N, 18.2. Found: C, 40.3; H, 3.6; N, 18.1.

Antiviral Evaluation. The compounds were assayed for antiviral activity in primary rabbit kidney cells by using a viral cytopathogenicity inhibition method as described previously.[11]

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Registry No. 1, 74131-09-2; 2, 91593-18-9; 3, 111160-27-1; 4, 80991-41-9; 5, 109389-25-5; 6, 111160-28-2; 7, 111160-31-7; (*E*)-8, 111160-29-3; (*Z*)-8, 111160-30-6; Ac-dThd-Ac, 6979-97-1; H-dThd-H, 50-89-5; 1,2,4-triazole, 288-88-0; (*E*)-5-(2-bromovinyl)-2′-deoxyuridine, 69304-47-8.

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N^6-(Arylalkyl)adenosines. Identification of N^6-(9-Fluorenylmethyl)adenosine as a Highly Potent Agonist for the Adenosine A_2 Receptor

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Several N^6-(arylalkyl)adenosines related to N^6-benzyladenosine were synthesized, and their A_1 and A_2 adenosine receptor binding affinities were determined. The annulated derivative N^6-(1-naphthylmethyl)adenosine resulted in a very potent A_2 agonist (A_1 K_i = 24 nM, A_2 K_i = 9.1 nM), whereas N^6-(9-anthracenylmethyl)adenosine was virtually inactive (A_1 K_i = 9000 nM, A_2 K_i = 29000 nM). Interestingly, the structurally similar N^6-(9-fluorenylmethyl)adenosine was the most potent A_2 agonist reported to date, with a K_i of 4.9 nM in A_2 binding and 5.1 nM in A_1 binding. The homologues N^6-9-fluorenyladenosine and N^6-[2-(9-fluorenyl)ethyl]adenosine showed little or no activity at either adenosine receptor. Effects of these agents on heart rate and coronary flow in the isolated rat heart paralleled their A_1 and A_2 binding affinities, respectively. These data suggest that for high affinity at the A_2 receptor a planar hydrophobic function at a certain distance and angle from the N^6 nitrogen is required.

Adenosine and adenosine receptor agonists have been shown to produce a variety of pharmacological effects, including vasodilation, negative inotropy, hypotension, inhibition of platelet aggregation, anticonvulsant activity, inhibition of locomotor activity, inhibition of neurotransmitter release, and antilipolytic activity.[1] These actions are due to the activation of membrane-bound adenosine receptors, which are divided into two major subtypes: A_1 receptors, which inhibit adenylate cyclase, and A_2 receptors, which stimulate adenylate cyclase.[2,3] The two receptors have similar but distinguishable structure–activity relationships.[4] Receptor binding assays have been developed for the A_1 receptor and, more recently, the A_2 receptor.[5]

A significant number of agonists have been evaluated for potency in A_1 binding and A_1-mediated pharmacodynamic responses, revealing in part the structural requirements for high A_1 affinity and selectivity.[6,7] Numerous potent, A_1-selective agonists are known.[6-8] As yet, only a few potent and/or selective A_2 receptor agonists have been identified. 2-(Phenylamino)adenosine (CV-1808) (Figure 1) has been reported to be a selective coronary vasodilator,[9] and the A_2 selectivity of this compound has been confirmed in receptor binding.[5] NECA (1-(6-amino-9*H*-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide) possesses high potency in A_2 receptor binding[5] and as a coronary vasodilator,[10] but also has high affinity for the A_1 receptor.[5] N^6-Modified adenosines with high potency or selectivity for the A_2 receptor have not yet been reported. However, N^6-benzyladenosine and N^6-[(*R*)-1-methyl-2-phenylethyl]adenosine (*R*-PIA) have been found to possess appreciable affinities at the A_2 receptor in rat striatal membranes, with K_i values of 280 nM and 120 nM, respectively.[5] Although *R*-PIA is highly A_1 selective, N^6-benzyladenosine has almost equal affinity in A_1 and A_2 binding.[5] In light of the above findings, we have evaluated the A_2 affinities of other N^6-(arylalkyl)adenosines, including several novel agents with bi- or tricyclic aryl moieties. Of particular interest is N^6-(9-fluorenylmethyl)adenosine (1) (Figure 2), a highly potent adenosine A_2 receptor agonist with a K_i value of 4.9 nM in [^{3}H]NECA binding.

Table I. Physical–Chemical Properties of Novel N^6-Substituted Adenosines

compd	mp,[a] °C	(formula) anal.
1	139–142	($C_{24}H_{23}N_5O_4$) C, H, N
4	129–136	($C_{25}H_{23}N_5O_4$) C, H, N
5	210–212	($C_{23}H_{21}N_5O_4$) C, H, N
6	120–123	($C_{25}H_{25}N_5O_4$) C, H, N
7	104–106	($C_{24}H_{23}N_5O_5$) C, H, N
8	89–95	($C_{26}H_{27}N_5O_4$) C, H, N

[a] Melting points are uncorrected.

Chemistry. Adenosine analogues were synthesized at Warner-Lambert/Parke-Davis according to standard chemical procedures[11,12] in which 6-chloropurine riboside

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Table II. N^6-(Arylalkyl)adenosines and Reference Agents. Affinities in A_1 and A_2 Adenosine Receptor Binding Assays and Potencies in the Isolated Rat Heart

compd	K_i,[a] nM		ratio: A_2/A_1	EC_{25},[b] nM	
	A_1	A_2		rate	flow
		Examples			
1	5.1	4.9	0.96	100	2
2	24	9.1	0.38	400	1.2
3	12.3	230	19	110	30
4	9000	29000	3.2		
5	6200	42000	6.7		
6	320	4300	13	>3000	400
7	62	48	0.77	1200	2
8	160	390	2.4		
		Reference Agents			
2-(phenylamino)ado[c]	590	120	0.21	7000	3
NECA	6.3	12	1.9	21	1.1
N^6-benzylado	120	280	2.4	2300	35
N^6-(2,2-diphenylethyl)ado	6.8	25	3.7	90	10
2-chloroado	9.3	63	6.8	170	13
N^6-(2-phenylethyl)ado	13	160	13	300	70
R-PIA	1.2	120	100	13	10
N^6-cyclopentylado	0.59	460	780	3	>100

[a] A_1 and A_2 affinities of compounds were determined in [^{3}H]-N^6-cyclohexyladenosine binding to rat whole brain membranes and [^{3}H]-NECA binding to rat striatal membranes, respectively, as previously described.[5] Values are means of three or more independent determinations. Affinities of reference agents are taken (with permission) from ref 5 and 15. [b] EC_{25} values for reduction of heart rate (A_1 response) and enhancement of coronary flow (A_2 response) in the rat isolated heart preparation were determined as previously described.[17] [c] Adenosine is abbreviated ado.

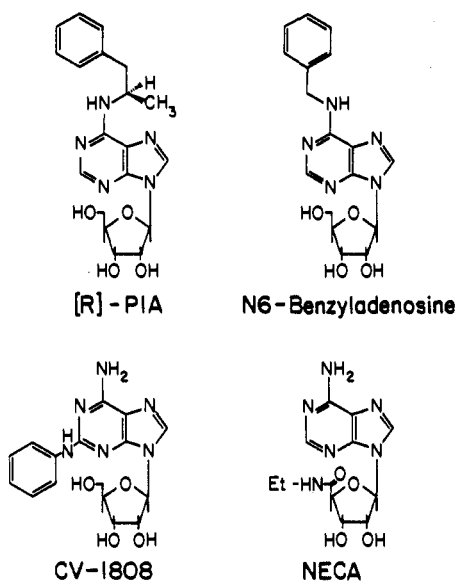

Figure 1.

was reacted with the appropriate amine in the presence of triethylamine in refluxing ethanol. All the side-chain amines are known and were prepared in a conventional manner either by catalytic hydrogenation of the readily available nitrile (compounds **3**, **4**, **6**, and **8**) or from commercially available carboxylic acid derivatives (compounds **1** and **7**). Physical properties (^{1}H NMR, IR, mass spectra, and elemental analysis) were consistent with the chemical structures (Table I).

Receptor Binding. N^6-(1-Naphthylmethyl)adenosine (**2**) showed significant improvement in affinities at both A_1 and A_2 receptors compared to N^6-benzyladenosine, with K_i values of 24 and 9.1 nM, respectively (Table II). This compound was equal to NECA in A_2 affinity, but possessed fivefold higher A_2 selectivity; it has been previously shown

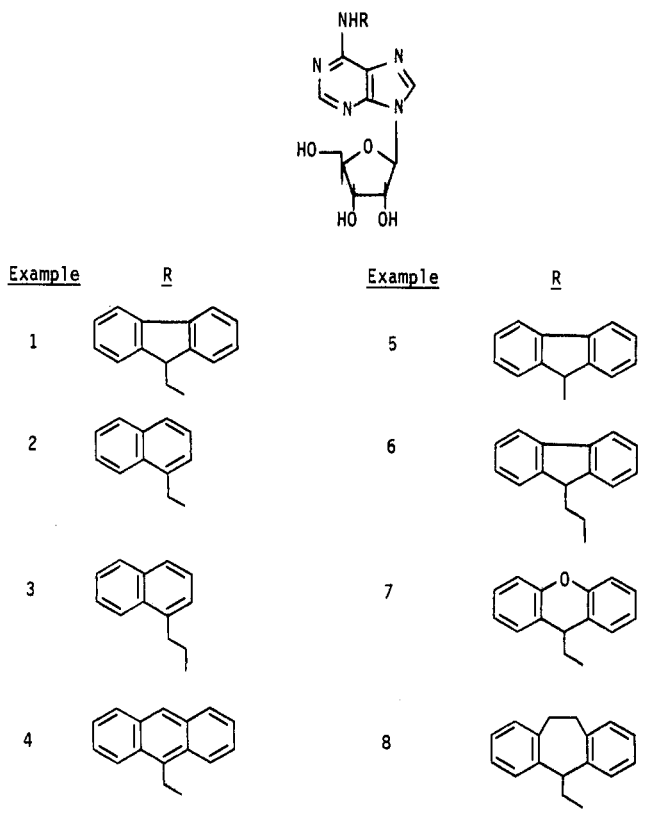

Figure 2.

to be a potent coronary vasodilator[13,14] and a moderately potent inhibitor of A_1 binding.[6] Lengthening the methyl spacer to ethyl (compound **3**) rendered the naphthyl derivative A_1 selective with moderate affinity at the A_2 receptor. The A_1 binding affinities and coronary vasodilator potencies of **3** have been reported.[6,14] Interestingly, appending an additional phenyl moiety onto **2** results in

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N^6-(9-anthracenylmethyl)adenosine (4), which is virtually devoid of affinity at A_1 and A_2 receptors. These data suggest that for high affinity at the A_2 receptor a planar hydrophobic function at a certain distance from the N^6 nitrogen is required. However, additional steric bulk such as in the case of 4 is not tolerated.

Another compound of interest in defining the geometry of the aryl-binding portion of the A_2 receptor is N^6-(9-fluorenylmethyl)adenosine (1). This compound exhibited high absolute potencies at both A_1 and A_2 receptors, with K_i values of 5.1 and 4.9 nM, respectively; its A_2 potency was twice that of NECA, making it the most potent A_2 agonist yet reported. A comparison between the highly active 1 and the inactive 4 provides some information about the geometry of the interaction between the adenosine receptor and the N^6 side chain. Like 4, 1 possesses a planar tricyclic aryl moiety, and in both compounds a single-carbon spacer forms a bridge between the purine N^6 and the central ring of the tricyclic group. The most prominent structural difference between 1 and 4 is the fact that the spacer carbon is coplanar with the tricyclic ring in 4, but is out of the plane at an angle of about 50° in 1. The angle of the tricyclic ring relative to N^6 therefore must be important. Other factors, including the relative positions of the two outside aryl rings and the π conjugation of the central ring, may also contribute to the difference between 1 and 4. Compound 1 can also be considered a cyclized analogue of N^6-(2,2-diphenylethyl)adenosine (CI-936), an adenosine agonist that has been reported to possess high affinity for A_2 receptors.[15] Compared to 1, CI-936 is almost equally potent in A_1 binding (K_i = 6.8 nM), but only $^1/_5$ as potent in A_2 binding (K_i = 25 nM).

Examples 5 and 6, the lower and higher spacer homologues of 1 respectively, showed little or no activity at either adenosine receptor, confirming the importance of distance and angle relative to N^6. It is important to note that all the compounds discussed so far have a planar aryl group in the N^6 side chain. To better understand the significance of this, we prepared analogues of 1 in which spacer atoms were incorporated to introduce nonplanarity at the aryl moiety, such as in examples 7 and 8. Both compounds once again showed appreciable loss of affinity, although not to the extent seen with 4.

Coronary Flow and Heart Rate. Using isolated heart preparations, one can identify adenosine receptor mediated responses by selective effects on coronary flow and heart rate. Previous studies provide evidence that the adenosine receptor mediated decrease in heart rate is due to A_1 receptor activation and that the vasodilation is mediated through A_2 receptor activation.[16]

We have utilized the rat isolated heart preparation to define, on a pharmacologic basis, the receptor selectivity of the adenosine agonists discussed in this paper. For most compounds, the pharmacologic potency of the coronary flow effects correlated closely with receptor binding affinity (Table II). This is illustrated by examples 1, 2, and 7, which have the highest A_2 binding affinities and are the most potent vasodilators on the basis of their coronary flow EC_{25} values. The vasodilator potencies of these three compounds are almost equal to that of NECA, the most potent reference agent. The greater activity of 2 in the buffer-perfused rat heart (potency 8 times that of *R*-PIA) compared to the blood-perfused dog heart (potency $^1/_2$ that of *R*-PIA) is probably due to binding of the very hydrophobic 2 to albumin in the latter preparation.[14]

In summary, this paper describes the first N^6-substituted adenosine derivatives with high potency at the adenosine A_2 receptor. The most potent agent, N^6-(9-fluorenylmethyl)adenosine, is approximately equal to NECA in both A_2 binding affinity and coronary vasoactivity.

Experimental Section

Melting points were estimated on an electrothermal melting point apparatus and are uncorrected. Analytical thin-layer chromatography (TLC) was done with precoated glass plates (EM Reagents silica gel 60 F-254). ^{1}H NMR spectra was obtained on a Varian EM-390 or Varian XL-200 spectrometer. Mass spectra were recorded on a Finnegan 4500 mass spectrometer with an 11/250 data system. Solvents and reagents were commercially available unless otherwise noted and were used directly. Elemental analyses were determined at Parke-Davis Pharmaceutical Research Division.

Synthesis of N^6-Substituted Adenosines. All the adenosine analogues were synthesized by reacting 6-chloropurine riboside with the appropriate amine in the presence of triethylamine in refluxing ethanol.[11,12] Workup consisted of crystallizing the product from ethanol upon cooling. Nucleosides that did not crystallize from the reaction mixture were purified by evaporation of ethanol followed by treatment with cold water, which precipitated the nucleoside, which was filtered, dried, and crystallized from methanol/chloroform or chloroform/hexane. The side-chain amines were prepared either by catalytic hydrogenation of the readily available nitrile (entries 3, 4, 6, and 8) or from the commercially available carboxylic acid derivative (compounds 1 and 7). The amines for compounds 2 and 5 were obtained commercially.

Receptor Binding Assays. Affinities of N^6-(arylalkyl)-adenosines were determined in A_1 receptor binding using [^{3}H]-N^6-cyclohexyladenosine in rat whole brain membranes, and in A_2 binding using [^{3}H]NECA in rat striatal membranes in the presence of 50 nM N^6-cyclopentyladenosine.[5]

Coronary Flow and Heart Rate Measurements. Heart rate and coronary vascular activities were measured in an isolated rat heart preparation perfused by the Langendorff method as previously described.[17] In brief, male Sprague–Dawley normotensive rats were anesthetized with sodium pentobarbitol (50 mg/kg, ip) and heparinized (2000 units, ip) to prevent blood clotting. Hearts were rapidly isolated and perfused with a modified Krebs Henseleit bicarbonate buffer. Coronary perfusion pressure was maintained constant at 70 mmHg throughout the experiment. Heart rate was determined from the electrocardiogram recorded from the base of the heart. Vascular activity was assessed by measuring coronary flow with a calibrated perfusion pump. The adenosine receptor agonists were prepared in NaOH solution at pH 10.0. The small volumes of NaOH used did not affect the pH (7.36–7.42) of the perfusion buffer. In 60-min stability experiments, heart rate varied less than 5% and coronary flow less than 11% from control in an average of six hearts. On the basis of receptor binding affinities, agonists were tested at four concentrations at half-log increments over the range of 1 nM to 1 μM. Effects of the adenosine receptor agonist on heart rate and coronary flow are expressed as percent change from control and given as EC_{25} (25% change from control) values (Table II). The EC_{25} values were graphically determined on the average effect on two hearts for each compound.

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Registry No. 1, 103450-84-6; 2, 21924-65-2; 3, 101565-83-7; 4, 94617-16-0; 5, 103450-83-5; 6, 103450-85-7; 7, 103450-86-8; 8, 111209-71-3.

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