

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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(to J.R.S.), G. D. Searle & Co Ltd. for financial assistance, Anita Van Lierde for excellent technical help with the antiviral assays, and Christiane Callebaut for fine editorial assistance.

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.

N⁶-(Arylalkyl)adenosines. Identification of N⁶-(9-Fluorenylmethyl)adenosine as a Highly Potent Agonist for the Adenosine A₂ Receptor

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Several N⁶-(arylalkyl)adenosines related to N⁶-benzyladenosine were synthesized, and their A₁ and A₂ adenosine receptor binding affinities were determined. The annulated derivative N⁶-(1-naphthylmethyl)adenosine resulted in a very potent A₂ agonist (A₁ K_i = 24 nM, A₂ K_i = 9.1 nM), whereas N⁶-(9-anthracenylmethyl)adenosine was virtually inactive (A₁ K_i = 9000 nM, A₂ K_i = 29 000 nM). Interestingly, the structurally similar N⁶-(9-fluorenylmethyl)adenosine was the most potent A₂ agonist reported to date, with a K_i of 4.9 nM in A₂ binding and 5.1 nM in A₁ binding. The homologues N⁶-9-fluorenyladenosine and N⁶-[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 A₁ and A₂ binding affinities, respectively. These data suggest that for high affinity at the A₂ receptor a planar hydrophobic function at a certain distance and angle from the N⁶ 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 antipolytic activity.¹ These actions are due to the activation of membrane-bound adenosine receptors, which are divided into two major subtypes: A₁ receptors, which inhibit adenylate cyclase, and A₂ 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 A₁ receptor and, more recently, the A₂ receptor.⁵

A significant number of agonists have been evaluated for potency in A₁ binding and A₁-mediated pharmacodynamic responses, revealing in part the structural requirements for high A₁ affinity and selectivity.^{6,7} Numerous potent, A₁-selective agonists are known.⁶⁻⁸ As yet, only a few potent and/or selective A₂ receptor agonists have been identified. 2-(Phenylamino)adenosine (CV-1808) (Figure 1) has been reported to be a selective coronary vasodilator,⁹ and the A₂ selectivity of this compound has been confirmed in receptor binding.⁵ NECA (1-(6-amino-9H-purin-9-yl)-1-deoxy-N-ethyl-β-D-ribofuranuronamide) possesses high potency in A₂ receptor binding⁵ and as a coronary vasodilator,¹⁰ but also has high affinity for the A₁ receptor.⁵ N⁶-Modified adenosines with high potency or selectivity for the A₂ receptor have not yet been reported. However, N⁶-benzyladenosine and N⁶-[(R)-1-methyl-2-phenylethyl]adenosine (R-PIA) have been found to possess appreciable affinities at the A₂ receptor in rat striatal membranes, with K_i values of 280 nM and 120 nM, respectively.⁵ Although R-PIA is highly A₁ selective, N⁶-benzyladenosine has almost equal affinity in A₁ and A₂ binding.⁵ In light of the above findings, we have evaluated

Table I. Physical-Chemical Properties of Novel N⁶-Substituted Adenosines

compd	mp, °C	(formula) anal.
1	139-142	(C ₂₄ H ₂₃ N ₅ O ₄) C, H, N
4	129-136	(C ₂₅ H ₂₃ N ₅ O ₄) C, H, N
5	210-212	(C ₂₃ H ₂₁ N ₅ O ₄) C, H, N
6	120-123	(C ₂₅ H ₂₅ N ₅ O ₄) C, H, N
7	104-106	(C ₂₄ H ₂₃ N ₅ O ₅) C, H, N
8	89-95	(C ₂₆ H ₂₇ N ₅ O ₄) C, H, N

^a Melting points are uncorrected.

the A₂ affinities of other N⁶-(arylalkyl)adenosines, including several novel agents with bi- or tricyclic aryl moieties. Of particular interest is N⁶-(9-fluorenylmethyl)adenosine (1) (Figure 2), a highly potent adenosine A₂ receptor agonist with a K_i value of 4.9 nM in [³H]NECA binding.

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

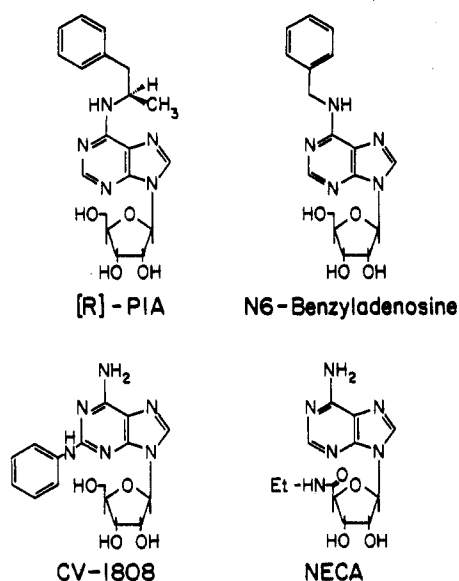
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Table II. *N*⁶-(Arylalkyl)adenosines and Reference Agents. Affinities in A₁ and A₂ Adenosine Receptor Binding Assays and Potencies in the Isolated Rat Heart

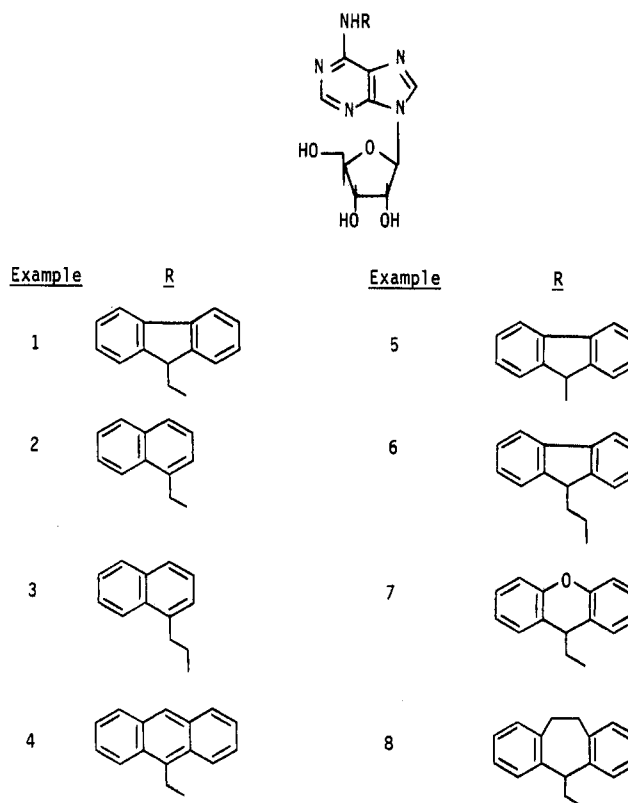
compd	K_i^a nM		ratio: A_2/A_1	EC_{25}^b nM	
	A_1	A_2		rate	flow
Examples					
1	5.1	4.9	0.96	100	2
2	24	9.1	0.38	400	1.2
3	12.3	230	19	110	30
4	9000	29 000	3.2		
5	6200	42 000	6.7		
6	320	4300	13	>3000	400
7	62	48	0.77	1200	2
8	160	390	2.4		
Reference Agents					
2-(phenylamino)ado ^c	590	120	0.21	7000	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 A₁ and A₂ affinities of compounds were determined in [³H]-*N*⁶-cyclohexyladenosine binding to rat whole brain membranes and [³H]-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 EC₂₅ values for reduction of heart rate (A₁ response) and enhancement of coronary flow (A₂ 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 (¹H 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 A₁ and A₂ 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 A₂ affinity, but possessed fivefold higher A₂ selectivity; it has been previously shown

**Figure 2.**

to be a potent coronary vasodilator^{13,14} and a moderately potent inhibitor of A₁ binding.⁶ Lengthening the methyl spacer to ethyl (compound 3) rendered the naphthyl derivative A₁ selective with moderate affinity at the A₂ receptor. The A₁ 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 A₁ and A₂ receptors. These data suggest that for high affinity at the A₂ receptor a planar hydrophobic function at a certain distance from the N⁶ 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 A₂ receptor is *N*⁶-(9-fluorenylmethyl)adenosine (1). This compound exhibited high absolute potencies at both A₁ and A₂ receptors, with K_i values of 5.1 and 4.9 nM, respectively; its A₂ potency was twice that of NECA, making it the most potent A₂ 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 N⁶ 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 N⁶ 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 N⁶ 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 A₂ receptors.¹⁵ Compared to 1, CI-936 is almost equally potent in A₁ binding (K_i = 6.8 nM), but only 1/5 as potent in A₂ 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 N⁶. It is important to note that all the compounds discussed so far have a planar aryl group in the N⁶ 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 A₁ receptor activation and that the vasodilation is mediated through A₂ 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 A₂ binding affinities and are the most potent vasodilators on the basis of their coronary flow EC₂₅ 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 A₂ receptor. The most potent agent, *N*⁶-(9-fluorenylmethyl)adenosine, is approximately equal to NECA in both A₂ 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). ¹H 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 A₁ receptor binding using [³H]-*N*⁶-cyclohexyladenosine in rat whole brain membranes, and in A₂ binding using [³H]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 pentobarbital (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 μ M. Effects of the adenosine receptor agonist on heart rate and coronary flow are expressed as percent change from control and given as EC₂₅ (25% change from control) values (Table II). The EC₂₅ 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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