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

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The reaction of aliphatic aldehydes and ketones with 2-hydrazinoadenosine under relatively mild conditions (at room temperature or in refluxing methanol) formed 2-(*N'*-alkylidenehydrazino)-adenosines, **5–22**, in good yields. Two kinds of adenosine receptors regulate cardiac and coronary physiology. In supraventricular tissues an A_1AR coupled to muscarinic K channels mediates the negative chronotropic, dromotropic, and inotropic actions of adenosine, and an inhibitory A_1AR coupled to adenylate cyclase mediates the "antiadrenergic" action of adenosine. One or more kinds of A_2 receptors mediate coronary vasodilation. Bioassays employing a guinea pig heart Langendorff preparation showed that **5–22** weakly retard impulse conduction through the AV node (negative dromotropic effect), but several analogues were very active coronary vasodilators. The coronary vasoactivity of the (*n*-alkylidene- and of the (isoalkylidenehydrazino)adenosines paralleled the length of the alkyl chain, the EC_{50}s of the of the most active *n*-pentylidene (**8**) and isopentylidene (**18**) congeners being 1 nM. The EC_{50}s of the cyclohexylmethylene (**9**), cyclohexylethylidene (**10**), and cyclohex-3-enylmethylene (**12**), analogues were likewise <1 nM, but the cyclohex-1-enylmethylene congener **12** was 10 times less active than **9**. The unselective adenosine receptor antagonist 8-(*p*-sulfophenyl)theophylline (0.1 mM) raised the EC_{50}s of the negative dromotropic effects of **8**, **9**, and **18** by 5–28-fold and the EC_{50}s of coronary vasodilation of 22–90-fold. Catalytic reduction of **9** increased the hydrophobicity and changed the UV spectrum, suggesting reduction of the —CH=N— bond. The product darkened on exposure to air and so was not characterized further. A new method for preparing 2',3',5'-tri-*O*-acetyl-2,6-dichloropurine riboside, a precursor in the synthesis of 2-hydrazinoadenosine, consists of the addition of *tert*-butyl nitrite to a mixture of 2',3',5'-tri-*O*-acetyl-6-chloroguanosine and CuCl in $CHCl_3$ saturated with Cl_2.

Several kinds of adenine C-2 substituents selectively increase the potency of adenosine at the A_2 adenosine receptor (A_2AR). Such substituents may be alkyl aryl, or aralkyl groups, and they may be attached to the adenine base directly,[1-3] that is, through a C–C bond, or through an amino,[4-6] oxo,[7,8] or thio[9,10] linkage. The (aralkylamino)-adenosines and their aralkoxy isosteres have interesting biological characteristics,[11-13] but they are rather difficult to synthesize. A considerable part of the difficulty owes to the low reactivity of leaving groups attached to C-2 such as Cl or SO_2CH_3, which amines and alkoxides can displace, but with some difficulty, requiring temperatures in excess of 100 °C in the case of amines[6] or reaction times of several days in the case of alkoxides.[7] However, unlike amines and alkoxides, hydrazine readily displaces the chloro substituent from 2-chloroadenosine to give 2-hydrazinoadenosine at high yields.[14]

The present report describes the synthesis and some cardiovascular actions of 2-(*N'*-alkylidenehydrazino)-adenosines generated by the condensation of 2-hydra-

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zinoadenosine with aldehydes or ketones. The 2-(*N'*-alkylidenehydrazino)adenosines are nitrogen isosteres of the 2-(alkylamino)adenosines and the 2-alkoxyadenosines, two classes of adenosine analogues that include some very potent and selective agonists at the A_{2a}AR of rat striatum membranes as well as very active coronary and systemic vasodilators.[6-8,13] There is precedent for the use of aldehydes and ketones as synthons for preparing adenosine analogues. The Schiff bases formed by the reaction of 2',3'-*O*-isopropylideneadenosine with a variety of aldehydes and certain cycloalkanones can be isolated, but such compounds readily hydrolyze in water and so they are not useful as adenosine receptor agonists.[15] However, catalytic hydrogenation of those Schiff bases afforded the congeneric N^6-(ar)alkyl-adenosines. The present report shows that aliphatic aldehydes and ketones react with 2-hydrazinoadenosine rapidly and under relatively mild conditions to form 2-(*N'*-alkylidenehydrazino)adenosines in good yields. The large number of aldehydes and ketones that are either available commercially or that are easily accessible, for example, by oxidation of the cognate alcohols, add versatility to the simplicity of this approach.

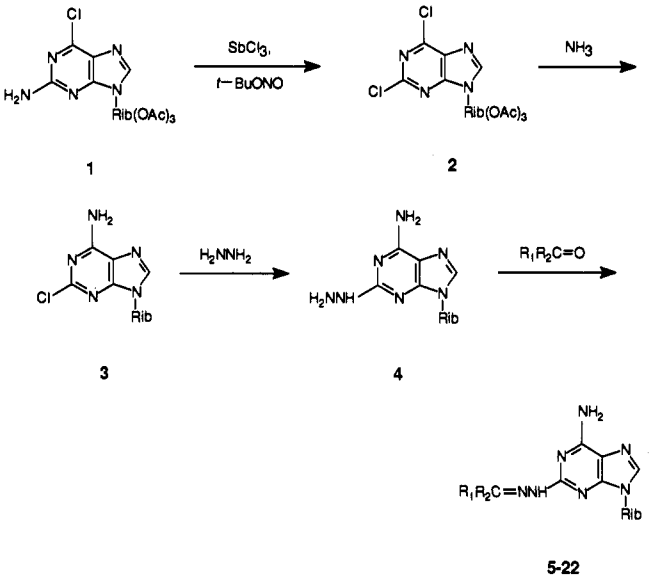
The project required a rather large supply of 2-hydrazinoadenosine, and so some effort was spent in exploring ways to improve the synthesis of 2',3',5'-tri-*O*-acetyl-2,6-dichloropurine riboside, the immediate precursor of 2-chloroadenosine. The diazotization of suitably protected 6-chloroguanosine dissolved in a polyhalomethane is a general route to 2-haloadenosines.[16] In the case of chlorination, the yield depends on the concentration of Cl, and so the addition of salts such as $SbCl_3$ is used to improve yields.[16] However, the product must be separated from a colloidal suspension of antimony salts remaining at the end of the reaction, which requires a slow and tedious filtration step. The present study shows that Cl_2 is a satisfactory, cheaper, and more convenient replacement for $SbCl_3$.

Chemistry

The Sandmeyer reaction applied to 2',3',5'-tri-*O*-acetyl-6-chloroguanosine, **1**, is a rapid and simple route to 2',3',5'-tri-*O*-acetyl-2,6-dichloropurine riboside, **2**, the precursor of the 2-chloroadenosine, **3**, needed for the synthesis of 2-hydrazinoadenosine, **4** (Scheme I). A solution of **1** in $CHCl_3$ containing CuCl was saturated with Cl_2, and the addition of Cl_2 continued during the cautious dropwise addition of *tert*-butyl nitrite. The strongly exothermic reaction went to completion in 10–15 min. Workup consisted simply of reducing the excess Cl_2 with $NaHSO_3$, washing with $CHCl_3$ solution with water, and filtration through Celite to remove a small amount of colloidal precipitate.

Treating a suspension of **4** in methanol with 1.1 equiv of an aldehyde or aliphatic ketone generated the hydrazones **5–22**. Most of the 2-(*N'*-alkylidenehydrazino)-adenosines did not crystallize, but purification by reverse-phase liquid chromatography employing elution by

Scheme I



gradients by methanol/water yielded pure products (Table I). Catalytic reduction of **9** over Pd–C changed both its mobility on reverse-phase HPLC and its UV spectrum. The reduction product was retained slightly longer than **9** on C-18 silica eluted with 1:1 methanol/water. Whereas the UV spectrum of **9** exhibited a maximum at 273 nm, that of the reduction product resembled the spectrum of **4**, in having a maximum at 259 nm and a shoulder at 282 nm. Although the similarity of the UV spectra raises the possibility that reduction cleaved the —CH=N— bond to regenerate **4**, the reduction product was much more hydrophobic than **4**, which argues instead that the reduction product retained the cyclohexanemethyl group. Unfortunately, the reduction product decomposed on exposure to air and so was not characterized further.

Cardiovascular Activity

A guinea pig heart Langendorff preparation served for the simultaneous bioassay of the retardation of impulse conduction through the AV node and of coronary vasoactivity (Table II). As expected from the low activity of 2-aminoadenosine, 2-hydrazinoadenosine, **4**, exhibited little negative chronotropic vasodilator activity and, accordingly, was among the least selective of the coronary vasodilators.

Analogues **5–22** had rather weak negative dromotropic activity, the EC_{50}s ranging between 3.5 and 66 μM. An alkyl substituent increased the activity of **4** by at most 6-fold, and some analogues (**8**, **12**, **22**) were only equal to or even slightly less active than **4**. Agonist activity did not correlate with either alkyl chain length, the presence of a cyclo- or bicycloalkyl group, or with the presence of double bonds within the cycloalkyl group.

Several of the 2-(*N'*-alkylidenehydrazino)adenosines were quite active coronary vasodilators. In both the *n*-alkylidene series (**5–8**) and also the isoalkylidene series (**16–18**), coronary vasoactivity increased in parallel with the length of the alkyl chain. The EC_{50}s of the two most potent members of each series, the *n*- and isopentylidene isomers, **8** and **18**, were 1.0 and 0.4 nM, respectively. A cycloalkyl group also improved coronary vasoactivity. The EC_{50}s for the cyclohexylmethylene (**9**), cyclohexylethylidene (**10**), and cyclohex-3-enylmethylene (**13**), analogues were all <1 nM. Interestingly, the 1-cyclohexene analogue

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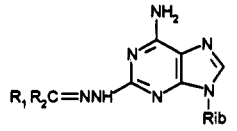
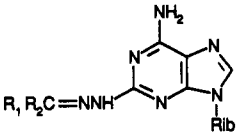
Table I. Physical and Analytical Data of 2-(N'-Alkylidenehydrazino)adenosines



no.	R_1, R_2	% yield	purification[a]	mp, °C	formula	anal.
5	CH_3, H	92	A	163	$C_{12}H_{19}N_7O_4$	C,H,N
6	C_2H_5, H	88	L, 40/60	155	$C_{13}H_{21}N_7O_4$	C,H,N
7	C_3H_7, H	51	L, 40/60	146	$C_{14}H_{23}N_7O_4$	C,H,N
8	C_4H_9, H	73	H, 40/70	135–6	$C_{15}H_{25}N_7O_4$	C,H,N
9	c-C_6H_{11}, H	66	B	154–7	$C_{17}H_{25}N_7O_4$	C,H,N
10	c-$C_6H_{11}CH_2$, H	51	L, 60/75	136–7	$C_{18}H_{27}N_7O_4$	C,H,N
11	c-$C_6H_{11}(CH_2)_2$, H	92	L, 60/75	135–7	$C_{19}H_{27}N_7O_4$	C,H,N
12	1-cyclohexene, H	56	L, 40/70	167–9	$C_{17}H_{23}N_7O_4$	C,H,N
13	3-cyclohexene, H	69	H, 60/80	149–50	$C_{17}H_{23}N_7O_4 \cdot 0.5H_2O$	C,H,N[b]
14	*endo*-2-norborn-5-ene, H	59	L, 40/75	165–7	$C_{18}H_{23}N_7O_4$	C,H,N
15	myrtenyl,[c] H	69	B	232	$C_{20}H_{27}N_7O_4$	C,H,N
16	CH_3, CH_3	65	filter, dry	274–5	$C_{13}H_{19}N_7O_4$	C,H,N
17	$(CH_3)_2CH$, H	51	L, 40/75	143–5	$C_{14}H_{21}N_7O_4$	C,H,N
18	$(CH_3)_2CHCH_2$, H	65	H, 40/70	145–6	$C_{15}H_{22}N_7O_4$	C,H,N
19	n-C_3H_7, CH_3	43	B	212–3	$C_{15}H_{23}N_7O_4$	C,H,N
20	C_2H_5, C_2H_5	33	H, 50/70	145–7	$C_{15}H_{23}N_7O_4$	C,H,N
21	$(C_2H_5)_2CH$, H	55	H, 60/80	154	$C_{16}H_{25}N_7O_4$	C,H,N
22	$(CH_3)_3C$, H	49	B	161	$C_{15}H_{23}N_7O_4$	C,H,N

[a] Abbreviations are A, wash residue with hexane; B, crystallize from MeOH/H_2O; H, reverse-phase HPLC with MeOH/H_2O gradient, numbers being the initial and final percent MeOH; L, reverse-phase LPLC with MeOH/H_2O gradient, numbers being the initial and final percent MeOH. [b] N: calcd, 24.61; found, 25.11. [c] Myrtenyl is 7,7-dimethylbicyclo[3.1.1]hept-2-en-3-yl.

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



		$-\log EC_{50}$, M[a]		
no.	R_1, R_2	Stim-QRS[b]	Coronary[c]	A_1/A_2
4	2-hydrazinoadenosine	4.70 ± 0.32	7.10 ± 0.17	300 ± 110
5	CH_3, H	5.44 ± 0.75	7.12 ± 0.04	210 ± 80
6	C_2H_5, H	4.87 ± 0.07	7.88 ± 0.15	1100 ± 270
7	n-C_3H_7, H	4.94 ± 0.06	8.54 ± 0.10	4100 ± 760
8	n-C_4H_9, H	4.60 ± 0.04	8.99 ± 0.20	33000 ± 17000
9	c-C_6H_{11}, H	5.45 ± 0.05	9.59 ± 0.12	16000 ± 5300
10	c-$C_6H_{11}CH_2$, H	5.01 ± 0.01	9.16 ± 0.09	15000 ± 3400
11	c-$C_6H_{11}(CH_2)_2$, H	4.18 ± 0.04	8.75 ± 0.11	43000 ± 14000
12	1-cyclohexene, H	4.76 ± 0.05	8.46 ± 0.08	5700 ± 1800
13	3-cyclohexene, H	5.14 ± 0.14	9.49 ± 0.12	26000 ± 10000
14	*endo*-2-norborn-5-ene, H	4.69 ± 0.04	8.48 ± 0.08	6000 ± 1700
15	myrtenyl,[d] H	4.59 ± 0.14	8.13 ± 0.06	3800 ± 880
16	CH_3, CH_3	4.92 ± 0.19	7.30 ± 0.06	260 ± 66
17	$(CH_3)_2CH$, H	4.90 ± 0.01	8.13 ± 0.06	1700 ± 220
18	$(CH_3)_2CHCH_2$, H	4.68 ± 0.06	9.33 ± 0.08	50000 ± 12000
19	n-C_3H_7, CH_3	4.78 ± 0.08	8.70 ± 0.03	8600 ± 1200
20	C_2H_5, C_2H_5	5.05 ± 0.05	6.75 ± 0.12	54 ± 12
21	$(C_2H_5)_2CH$, H	4.96 ± 0.11	8.24 ± 0.06	2200 ± 730
22	$(CH_3)_3CH$, H	4.60 ± 0.09	7.46 ± 0.07	850 ± 270

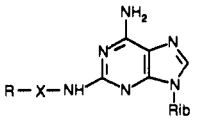
[a] Data are mean ± SEM of assays in four hearts. [b] Prolongation of the stimulus–QRS interval, an index of activity at an A_1AR. [c] Coronary vasodilation, mediated by an A_2AR. [d] Myrtenyl is 7,7-dimethylbicyclo[3.1.1]hept-2-en-3-yl.

12 was 13 times less vasoactive than 9. The reduced flexibility of the 2-substituent of 12 and a diminished ability to interact with the A_2AR is one explanation for why 12 is less active than 9 or 13. The high activity of analogues 8–11, 13, and 18 indicate that the rigidity of the —CH=N— double bond does not seriously impair the interaction of those agonists with the receptor. However, in 12 the cyclohexene —C1=C2— and —CH=N— bonds are conjugated and thus tend to lie in the same plane, which significantly reduces the flexibility of that portion of the molecule and, consequently, may affect its ability to interact with thereceptor. The C-2 substituent of the 3-cyclohexene congener 13 also has a pair of double bonds, but these are not conjugated, perhaps explaining why it is as active as 9.

In contrast to the increase in vasoactivity caused by lengthening the alkyl chains of the *n*-alkylidene and isoalkylidene nucleosides, a similar chain lengthening decreased the vasoactivity of the cyclohexylalkylidene analogues, the potency ranking being 9 > 10 > 11.

Analogoues 19 and 20 are *sec*-alkylidene nucleosides formed by the reaction of 4 with 2-pentanone or 3-pentanone. The 1-methylbutylidene analogue 19 is 2 orders of magnitude weaker than the *n*-propylidene analogue 6. Such a result could mean that (a) the methyl residue (R_2) of 19 does not sterically hinder interaction with the receptor

Table III. Comparison of 9 with CGS 22492 and 12 with CGS 22,989



analogue	R	X	-log EC_{50}, M stim-QRS[a]	-log EC_{50}, M coronary[a]
CGS 22,492	cyclohexane	$(CH_2)_2$	4.91 ± 0.02	8.72 ± 0.04
9		CH=N	5.45 ± 0.05[b]	9.59 ± 0.12[b]
CGS 22,989	1-cyclohexene	$(CH_2)_2$	4.64 ± 0.06	8.65 ± 0.04
12		CH=N	4.76 ± 0.05[c]	8.46 ± 0.08[c]

[a] Data are mean ± SEM of assays in four hearts. [b] Significantly greater than CGS 22,492, $p < 0.005$. [c] Not significantly different from CGS 22,989.

and (b) the low vasoactivity of 20 owes to the shorter length of the propylidene residue and steric hindrance exerted by the ethyl residue (R_1).

The 2-ethylbutylidene analogue 21 has to more methylene residues than the 2-propylidene nucleoside 16 but one fewer methylene than the cyclohexylmethylene analogue 9. The sizes and, perhaps, the hydrophobicity of these alkyl groups could explain the vasoactivity ranking 9 > 21 > 16. The 2,2-dimethylpropylidine analogue 22 is substantially less active than the isobutyl (2-methylpropylidene) congener 17, suggesting that the bulk imparted by the extra methyl group of 22 hinders interaction with the receptor.

Analogues 8–11, 13, and 18 were highly selective coronary vasodilators; the activity ratio, EC_{50}(stimulus–QRS prolongation)/EC_{50}(coronary vasodilation), ranging between 15 000 and 50 000. Even though the assessment of selectivity by bioassay greatly overestimates selectivity as measured directly by radioligand binding assays,[18] such results nevertheless indicate substantial selectivity for coronary vasoactivity. Analogues 8, 9, and 18 served for tests of the hypothesis that adenosine receptor mediated the negative dromotropic and coronary vasodilator activities. The unselective adenosine receptor antagonist 8-(*p*-sulfophenyl)theophylline raised the EC_{50}s of the negative dromotropic effects of 8, 9, and 18 by 7-, 5-, and 28-fold, respectively, and the EC_{50}s of coronary vasoactivity by 90-, 22-, and 40-fold, respectively. Such results suggest that these representative analogues produce their cardiovascular actions through A_1 and A_2 adenosine receptors.

The 2-(*N'*-alkylidenehydrazino)adenosines are isosteres of the 2-(alkylamino)adenosines[6] and the 2-alkoxyadenosines,[7] two classes of adenosine analogues that include some very potent and selective coronary vasodilators. The availability for bioassay of CGS 22,492 and CGS 22,989, which are 2-[(2-cycloalkylethyl)amino] isosteres of 9 and 12, respectively, and the results[7] of our previous assays of 2-alkoxyadenosines that are isosteres of 5–11, 14–18, 20, and 21 permit direct comparisons of negative dromotropic and coronary vasodilator activity. Both the negative dromotropic and coronary vasodilator activities of 9 were significantly greater than those of CGS 22,492, though by only 3.5- and 7.4-fold, respectively (Table III). However, 12 and CGS 22,989 were equiactive in producing either AV block or coronary vasodilation. The negative dromotropic activities of the 2-(*N'*-alkylidenehydrazino)adenosines differed from those of the 2-alkoxyadenosines; analogues 5–7, 16–18, 20, and 21 were more potent than their 2-alkoxy isosteres, but in the case of analogues 8–11, 14, and 15 the relative activities were reversed. In either case the differences in activities were small, usually only 2–9-fold. Figure 1 compares the coronary vasoactivities of the 2-alkoxyadenosines with those of the corresponding 2-(*N'*-alkylidenehydrazino)adenosines. In every instance the 2-(*N'*-alkylidenehydrazino)adenosine was either equipotent or significantly more potent than the alkoxy isostere, but here, too, the differences were only modest, 8-fold or less. Although limited, such evidence suggests that the substitution of a —CH=NNH— for either a $—CH_2CH_2NH—$ or a $—CH_2CH_2O—$ linkage affects cardiovascular activity very slightly.

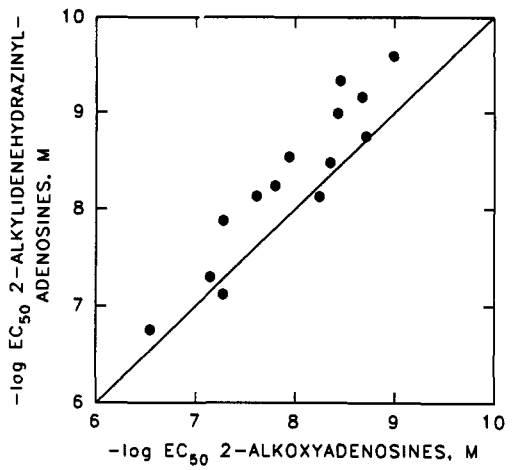


Figure 1. Comparison of the coronary vasoactivities of 2-(*N'*-alkylidenehydrazino)adenosines (ordinate) with those of isosteric 2-alkoxyadenosines (abscissa). Note that most data points lie near or above the line of identity, indicating that the 2-(*N'*-alkylidenehydrazino)adenosines are at least as vasoactive as the 2-alkoxyadenosines. See the text for additional discussion.

In summary, the reaction of 2-hydrazinoadenosine with aldehydes or ketones is a simple path to a large variety of 2-(*N'*-alkylidenehydrazino)adenosines. In the isolated guinea pig heart the *n*-pentylidene (8), 3-methylbutylidene (18), cyclohexylmethylene (9), and cyclohex-3-enylmethylene (13) derivatives strongly promote coronary vasodilation ($EC_{50} \leq 1$ nM) but only weakly retard AV node conduction. That 2-[*N'*-(cyclohex-1-enylmethylene)hydrazino]adenosine, 12, is substantially less vasoactive than either 9 or 13 suggests that rigidity introduced into the alkyl substituent by the conjugation of the cyclohexene and methylidine double bonds prevents optimum interaction with the receptor. Limited comparisons of the 2-(*N'*-alkylidenehydrazino)adenosines with 2-(alkylamino)adenosines and 2-alkoxyadenosines suggest that the three classes of nucleosides are approximately equiactive as coronary vasodilators.

Experimental Section

Except for 1-cyclohexenecarboxaldehyde, the ketones and aldehydes were available commercially or were prepared by the oxidation of the corresponding alcohols by pyridinium chlorochromate.[19] A minor modification of a literature procedure[20] described below furnished 1-cyclohexenecarboxaldehyde. The purification of nucleosides by preparative reverse-phase chro-

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matography employed either low-pressure elution of tandem 1.5 × 12 in. columns packed with 40–60 μm C-18 silica or high-pressure elution on a Rainin Auto-Prep, fitted with a 1 × 25 cm column of C-18 silica. Both techniques employed elution by gradients of CH_3OH/H_2O as described in Table I. Melting points were measured on a Thomas-Hoover apparatus and are uncorrected. MHW Laboratories (Tucson, AZ) performed the elemental analyses. The ^1H NMR spectrum of each compound in DMSO-d_6, referred to a TMS interval standard, was consistent with the putative structure. Analytical reverse-phase HPLC showed that product accounted for >99% of the UV-absorbing material in samples submitted for bioassay.

2',3',5'-Tri-*O*-acetyl-2,6-dichloropurine Riboside (2). A 1-L flask fitted with a gas inlet tube, magnetic stirring bar, and pressure-equalizing dropping funnel containing *tert*-butyl nitrite (10 mL, 84 mmol) was charged with 1 (10 g, 22.4 mmol), CuCl (2.5 g, 25.3 mmol), and 250 mL of $CHCl_3$. Under magnetic stirring and at room temperature, anhydrous Cl_2 was introduced in a slow stream until an increase in the rate of bubbling indicated incipient saturation. The addition of Cl_2 continued during the cautious dropwise addition of *tert*-butyl nitrite (*exothermic*!) at a rate that maintained gentle reflux. Stirring continued for an additional 10 min and then the dark mixture was poured over ice. Solid $Na_2S_2O_3$ added to the vigorously stirred mixture (*exothermic*!) reduced excess Cl_2 and turned the mixture light yellow. Filtering the organic phase through Celite, drying ($MgSO_4$), evaporation, and crystallization from methanol yielded 8.5 g (81%) of **2**.

2-Hydrazinoadenosine (4). Stirring a solution of **3** (20 g, 66.3 mmol) in 100 mL of hydrazine hydrate for 10 h caused the disappearance of **3**. Diluting the solution with 700 mL of 2-propanol precipitated a gum that was taken up in 200 mL of water and stirred overnight. Crystalline product collected by filtration was washed and dried, yielding 16.5 g (84%) of off-white crystals. Concentration of the mother liquor to less than 50 mL yielded an additional 1.3 g of product for an overall yield of 17.8 g (90%).

1-Cyclohexenecarboxaldehyde. A mixture of cyclohexanecarboxaldehyde (16.8 g, 0.15 mol) and $CaCO_3$ (30 g, 0.3 mol) in 150 mL of CH_2Cl_2 was magnetically stirred at room temperature during the dropwise addition of a solution of Br_2 (24.8 g, 0.155 mol) in 25 mL of CH_2Cl_2 at a rate that maintained gentle reflux. The pale orange mixture was stirred for an additional hour and filtered and the salt washed with CH_2Cl_2. The organic layer was shaken with 150 mL of saturated $Na_2S_2O_4$, washed once with water, and dried over $MgSO_4$. The residual syrup after evaporation was mixed with 75 mL of diisopropylethylamine and heated at reflux overnight, at which time ^1H NMR showed that the intensity of the allylic resonance at 6.83 ppm equaled that of the aldehyde proton at 9.47 ppm. The product was filtered off and the cake of salts was pulverized and extracted with ether. The combined filtrates were treated with 25% $NaHSO_3$, and then more ether was added to complete the precipitation of the bisulfite adduct, which was filtered off, washed with ether, and dried to yield 20.3 g (71%) of white powder.

2-(*N'*-Alkylidenehydrazino)adenosines. General Method. Heating at reflux a mixture of **4** (1.5 g, 5.05 mmol) and 6.1 mmol of aliphatic aldehyde in 50 mL methanol resulted in the disappearance of starting material after 2–24 h. Evaporation of solvent and trituration of the residue with hexane prepared the product for purification by means of medium-pressure reverse-phase chromatography or by reverse-phase HPLC, employing the conditions set forth in Table I. The reaction of **4** with aldehydes boiling at <65 °C proceeded at room temperature, going to completion in 24–48 h.

Assays of Cardiovascular Activity. The use of experimental animals in this investigation conformed with 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 catheter 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 inline electromagnetic flowmeter in the aortic perfusion cannula. We increased the rate of nucleoside infusion stepwise, but at rates not exceeding 0.6 mL/min, until the appearance of a 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,[21] 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 that 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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