ketone (analogues **25–27**) affords the title compounds in good yields (Scheme I). In most instances the product crystallized out of the reaction mixture on cooling; in two, analogues **3** and **4**, medium-pressure

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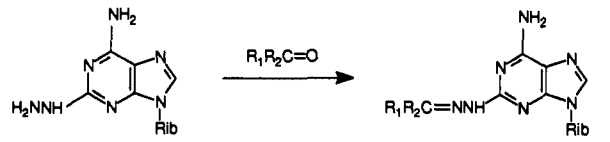
Scheme I



1

2-24 R_2=H

25-27 R_2=CH_3

reverse-phase chromatography was needed to obtain pure product. Unlike the 2-(*N'*-alkylidenehydrazino)adenosines, which appear to undergo catalytic reduction to unstable 2-(*N'*-alkylhydrazino)adenosines, analogue **2** was resistant to catalytic reduction over Pd–C. Perhaps the resistance to hydrogenation owes to the stability conferred by the conjugation of the —C=N— bond with the aromatic system of the benzene ring.

Scheme II depicts the preparation of the hydrazinoadenosine analogue of CGS 21,680. The reaction under conditions used to prepare 4-[2-(methoxycarbonyl)ethyl]benzaldehyde,[9] from *tert*-butyl acrylate with 4-bromobenzaldehyde, **28**, proceeded smoothly to yield yellow crystals of 4-[2-(*tert*-butoxycarbonyl)ethenyl]benzaldehyde, **29**. Analogue **30**, the product of the reaction of **1** and **29**, was hydrogenated over Pd–C to yield **31**, which was saponified by stirring in 1 N KOH to generate the deep red anion of **32**. Neutralization afforded **32** as the colorless acid. Table I lists the properties of analogues **2–27**, **31**, and **32**.

Because the carboxamide group contributes to the agonist potency of CGS 21,680,[3,6] a comparison of the activity of **32** with that of CGS 21,680 is inappropriate. The synthesis of the adenosine analogue of CGS 21,680 and its *tert*-butyl ester for comparison with **31** and **32** entailed some modifications in the preparation of *tert*-butyl 3-[4-(2-aminoethyl)phenyl]propionate, **35**, the amine that becomes the C-2 substituent of **37** (Scheme III). The literature method[6] generated **35** from *tert*-butyl 4-(cyanomethyl)cinnamate, **33**, by a single catalytic hydrogenation of 7 h over Pd–C in the presence of HCl. Workup consisted of filtration, evaporation of solvent, dissolving the residue in Et_2O, and washing the solution with $NaHCO_3$, drying over K_2CO_3, and evaporation. In our hands this approach generated large amounts of an insoluble byproduct and so we investigated alternative routes to **35**. Hydrogenation over Pd–C in the absence of HCl selectively reduced the allylic double bond of **33** to give the nitrile **34**. The catalyst was filtered off and the filtrate was hydrogenated for 2.5–3 h over Raney nickel T-1 in the presence of NH_4OH. Filtration of the catalyst and vacuum evaporation afforded the crude amine, **35**, which was reacted[4] immediately with 2-chloroadenosine, **36**, to yield **37** and, after saponification, **38**.

Cardiovascular Activity

A guinea pig heart Langendorff preparation served for the simultaneous bioassay of slowing of cardiac impulse conduction through the AV node[10] and coronary vasodilation.[11] Table II lists the results of those assays.

The negative dromotropic activity of the index analogue, 2-(*N'*-benzylidenehydrazino)adenosine, **2**, was low, the mean EC_{50} being 83 μM. Any modification, such as lengthening the alkyl chain (analogues **3** and **4**), ring substitution (analogues **5–17**), or replacement of the phenyl group with an aromatic heterocycle (analogues **17–22**) or with a naphthyl group (analogues **23** and **24**), improved activity. However, the most potent analogue, 2-[*N'*-(1-naphthylmethylene)hydrazino]adenosine, **23**, was only 2 orders of magnitude more potent than **2**. Neither the type nor the ring position of a phenyl substituent nor the kind of heterocyclic group correlated with activity. The five analogues with the greatest lateral bulk distal to the methylene group (analogues **24–27**) had the highest activity, which suggests that 2-substituent interacts with a relatively capacious part of the receptor.

All analogues were significantly more active as coronary vasodilators than as inhibitors of AV node conduction. The selectivity ratios, the EC_{50} of negative dromotropic activity divided by the EC_{50} of coronary vasoactivity, ranged between a low of 110 (analogue **14**) and a high of 43 000 (analogue **2**). Whereas ring substitutions and other modifications of the aryl moiety increased negative dromotropic activity, the converse was true as regards coronary vasoactivity. Except for 2-[*N'*-(4-methoxybenzylidene)hydrazino]adenosine, **16**, which was only one-third more active than **2**, all of the phenyl ring modifications reduced activity by up to 60-fold (analogue **14**). The coronary vasoactivity of the benzylidene derivatives **5–16** depended on both the type of substituent and its position in the benzene ring. For ortho and meta substituents the vasoactivity ranking was similar, namely $OCH_3 \leq CH_3 \leq Cl < F = H$, decidedly different from the ranking for para substituents, which was $Cl < CH_3 = F = H < OCH_3$. For each type of substituent the potency ranking increased ortho < meta ≤ para.

The reaction of **1** with acetophenone, *p*-fluoroacetophenone, or with 2-acetylthiophene yielded analogues **25–27**, which are the 1-ethylidene homologues of **2**, **10**, and **20**. At the receptor modulating AV node conduction, each of the three ethylidene analogues was somewhat more active than its methylene congener, the differences ranging between 3- and 28-fold. With respect to coronary vasodilation, the reverse was true, the activities of the ethylidine analogues being 25–82% lower than of the methylene homologues. As a result of these opposite effects at the two receptors, the additional methyl groups of **25–27** greatly reduced the selectivity ratio.

Three representative analogues, **2**, **16**, and **24**, served to test for antagonism of the negative dromotropic and coronary vasodilator activities by dialkylxanthines. The unselective antagonist 8-(*p*-sulfophenyl)theophylline raised the EC_{50}s of negative dromotropsim by 3-, 18-, and 7-fold and those of coronary vasoactivity by 11-, 3-, and 30-fold, respectively. The increases in EC_{50} owed to rightward shifts of the dose–respone curves. Such results suggest that adenosine receptors mediate the actions of these analogues.

Some of the 2-(*N'*-aralkylidenehydrazino)adenosines are nitrogen isosteres of 2-aralkoxyadenosines described in an earlier report.[4] Direct comparisons (Figure 1) showed

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Scheme II

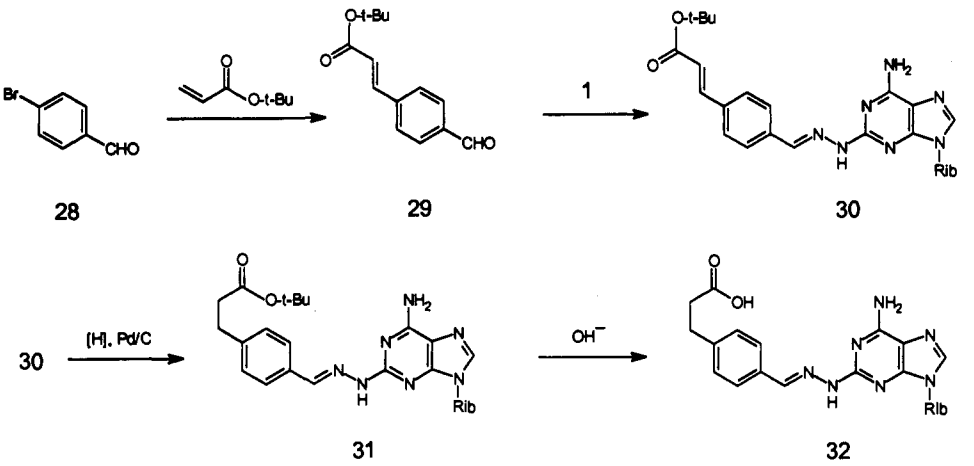
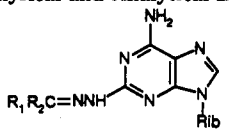


Table I. 2-(*N'*-Aralkylidenehydrazino)adenosines. Physical and Analytical Data



no.	R_1, R_2	purification[a]	% yield	mp, °C	formula	anal.
2	C_6H_5, H	A	70	171–3	$C_{17}H_{19}N_7O_4 \cdot 0.5H_2O$	C,H,N
3	$C_6H_5CH_2$, H	L, 50/70	60	141–2	$C_{18}H_{21}N_7O_4$	C,H,N
4	$C_8H_5(CH_2)_2$, H	L, 50/70	38	89–91	$C_{19}H_{23}N_7O_4$	C,H,N
5	2-FC_6H_4, H	A	59	203–4	$C_{17}H_{18}FN_7O_4$	C,H,N,F
6	3-FC_6H_4, H	A	74	165–7	$C_{17}H_{18}FN_7O_4$	C,H,N,F
7	4-FC_6H_4, H	A	66	154–6	$C_{17}H_{18}FN_7O_4$	C,H,N,F
8	2-ClC_6H_4, H	A	78	283–4	$C_{17}H_{16}ClN_7O_4$	C,H,N,Cl
9	3-ClC_6H_4, H	B	78	196	$C_{17}H_{18}ClN_7O_4$	C,H,N,Cl
10	4-ClC_6H_4, H	A	85	253–5	$C_{17}H_{18}ClN_7O_4$	C,H,N,Cl[b]
11	2-$CH_3C_6H_4$, H	A	74	170–1	$C_{18}H_{21}N_7O_4 \cdot 0.75H_2O$	C,H,N
13	3-$CH_3C_6H_4$, H	B	79	193–6	$C_{18}H_{21}N_7O_4$	C,H,N
13	4-$CH_3C_6H_4$, H	A	75	238–40	$C_{18}H_{21}N_7O_4$	C,H,N
14	2-$CH_3OC_6H_4$, H	A	72	157–8	$C_{18}H_{21}N_7O_5 \cdot H_2O$	C,H,N
15	3-$CH_3OC_6H_4$, H	C	75	175	$C_{18}H_{21}N_7O_5 \cdot 0.75H_2O$	C,H,N
16	4-$CH_3OC_6H_4$, H	A	75	258–61	$C_{18}H_{21}N_7O_5 \cdot 0.25H_2O$	C,H,N
17	2-C_5H_4N, H	A	85	189–92	$C_{16}H_{18}N_8O_4$	C,H,N
18	3-C_5H_4N, H	A	82	280–2	$C_{16}H_{18}N_8O_4$	C,H,N
19	4-C_5H_4N, H	A	72	282–5	$C_{16}H_{18}N_8O_4$	C,H,N
20	2-C_4H_3S, H	A	81	187–90	$C_{15}H_{17}N_7O_4S \cdot H_2O$	C,H,N,S
21	3-C_4H_3S, H	A	84	165–7	$C_{15}H_{17}N_7O_4 \cdot H_2O$	C,H,N,S
22	4-BrC_4H_2S, H	A	70	164	$C_{15}H_{16}BrN_7O_4S$	C,H,N,S,Br
23	1-$C_{10}H_7$, H	B	89	173–5	$C_{21}H_{21}N_7O_4 \cdot H_2O$	C,H,N
24	2-$C_{10}H_7$, H	B	91	243–5	$C_{21}H_{21}N_7O_4 \cdot 0.67H_2O$	C,H,N
25	C_6H_5, CH_3	A	89	158–60	$C_{18}N_{20}N_7O_4$	C,H,N
26	4-FC_6H_4, CH_3	A	73	275–7	$C_{18}H_{20}FN_7O_4$	C,H,N,F
27	2-C_4H_3S, CH_3	A	81	145–7	$C_{16}H_{19}N_7O_4S$	C,H,N,S
31	4-($HOOCCH_2CH_2$)C_6H_4, H	A	95	208–9	$C_{24}H_{31}N_7O_4 \cdot 0.33H_2O$	C,H,N
32	4-($HOOCCH_2CH_2$)C_6H_4, H	A	89	182	$C_{20}H_{23}N_7O_6$	C,H,N

[a] Method A, recrystallized from MeOH/H_2O; B, recrystallized from MeOH; C, recrystallized from MeOH/Et_2O, L, reverse-phase LPLC with elution by a methanol/water gradient, the numbers referring to the initial and final percent MeOH. [b] N: Calcd, 22.39, found, 22.91.

that the coronary vasoactivity of the benzylidene analogue **2** equaled that of its isostere, 2-phenethoxyadenosine. However, the phenethylidene and phenylpropylidene homologues **3** and **4** were significantly more vasoactive than the corresponding phenylalkoxy isosteres. By contrast, every one of the (arylmethylene)hydrazino analogues, **5–16**, **20**, **21**, **23**, and **24**, was less vasoactive than its arylethoxy isostere. Steric hindrance arising from the rigidity caused by the conjugation of the —CH=N— double bond with the double bonds of the aromatic residue could explain why the arylmethylene analogues are less active than their arylethoxy isosteres and also could explain why **3** and **4**, which lack such a conjugated double bond system, are more active than their oxygen isosteres.

The C-2 substituent of **32** is a nitrogen isostere of the C-2 substituent of CGS 21,680, the prototypical A_2AR agonist. In the guinea pig heart Langendorff preparation the EC_{50} of coronary vasodilation by CGS 21,680 was 0.74 nM and the selectivity ratio was 33 000,[5] but the corresponding data for **32** were 6.6 and 4900 nM, respectively. Thus, **32** is not a suitable substitute for CGS 21,680. Because the C-5 amide group contributes significantly to the affinity of CGS 21,680 for the A_2AR, **32** should not be compared with CGS 21,680. Rather, the activities of **31** and **32** should be compared to those of **37** and **38**, which also lack C-5′ amide groups. The two comparisons (Table III) show that the coronary vasoactivity of each of the 2-(*N'*-benzylidenehydrazino)adenosines equals that of the corresponding 2-[(2-arylethyl)amino]adenosines. That evidence, although limited, suggests the two kinds of C-2 substituent are equivalent and supports the conclusion that the C-5′ amide group is a major determinant of the

Scheme III

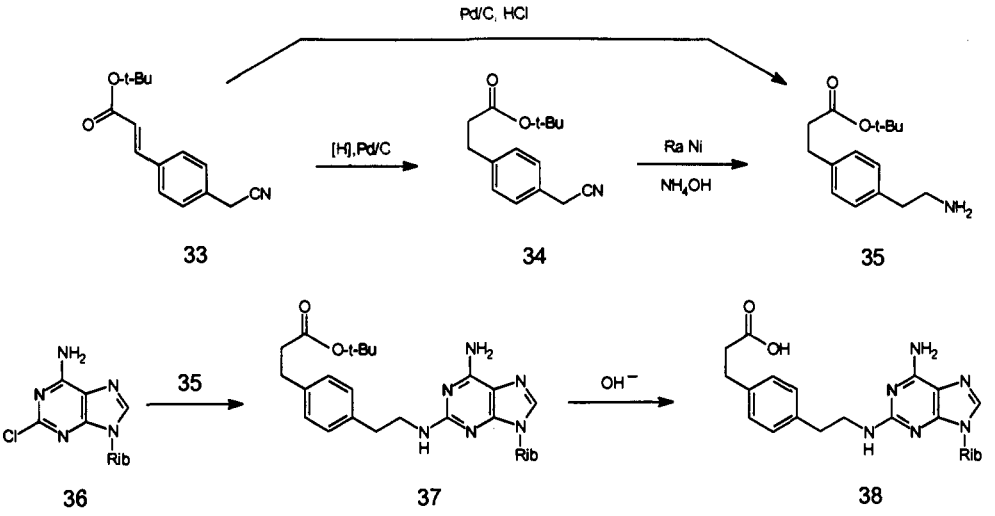
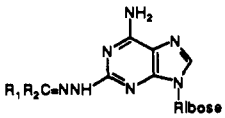


Table II. 2-(N'-Aralkylidenehydrazino)adenosines. Cardiac and Coronary Activity



no.	R_1, R_2[a]	−log EC_{50}, M: stim–QRS[b]	−log EC_{50}, M: coronary[c]	selectivity ratio, A_1/A_2
1, 2-hydrazinoadenosine		4.70 ± 0.32	7.10 ± 0.17	300 ± 110
2	C_6H_5, H	4.08 ± 0.15	8.64 ± 0.06	43000 ± 13000
3	$C_6H_5CH_2$, H	4.08 ± 0.17	8.04 ± 0.04	11000 ± 3100
4	$C_6H_5(CH_2)_2$, H	4.18 ± 0.07	8.71 ± 0.16	37000 ± 10000
5	2-FC_6H_4, H	4.38 ± 0.12	8.43 ± 0.02	12000 ± 4700
6	3-FC_6H_4, H	5.04 ± 0.34	8.47 ± 0.07	8000 ± 4600
7	4-FC_6H_4, H	4.90 ± 0.22	8.61 ± 0.10	8,500 ± 3400
8	2-ClC_6H_4, H	4.31 ± 0.11	8.12 ± 0.02	6800 ± 1200
9	3-ClC_6H_4, H	4.31 ± 0.04	8.34 ± 0.05	11000 ± 1400
10	4-ClC_6H_4, H	4.85 ± 0.18	8.35 ± 0.12	5500 ± 2600
11	2-$CH_3C_6H_4$, H	4.36 ± 0.23	7.92 ± 0.06	4900 ± 1900
13	3-$CH_3C_6H_4$, H	4.77 ± 0.15	8.36 ± 0.08	4700 ± 1400
13	4-$CH_3C_6H_4$, H	4.40 ± 0.08	8.49 ± 0.06	14000 ± 4800
14	2-$CH_3OC_6H_4$, H	4.91 ± 0.14	6.86 ± 0.08	110 ± 41
15	3-$CH_3OC_6H_4$, H	4.71 ± 0.25	7.91 ± 0.08	2300 ± 1000
16	4-$CH_3OC_6H_4$, H	4.64 ± 0.07	8.76 ± 0.05	14000 ± 2000
17	2-C_5H_4N, H	4.38 ± 0.07	8.24 ± 0.15	9400 ± 4400
18	3-C_5H_4N, H	4.49 ± 0.06	7.83 ± 0.10	2700 ± 1000
19	4-C_5H_4N, H	4.58 ± 0.12	7.96 ± 0.05	2800 ± 830
20	2-C_4H_3S, H	4.38 ± 0.20	7.86 ± 0.02	4400 ± 2100
21	3-C_4H_3S, H	4.22 ± 0.07	7.81 ± 0.05	4400 ± 1300
22	4-Br-2-C_4H_2S, H	4.24 ± 0.07	8.28 ± 0.07	12000 ± 2200
23	1-$C_{10}H_7$, H	6.08 ± 0.17	8.02 ± 0.05	110 ± 34
24	2-$C_{16}H_7$, H	5.58 ± 0.27	8.38 ± 0.12	770 ± 260
25	C_6H_5, CH_3	5.52 ± 0.27	7.90 ± 0.07	380 ± 230
26	4-FC_6H_5, CH_3	5.38 ± 0.24	8.49 ± 0.01	1800 ± 1100
27	2-C_4H_3S, CH_3	5.05 ± 0.13	7.55 ± 0.04	350 ± 100

[a] R_1 is as indicated, R_2 is H (analogues 2–24) unless noted otherwise (analogues 25–27). [b] Prolongation of the stimulus-QRS interval of the ECG, an index of activity at the A_1AR in the cardiac AV note. [c] Coronary vasodilation, an index of activity at the A_2AR in coronary arteries. [d] Selectivity ratio, EC_{50}(stimulus–QRS prolongation)/EC_{50}(coronary vasodilation). Data are mean ± SEM of measurements on four hearts.

potency of CGS 21,680. The high selectivity of **38** for coronary vasoactivity owes to its very weak negative dromotropic activity.

In summary, 2-hydrazinoadenosine reacts readily with aromatic aldehydes and ketones to yield 2-(N'-aralkylidenehydrazino)adenosines. Such analogues weakly retard impulse conduction through the cardiac atrioventricular node. The 2-[N'-(arylmethylene)hydrazino]adenosines **2** and **5–24** are strong coronary vasodilators, but not as strong as the isosteric 2-(arylethoxy)adenosines. Conjugation of the —C=N— double bond with those of the aryl group may confer rigidity on the C-2 substituent of these analogues that prevents optimum interaction with the A_2-AR. The 2-phenethylidene and 2-phenylpropylidene analogues **3** and **4** lack such a conjugated double bond system and are more vasoactive than their oxygen isosteres.

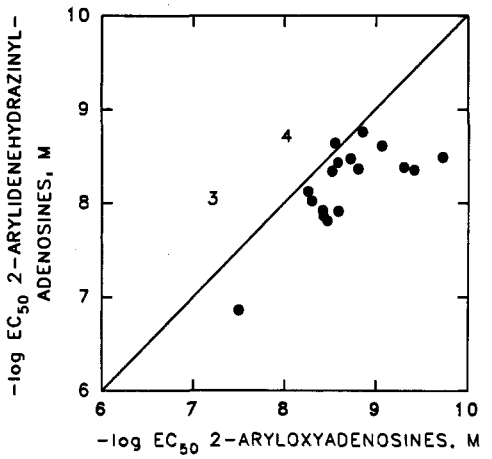


Figure 1. Comparison of the coronary vasoactivities of analogues **3** and **4** and some 2-(N'-benzylidenehydrazino)adenosines (●) with those of isosteric 2-aralkoxyadenosines. Note that with the exception of **3** and **4**, all data points lie on or below the line of identity, evidence that the 2-(N'-benzylidenehydrazino)adenosines are equal to or weaker than the 2-arylethoxyadenosines as coronary vasodilators. See the text for additional discussion.

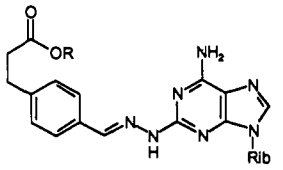
Experimental Section

Chemistry. Melting point measurements employed a Thomas-Hoover apparatus and are uncorrected. The ^1H NMR spectra of solutions of compounds in DMSO-d_6 were recorded on a Varian EM360L spectrometer, and resonances are reported as chemical shifts (δ) in ppm relative to a TMS internal standard. Those spectra were consistent with the putative structure of the compound under examination. MHW Laboratories (Tucson, AZ) performed the elemental analyses. Reverse-phase HPLC on C-18 silica revealed that product accounted for >98% of the material in nucleoside samples submitted for bioassay. Aldehydes and ketones were available commercially or were prepared by oxidation of the corresponding alcohol.[12]

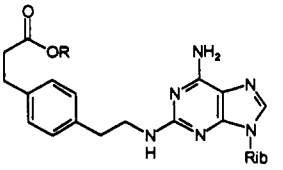
Synthesis of 2-(N'-Aralkylidenehydrazino)adenosines. General Method. Heating at reflux a mixture of **1** (1 g, 3.36

(12) Corey, E. J.; Suggs, J. W. Pyridinium chlorochromate. An efficient reagent for oxidation of primary and secondary alcohols to carbonyl compounds. *Tetrahedron Lett.* **1975**, 2647–2650.

Table III. Comparisons of the Cardiovascular Activities of 2-(*N'*-Aralkylidenehydrazino)adenosines **31** and **32** with 2-[(2-Arylethyl)amino]adenosines **37** and **38**



31, 32



37, 38

		-log EC_{50}, M				
no.	R	stim–QRS	p[a]	coronary	p	A_1/A_2
31	*t*-Bu	4.92 ± 0.04		8.52 ± 0.14		5100 ± 2100
37	*t*-Bu	4.72 ± 0.05	NS	8.28 ± 0.07	NS	7400 ± 460
32	H	4.51 ± 0.05		8.18 ± 0.07		4900 ± 800
38	H	3.95 ± 0.19	NS	7.95 ± 0.15	NS	47000 ± 12000

[a] p, significance of difference of means; NS, not significant. Other abbreviations as in Table II.

mmol) and 1.1 equiv of aldehyde or ketone in 50 mL of methanol led to the disappearance of product in 6–24 h. Product that crystallized out on cooling was filtered off, washed with a little methanol, and dried. One recrystallization from methanol/water afforded analytically pure material for all products except **3** and **4**, which were purified by medium-pressure reverse phase chromatography as described in Table I.

4-[2-(*tert*-Butoxycarbonyl)ethenyl]benzaldehyde (29). A mixture of 4-bromobenzaldehyde, **28** (10 g, 54 mmol), *tert*-butyl acrylate (11 g, 86.4 mmol), $Pd(CH_3COO)_2$ (0.12 g, 0.54 mmol), and tri-*o*-tolylphosphine (0.64 g, 2.1 mmol) in 25 mL of triethylamine was refluxed overnight, poured onto ice, acidified, and extracted with ethyl acetate. Drying ($MgSO_4$) and vacuum evaporation left a bright yellow residue that was crystallized from ether/hexane to yield 9.8 g (78%) of chromatographically pure product. Mp: 108–9 °C. ^1H NMR (δ): 1.58 (s, 9 H, CH_3), 6.50 (d, 1 H, Ph C*H*=CH), 7.63 (d, 2 H, Ph H-3, H-5), 7.70 (d, 1 H, Ph CH=C*H*), 7.96 (d, 2 H, Ph H-2, H-6), 10.10 (s, 1 H, C*H*O). Anal. Calcd for $C_{14}H_{16}O_3 \cdot 0.75H_2O$: C, 68.48; H, 7.36. Found: C, 68.41; H, 7.18.

2-[*N'*-[4-[2-(*tert*-Butoxycarbonyl)ethyl]benzylidene]hydrazino]adenosine (31). Starting with 3.5 g (11.8 mmol) of **1** and 3.5 g (14.1 mmol) of **29**, the general method afforded 5 g (9.8 mmol, 83%) of orange crystalline product. A solution of the product (4 g, 7.8 mmol) in 100 mL of DMF containing 1 g of 5% Pd/C was shaken overnight in the presence of H_2 (50 psig). Catalyst was filtered off (Celite) and washed with DMF, and the solvent was evaporated in a Kugelrohr (35 °C, oil pump). The residue was crystallied from methanol/water to yield 3.8 g (95%) of product. ^1H NMR (δ): 1.40 (s, 9 H, CH_3), 2.70 (m, 4 H, —CH_2-CH_2—), 3.50–5.60 (m, 8 H, ribose), 5.86 (d, 1 H, anomeric), 7.07 (br s, 2 H, NH_2), 7.26 (d, 2 H, Ph H-3, H-5), 7.72 (d, 2 H, Ph H-2, H-6), 8.08 (unresolved singlets, 2 H, H-8 and C*H*=NNH), 10.80 (s, 1 H, CH=NN*H*).

2-[*N'*-[4-(2-Carboxyethyl)benzylidene]hydrazino]adenosine (32). A suspension of **26** (0.9 g, 1.75 mmol) in 20 mL of 1 N KOH was stirred at room temperature. As the starting material dissolved the solution became deep red. Acidification to pH 4 with 1 N HCl and extraction of the now colorless solution with ethyl acetate yielded, after drying ($MgSO_4$) and evaporation, crude product which was crystallined from methanol/water to give 0.7 g (89%) of white needles. ^1H NMR (δ): 2.73 (m, 4 H, CH_2CH_2COOH), 3.6–5.7 (m, 8 H, ribose), 5.90 (d, 1 H, anomeric), 7.11 (br s, 2 H, NH_2), 7.33 (d, 2 H, Ph H-3, H-5), 7.75 (d, 2 H, Ph H-2, H-6), 8.13 (unresolved singlets, 2 H, H-8 and C*H*=NNH), 10.89 (s, 1 H, CH=NN*H*), COO*H* not observed.

***tert*-Butyl 3-[4-(2-Aminoethyl)phenyl]propionate (35).** A solution of *tert*-butyl 4-(cyanomethyl)cinnamate[7] (15 g, 68 mmol) in 250 mL of absolute ethanol was shaken overnight with 2 g of 5% Pd–C at 3 atm of H_2. Filtration through Celite removed the catalyst, and the filtrate plus 10 mL of concentrated ammonia were immediately hydrogenated over 5 g of type T-1 Raney nickel.[13] The catalyst was filtered off, the solvent evaporated in vacuo, and the crude amine reacted immediately as described[4] to yield **37**.

Assays of Cardiovascular Activity. The use of experimental animals conformed to NIH Publication 85-23, *Guide for the Care and Use of Laboratory Animals*. A Langendorff guinea pig heart preparation paced at 260 beats/min via the left atrium served for assays of A_1AR and A_2AR agonist activity. The perfusion buffer consisted of (mM) NaCl (120), $NaHCO_3$ (27), KCl (3.7), KH_2PO_4 (1.3), $MgSO_4$ (0.64), $CaCl_2$ (1.3), pyruvate (2), and glucose (5). The buffer was saturated with 95% O_2/5% CO_2, equilibrated at 37 °C in a heat exchanger, and delivered at a pressure equivalent to 55 mmHg. Continuous drainage of the left ventricle by means of a catether inserted across the mitral valve insured that this cardiac chamber did no external work. An electrode in the right ventricle monitored the electrocardiogram. Timed collections of cardiac effluent in a graduated cylinder during the steady-state phase of the flow responses to analogue administration measured total coronary flow, which was also monitored by an in-line electromagnetic flowmeter in the aortic perfusion cannual. We increased the rate of nucleoside infusion stepwise until the appearance of second degree heart block. The quotient of the ratio of nucleoside infusion (mol/min) divided by coronary flow rate (L/min) equals agonist concentration in the perfusate. The EC_{50} of prolongation of the stimulus–QRS interval, the concentration of agonist needed to prolong the interval by 50% of the maximum response,[10] reflects activity at the A_1AR. Logit transformation of the coronary flow data and solution of the regression of logit (coronary flow) on log [analogue] for logit = 0 yielded an estimate of EC_{50} of coronary vasodilation, an index of A_2AR activity. Table II reports the mean ± SEM of the -log EC_{50} values from assays in four or more hearts. The quotient of the EC_{50} of stimulus–QRS prolongation divided by the EC_{50} of coronary vasodilation provided an index of selectivity. Values of the index <1 indicate selectivity for the A_1AR, and values >1, selectivity for the A_2AR. Table II reports the mean ± SEM of the A_1/A_2 activity ratios of individual experiments.

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