N^6-Substituted Adenosine Receptor Agonists: Potential Antihypertensive Agents[1]

B. K. Trivedi,* C. J. Blankley, J. A. Bristol, H. W. Hamilton, W. C. Patt, W. J. Kramer, S. A. Johnson, R. F. Bruns, D. M. Cohen, and M. J. Ryan

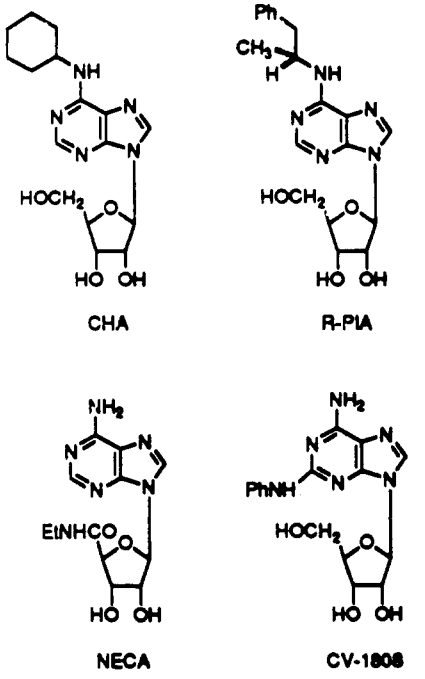
Departments of Chemistry and Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105. *Received August 20, 1990*

Adenosine is known to exert a wide range of pharmacological effects including hypotension. This effect of adenosine suggested that modified analogues of adenosine might provide useful antihypertensive agents. Thus, we prepared a series of novel N^6-benzocycloalkyladenosines and studied their receptor binding and antihypertensive activity. The structure–activity relationship study shows that the adenosine analogues having the hydrophobic phenyl moiety one carbon away from the C6-nitrogen have modest affinity and selectivity for the A_1 receptor, whereas those with the phenyl moiety two carbons away from the C6-nitrogen have excellent affinity and selectivity for the A_1 receptor. Many of these analogues showed excellent antihypertensive activity with a wide range of effects on heart rate. There is no direct correlation between the receptor binding affinities and antihypertensive activity; however, it is more closely associated with A_1 than A_2 affinity. The bradycardic effect of these agonists seems to be due to the A_1 affinity. From this set, compound **3** was further evaluated in secondary antihypertensive screens. It lowered the blood pressure dose dependently with effects lasting for over 20 h following administration of a 30 mg/kg dose. Compound **3** was also effective in lowering blood pressure in a renal hypertensive rat model. Thus, appropriately modified N^6-substituted adenosines represent a novel class of antihypertensive agents.

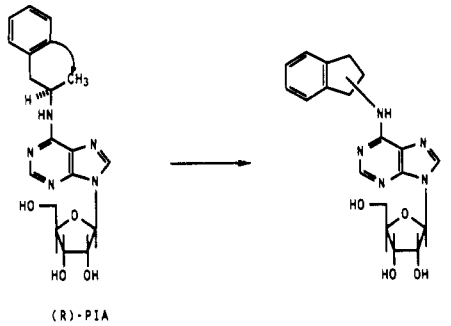
Adenosine is known to elicit its pharmacological effects via membrane-bound receptors designated as A_1 and A_2. Activation of A_1 receptors leads to inhibitory effects on adenylate cyclase whereas activation of A_2 receptors has stimulatory effects.[2-4] The two receptor subtypes have similar but distinguishable structure–activity relationships. Receptor binding assays have been developed for the A_1 receptor[5] and, more recently, for the A_2 receptor.[6] Among the wide variety of pharmacological effects such as modulation of neurotransmission, antilipolytic activity, negative inotropy, and vasodilation, hypotension is one of the prominent effects of adenosine. The latter effect of adenosine suggested that modified analogues with agonist activity might provide therapeutically useful antihypertensive agents.

Initially, we examined reference adenosine agonists N^6-cyclohexyladenosine (CHA), N^6-(*R*)-(phenylisopropyl)adenosine (*R*-PIA), adenosine-5′-*N*-ethylcarboxamide (NECA), and 2-(phenylamino)adenosine (CV-1808) in the spontaneously hypertensive rat (SHR) model of hypertension. Structures of these compounds are shown in Chart I. The reference agents caused dose-dependent decreases in blood pressure, accompanied by a reduction in heart rate (Figure 1). It was of interest to note that the A_1 selective agents (CHA, *R*-PIA) had the greatest bradycardic effects and the magnitude of this effect paralleled that for blood pressure reduction. NECA, a compound with equivalent potencies at A_1 and A_2 receptors, showed bradycardic effects at doses considerably higher than those eliciting hypotension. CV-1808, a compound with modest A_2 selectivity and considerably reduced A_1 potency, elicited moderate tachycardia in response to a blood pressure reduction, more reminiscent of the profile of vasodilating antihypertensives with other mechanisms of action; e.g., hydralazine and minoxidil. This and other evidence from the literature[7] support the idea that A_1 receptors possess direct cardiac negative chronotropic activity and offered the interesting possibility that a vasodilator antihypertensive with minimal reflex tachycardia could be designed if the proper balance of A_1 and A_2 receptor activity could be introduced into an adenosine analogue. A considerable number of adenosine derivatives

Chart I. Reference Adenosine Agonists



Scheme I



(1) This paper was presented in part to the Division of Medicinal Chemistry at the meeting of the Third Chemical Congress of North America in Toronto, Canada, 1988, MEDI-32.

(2) van Calker, D.; Muller, M.; Hamprecht, B. *J. Neurochem.* **1979**, *33*, 999-1005.
(3) Londos, C.; Cooper, D. M. F.; Wolff, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2551-2554.
(4) Hamprecht, B.; van Calker, D. *Trends Pharmacol. Sci.* **1985**, *6*, 153-154.
(5) Yeung, S. M. H.; Green, R. D. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *325*, 218-225.
(6) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331-346.
(7) Daly, J. W. *J. Med. Chem.* **1982**, *26*, 197-207.

0022-2623/91/1834-1043$02.50/0

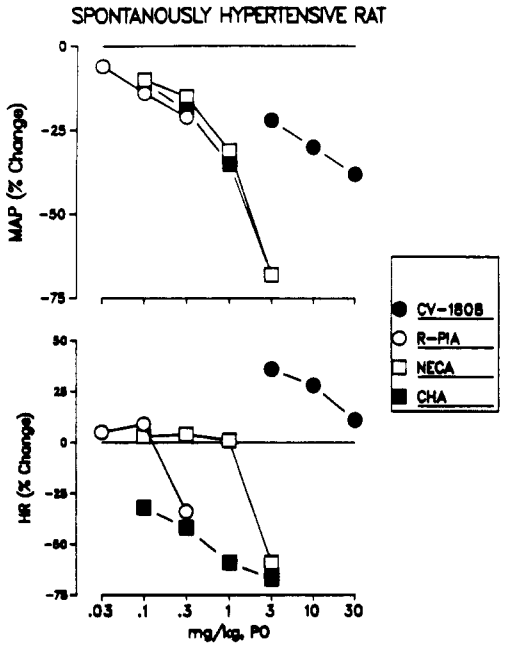


Figure 1. Antihypertensive effects of reference adenosine agonists.

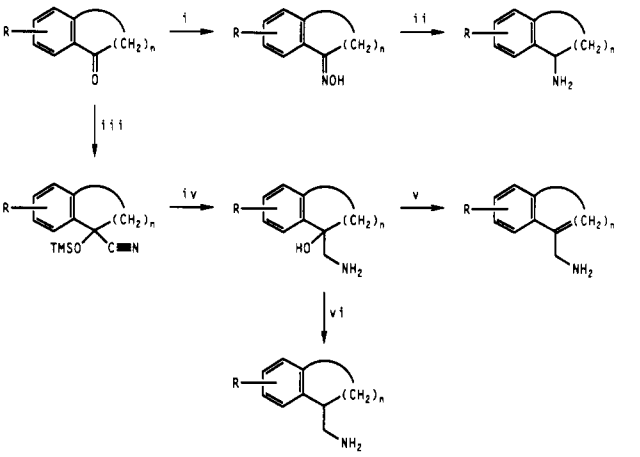
modified at N^6 were prepared which were found to possess a range of A_1/A_2 potency ratios in receptor binding assays. This paper reports the synthesis of a series of compounds conceived as constrained analogues of *R*-PIA, their receptor binding profiles, and their antihypertensive and heart rate effects in SHR.

Chemistry

Taking *R*-PIA as a starting point, we envisioned making rigid analogues by connecting the methyl carbon and the aromatic ring to give a series of easily accessible benzocycloalkyladenosine analogues (Scheme I). The requisite amines were either obtained commercially or prepared from available ketones as shown in Scheme II. Ketones were converted to the desired amines via catalytic hydrogenation of the corresponding oximes. Aminomethyl homologues were prepared by first reacting the ketone with trimethylsilyl cyanide (TMSCN) in the presence of ZnI_2 to give the corresponding cyanohydrin,[8,9] followed by reduction with lithium aluminum hydride to afford the ethanolamine. The latter could be converted to the unsaturated amine upon treatment with dilute acid, or to the saturated analogue by hydrogenolysis.

The chiral indane and tetralin amines were obtained by the resolution of the respective racemic amines using *N*-acetyl-D-leucine or D- and L-tartaric acid, respectively.[10,11] The 5-methoxy-1-indanylamine was resolved by using (*R*)-*N*-acetyl-(3,4-dimethoxyphenyl)alanine[12] in methanol as described in the Experimental Section. The 5-butoxy-1-indanylamine was resolved with dibenzoyl-L-tartaric acid as a resolving agent whereas the 7-methoxy-1-tetralinamine was resolved by D- and L-tartaric acid.[13] Each of these amines were in turn reacted with 6-chloropurine ribonucleoside in presence of triethylamine in refluxing ethanol to afford the corresponding nucleoside derivatives.[13,14]

Scheme II[a]



[a] (i) NH_2OH·HCl/NaOAc/EtOH; (ii) H_2, Pd/C; (iii) TMSCN/$AlCl_3$; (iv) LAH; (v) dilute HCl; (vi) H_2, Pd/C.

Receptor Binding

Each of the compounds was evaluated for A_1 and A_2 adenosine receptor binding activity. A broad range of potencies at both receptor subtypes was observed for the N^6-benzocycloalkyl analogues (Table II). A_1 activity (K_i) ranged from 3.6 nM for **55** to 7550 nM for **37**. Similarly, A_2 potency ranged from 44 nM for **23** to >100 000 nM for **16**. On average, this group of compounds is about 10–50-fold A_1 selective. The highest A_1 potencies were found among the 1-tetralinyl analogues, while the greatest A_2 potencies were found in the aminomethyl analogues, which lack a branch at the α-position and retain the embedded β-phenethyl moiety known to be optimal for A_2 potency. Aryl substituents in the 1-indanyl series had minimal effect on A_1 potency, but reduced A_2 affinity in proportion to increasing size. In the 1-tetralinyl series, this pattern was also observed for substitution at the 5- and 7-positions for A_1 receptor activity. A_2 potency was adversely affected by increasing size and by moving the substitution from the 5- to 7-position. In the case of a methoxy substituent peri to the N^6-position (**8**), activity at both receptors was severely compromised.

R-PIA is known to be more potent at both receptor subtypes than *S*-PIA. The present series also showed the same dependence on chirality at an α-branch position, with the exception of the 7-butoxyindan analogue pair (**16**, **17**). In the latter case, equal A_1 affinity was found for the two diastereomers.

Compound **45** had the greatest A_2/A_1 ratio and was slightly A_2 selective. All of the others were A_1 selective in degrees varying from 2-fold (**54**) to over 100-fold (**16**). Hydroxy substitution β to the C6-nitrogen resulted in considerably reduced potency at both receptor subtypes and, taken together with a similar result from the methoxy substituted analogue **8**, suggests the existence of an H-bonding interaction unfavorable to binding. This may either compromise the availability of the required N^6-H for binding to the receptor or alter the side-chain conformation in an unfavorable manner.

When the higher homologues were evaluated in the receptor binding assay, we found compound **45** to be the only A_2 selective agent among the compounds presented in this

(8) Evans, D. A.; Truesdale, L. K.; Carol, G. L. *J. Org. Chem.* **1974**, *39*, 914.
(9) Beeley, N. R. A.; Cremer, G.; Dorlhene, A.; Mompon, B.; Pascard, C. *J. Chem. Soc.* **1983**, 1046.
(10) Ghislandi, V.; Vercesi, D. *Bull. Chim. Pharm.* **1976**, *115*, 489–500.
(11) Ghislandi, V.; Vercesi, D. *Farmaco Ed. Sci.* **1971**, *26*, 474–486.
(12) Goel, O. P. US Patent 4,833,273, 1989.
(13) Trivedi, B. K.; Moos, W. H.; Hamilton, H. W.; Patt, W. C. US Patent 4,791,103, 1988.
(14) Fleysher, M. H. *J. Med. Chem.* **1972**, *15*, 187–191.

paper. This compound is structurally similar to the N^6-(1-naphthalenylmethyl)adenosine which is very potent at both A_1 (K_i = 24 nM) and A_2 (K_i = 9.4 nM) receptors. This may suggest that these two compounds occupy the same binding region in relationship to the N^6-nitrogen. This spatial arrangement must be different from that of other analogues, since for both compounds the β-carbon is sp_2 hybridized and thus the aromatic portion of the molecule is coplanar to the α-carbon.

Using these and other N^6-substituted adenosine analogues, we have done molecular modeling studies using the "active-analogue" approach[15] to map out the N^6 region of the A_2 receptor.[16] The model can rationalize the relative A_2 potencies of the nonaryl ring substituted analogues in this report quite satisfactorily, including some of the activity differences due to subtle structural changes, such as those between **50** and **45**, which show markedly different A_2 affinities.

Antihypertensive Activity

Compounds were then evaluated in SHR following a standard protocol described previously[17] at 10 mg/kg, and blood pressure and heart rate were continuously monitored for 10 h after dosing. Table II lists the maximal blood pressure and heart rate effects observed, as well as the times at which they occurred. As expected, many of the compounds showed excellent antihypertensive activity. Effects on heart rate ranged from strongly bradycardic; e.g., **10**, **28**, and **31**, to strongly tachycardic; e.g., **33** and **35**. The relationship between receptor binding affinities and oral antihypertensive activity is obscure. However, changes in heart rate are significantly associated with the A_2/A_1 ratio more than with either affinity alone. Of the two subtypes, A_1 potency appears to be the best correlate, i.e., greater A_1 potency is associated with a greater reduction in heart rate. This is shown by the following correlation coefficients: HR (% change)/log (A_2/A_1), r = 0.55 (p = 0.0001); HR/log A_1, r = 0.45 (p = 0.002); HR/log A_2, r = 0.08 (p = not significant). This includes compounds which show a significant reduction (>10 mmHg) in blood pressure.

The latter observation fits with our original expectation that a balance of A_1 and A_2 affinity could be found that would minimize heart rate effects in the presence of a substantial antihypertensive response. Unexpected was the observation that A_1 receptor affinity appears to be the primary determinant of antihypertensive activity. The A_2 receptor subtype has been shown to mediate coronary vasodilation[18] and might have been expected to be responsible for vasodilation in other vascular beds. Since A_1 receptor activities do not include vasodilation, the mechanism of blood pressure reduction must be sought among other A_1 actions. Strong possibilities are modulation of noradrenergic neurotransmission[19] and/or suppression of renin release.[20] This is supported by the observation that certain compounds described here, e.g., **3**, have been demonstrated to attenuate the reflex release of renin in response to potent vasodilation.[21]

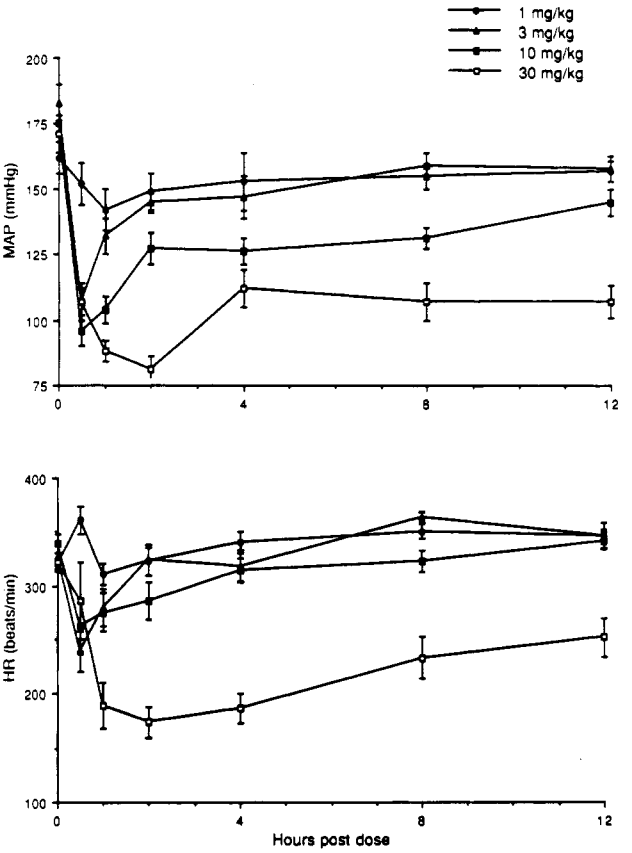


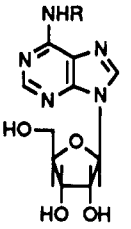
Figure 2. Dose response and time course of antihypertensive activity of **3** in SHR.

The cardiovascular profile of one group of compounds could not be predicted based on adenosine mechanism of action. Most of the alkoxytetralines, in contrast to the alkoxyindans, showed frank tachycardia in spite of having good A_1 potency and high A_1 selectivity. This is a consistent finding and, though we have no direct evidence, it seems likely that additional mechanisms may be coming into play with these compounds. The N^6-side chain amines bear close resemblance to known potent adrenergic and dopaminergic compounds, and it may be that the adenosine analogues of these compounds can interact significantly through mechanisms of this origin. In any case, this group provides a notably different profile from the other adenosine receptor agonists studied.

From this set of novel N^6-substituted adenosines, compound **3** was further characterized in terms of dose response, duration of action, etc. Compound **3** was initially evaluated in various receptor binding assays where it showed affinity for the adenosine A_1 and A_2 receptors with IC_{50} values of 22 and 412 nM, respectively (Table II). However, it was essentially inactive at the benzodiazepine, serotonin, muscarinic, dopamine, and α- and β-adrenergic receptors with IC_{50} values of >10 μM.[22] Compound **3** caused a reduction in blood pressure that was accompanied by little change in heart rate except at high doses. Once again, the A_1 selective agonist CHA caused substantial bradycardia at all doses, and the A_2 selective agonist, CV-1808, showed modest tachycardia. All of them dose

(15) Humblet, C.; Marshall, G. *Drug Dev. Res.* **1981**, *1*, 409–434.
(16) Ortwine, D. F.; Bridges, A. J.; Humblet, C.; Trivedi, B. K. In *Purines in Cell Signalling: Targets for New Drugs*; Jacobson, K., Daly, J., Manganiello, V., Eds.; Springer-Verlag: New York, 1990; pp 152–157.
(17) Smith, R. D.; Wood, T. J.; Tessman, B. K.; Olszewski, B.; Currier, G.; Kaplan, H. R., DHEW Publ. (NIH) (U.S.) No. 78-1473, 41 (1977).
(18) Daly, J. W.; Padgett, W.; Thompson, R. D.; Kasachi, S.; Bugni, W. J.; Olsson, R. A. *Biochemical Pharmacol.* **1986**, *35*, 2467–2481.
(19) Major, T. C.; Weishaar, R. E.; Taylor, D. G. *Eur. J. Pharmacol.* **1989**, *167*, 323–331.
(20) Itoh, S.; Carreteso, O.; Murray, R. *J. Clin. Invest.* **1985**, *76*, 1412–1417.
(21) Ryan, M. J.; Bjork, F. A.; Singer, R. M.; Evans, D. B.; Kaplan, H. R. *Fed. Proc.* **1986**, *45*, 914.
(22) Coughenour, L. Personal Communication.

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



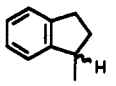
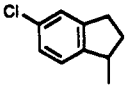
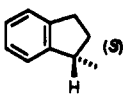
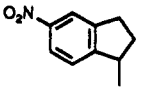
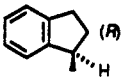
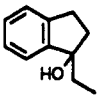
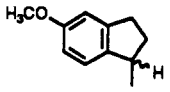
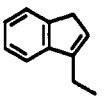
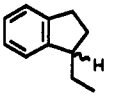
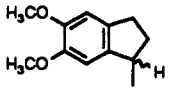
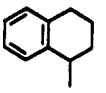
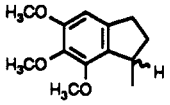
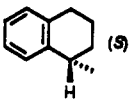
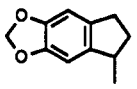
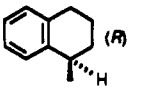
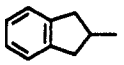
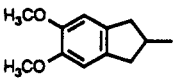
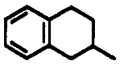
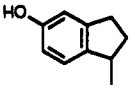
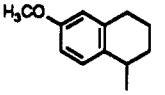
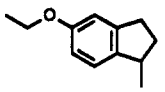
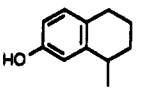
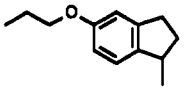
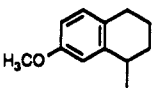
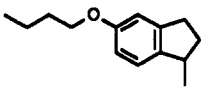
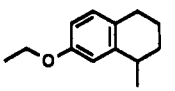
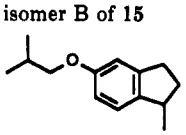
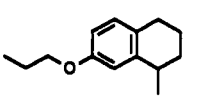
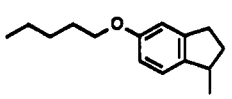
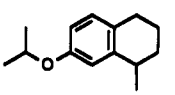
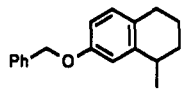
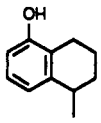
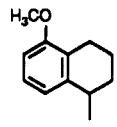
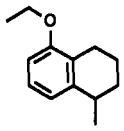
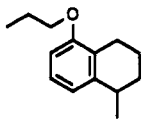
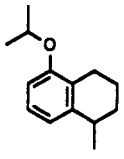
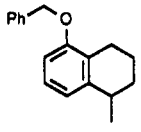
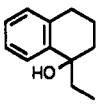
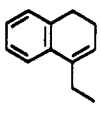
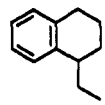
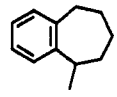
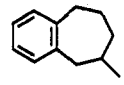
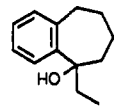
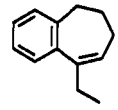
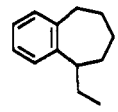
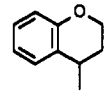
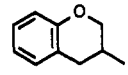
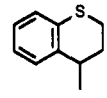
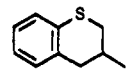
no.	R	mp, °C	formula	no.	R	mp, °C	formula
1		120–122	$C_{19}H_{21}N_5O_4$	20		148–150	$C_{19}H_{20}N_5O_4Cl$
2		123–126	$C_{19}H_{21}N_5O_4 \cdot 0.25CH_3OH$	21		123–125	$C_{19}H_{20}N_6O_6 \cdot 0.5H_2O$
3		185–187	$C_{19}H_{21}N_5O_4$	22		125–127	$C_{20}H_{23}N_5O_5 \cdot 0.12H_2O$
4		105–109	$C_{20}H_{23}N_5O_5 \cdot 0.3H_2O$	23		200–202	$C_{20}H_{21}N_5O_4$
5	isomer A of 4	152–155	$C_{20}H_{23}N_5O_5 \cdot 0.1H_2O$	24		137–139	$C_{20}H_{23}N_5O_4$
6	isomer B of 4	170–173	$C_{20}H_{23}N_5O_5 \cdot 0.5CH_3OH$				
7		113–116	$C_{21}H_{25}N_5O_6$	25		115–120	$C_{20}H_{23}N_5O_4 \cdot 0.15H_2O$
8		107–110	$C_{22}H_{27}N_5O_7 \cdot 0.5H_2O$	26		100–110	$C_{20}H_{23}N_5O_4$
9		211–213	$C_{20}H_{21}N_5O_6 \cdot 0.15H_2O$	27		108–115	$C_{20}H_{23}N_5O_4$
10		159–162	$C_{19}H_{21}N_5O_4$				
11		>240	$C_{21}H_{25}N_5O_6 \cdot 0.2H_2O$	28		130–132	$C_{20}H_{23}N_5O_4 \cdot 0.15H_2O$
12		118–120	$C_{19}H_{21}N_5O_5 \cdot 0.75H_2O$	29		117–120	$C_{21}H_{25}N_5O_5 \cdot 0.2H_2O$
13		110–111	$C_{21}H_{25}N_5O_5 \cdot 0.04CHCl_3$	30		145–147	$C_{20}H_{23}H_5O_5 \cdot 0.2CH_3OH$
14		166–168	$C_{22}H_{27}N_5O_5 \cdot 0.5H_2O$	31		95–110	$C_{21}H_{25}N_5O_5$
15		95–100	$C_{23}H_{29}N_5O_5$	32	isomer A of 29	128–130	$C_{21}H_{25}N_5O_5 \cdot 0.25H_2O$
				33	isomer B of 29	172–174	$C_{21}H_{25}N_5O_5 \cdot CH_3OH$
				34		128–134	$C_{22}H_{27}N_5O_5 \cdot 0.4CH_2Cl_2$
16	isomer A of 15	126–128	$C_{23}H_{29}N_5O_5 \cdot 0.3H_2O$				
17	isomer B of 15	149–151	$C_{23}H_{29}N_5O_5$				
18		106–108	$C_{23}H_{29}N_5O_5$	35		99–102	$C_{23}H_{29}N_5O_5$
19		95–97	$C_{24}H_{31}N_5O_5$	36		115–125	$C_{23}H_{29}N_5O_5 \cdot 0.1CH_2Cl_2$

Table I (Continued)

no.	R	mp, °C	formula
37		105–115	$C_{27}H_{29}N_5O_5 \cdot 0.1CH_2Cl_2$
38		136–138	$C_{20}H_{23}N_5O_5 \cdot 0.9H_2O$
39		87–90	$C_{21}H_{25}N_5O_5 \cdot 0.5C_3H_7OH$
40		130–142	$C_{22}H_{27}N_5O_5 \cdot 0.3CH_2Cl_2$
41		122–125	$C_{23}H_{29}N_5O_5$
42		125–130	$C_{23}H_{29}N_5O_5$
43		115–125	$C_{27}H_{29}N_5O_5 \cdot 0.25CH_2Cl_2$
44		110–115	$C_{21}H_{25}N_5O_5 \cdot 0.3C_2H_5OH$
45		131–133	$C_{21}H_{23}N_5O_4$
46		126–131	$C_{21}H_{25}N_5O_4 \cdot 0.6H_2O$
47		125–135	$C_{21}H_{25}N_5O_4 \cdot 0.6H_2O$
48		115–125	$C_{21}H_{25}N_5O_4 \cdot 0.25H_2O$
49		177–180	$C_{22}H_{27}N_5O_5 \cdot C_2H_5OH$
50		165–168	$C_{22}H_{25}N_5O_4 \cdot 0.6H_2O$
51		100–110	$C_{22}H_{27}N_5O_4 \cdot 0.6H_2O$
52		125–130	$C_{19}H_{21}N_5O_5 \cdot 0.4H_2O$
53		109–112	$C_{19}H_{21}N_5O_5 \cdot 0.2H_2O$
54		105–107	$C_{19}H_{21}N_5O_4S \cdot 0.7H_2O$
55		112–116	$C_{19}H_{21}N_5O_4S$

dependently lowered blood pressure. The antihypertensive effects of compound **3** were evident within 30 min after oral dosing and lasted about 8–12 h at lower doses. However, the blood pressure lowering effect lasted for over 20 h following administration of a 30 mg/kg dose (Figure 2). Compound **3** was further evaluated in a renal hypertensive rat model (two kidney one clip Goldblatt model) and was found to dose dependently reduce the blood pressure without affecting the heart rate.

Conclusion

In summary, we have identified several novel adenosine analogues with good antihypertensive activity in the spontaneously hypertensive rat model. We have also demonstrated that this biological activity is due to the interaction at the adenosine A_1 and A_2 receptors and the potency and/or selectivity of the agonists can be modulated by specific structural modification in the N^6-domain. Furthermore, this study shows that N^6-substituted adenosine derivatives, having the hydrophobic phenyl moiety one carbon away from the C6-nitrogen have modest affinity and selectivity for the A_1 receptor, whereas those with the phenyl moiety two carbons away from the C6-nitrogen (benzyl vs phenethyl) have excellent affinity and selectivity for the A_1 receptor at least in the endo-cyclic isomers (**1** vs **10**, **26** vs **28**, **52** vs **53**, **54** vs **55**, etc.). Thus one can "fine tune" the receptor potency and/or selectivity by specific structural modification and in turn design the preferable biological profile for novel adenosine agonists. Finally, we have also shown that several adenosine analogues are highly efficacious in the SHR and thus potentially represent a novel class of antihypertensive agents.

Experimental Section

Melting points are uncorrected. Analytical thin-layer chromatography (TLC) was done with precoated silica gel glass plates (EM 60F-254). ^1H NMR spectra were obtained on Varian XL-200 spectrometer. Mass spectra were recorded on a Finnigan 4500 spectrometer with an INCOS data system or a VG 7070 E/HR mass spectrometer with an 11/250 data system. Solvents and reagents were commercially available. Elemental analysis were determined at Parke-Davis.

Synthesis of N^6-Substituted Adenosines. All the adenosine analogues were synthesized by reacting 6-chloropurine ribonucleoside with an appropriate amine in the presence of triethylamine in refluxing ethanol. Workup consisted of either crystallizing the product from the ethanol solution or evaporation of ethanol followed by flash column chromatography on silica gel. The required amines were either commercially available or prepared from commercially available ketones via respective oximes. 1-Indanyl- and 1-tetralinamines were resolved using *N*-acetyl-D-leucine and -D- and -L-tartaric acid, respectively, as reported by

Table II. A_1 and A_2 Receptor Binding Affinities and Antihypertensive Activity of Adenosine Agonists

no.	K_i, nM			% change from control[a]	
	A_1	A_2	A_2/A_1	MBP[b]	HR[c]
1	45.8	1120	24.5	-22[d]	N/C
2	310.0	23000	74.0	N/C	N/C
3	22.2	412	18.6	-37[d]	-18
4	59.5	4350	73	-40[d]	-33
5	236	28260	119.7	N/C[e]	N/C
6	41.7	1450	34.8	-25[e]	-11
7	115	3002	26.2	-19[h]	N/C
8	1525	49710	32.6	N/C	N/C
9	50.5	4539	89.9	-21[g]	-21[g]
10	23.8	1642	68.9	-29	-47[g]
11	4.98	1383	278	-17[g]	-33[g]
12	42.3	769	18.2	-17[i]	-11[g]
13	33.4	7487	224	-33[g]	-34[g]
14	28.2	5302	188	-43[d]	-30
15	46.9	5922	126	-46[d]	-28
16	43.6	103200	2368	N/C	+15[k]
17	48.3	3946	81.7	-31[d]	-13
18	28	9038	323	-39[d]	-21[f]
19	104	12480	120	-47[d]	-20
20	41	1808	44	-24[h]	-24[h]
21	40.5	3548	87.6	-14[j]	N/C
22	68	666	9.8	-23	+17
23	32.4	44.2	1.36	-32[d]	+26[f]
24	5.19	66.7	12.9	-40[d]	-15
25	33.7	321	9.5	-51[d]	-24
26	299	3989	13.3	-28	N/C
27	22.9	163	7.1	-57[d]	-30
28	8.39	339	40.3	-47[d]	-44[f]
29	71.5	2947	41.2	-30	N/C
30	40.1	160	4.0	-17[e]	N/C
31	22.9	619	27	-56[d]	-40
32	2580	34060	13.2	N/C[e]	N/C
33	12.1	274	22.7	-44	+37[j]
34	68.6	5929	86.4	-39[d]	+32[g,f]
35	506	48290	95.5	N/C	+36[f]
36	425	43440	102	-41	+27[g,f]
37	7656	95100	12.4	-30[d]	+20
38	20.4	175	8.6	N/C[e]	N/C
39	23.7	218	9.2	-52[d]	-31[g]
40	41.2	396	9.6	-42[d]	N/C
41	81.1	327	4.0	-27[d,g]	+16[f]
42	87.9	642	7.3	-19	+24[g]
43	138	511	3.7	-14[n]	+20[k]
44	61.3	1922	31.4	N/C	N/C
45	99.8	53.4	0.53	-39[d]	+16[l]
46	17.3	91.7	5.2	N/C	+21[l]
47	17.8	76.9	4.27	-42	+28[n]
48	25.4	1234	48.7	-22[j]	-32[g]
49	275	2705	9.8	-14	N/C
50	404	1821	4.5	N/C	N/C
51	118	857	7.3	N/C	N/C
52	63.5	339	5.3	-21[d]	+21[f]
53	13.6	496	36.5	-38[d]	-33[f]
54	31.9	58.3	1.83	-23[d]	+29[l]
55	3.6	229	63.9	-21[d,g]	-33[g,f]

[a] Compounds were dosed orally at 10 mg/kg. Changes in blood pressure and heart rate were recorded for a 10-h period. The values given are the maximum effects at 1 h postdose unless otherwise noted. N/C = no change (<10%). [b] MBP = mean blood pressure. [c] HR = heart rate. [d] The blood pressure lowering effect is significant throughout the 10-h period (>10%). [e] Dose = 3 mg/kg. [f] The heart rate effect is significant throughout the 10-h period (>10%). [g] Four hours postdose. [h] Two hours postdose. [i] Three hours postdose. [j] Five hours postdose. [k] Nine hours postdose. [l] Seven hours postdose. [m] Six hours postdose. [n] Eight hours postdose.

Gishlandi and his co-workers.[10,11]

General Method for the Preparation of Compounds of Scheme II. *N*-[(1,2,3,4-Tetrahydro-1-naphthalenyl)-methyl]adenosine (46). A reaction mixture of 1,2,3,4-tetrahydro-1-naphthalenemethanamine (1.75 g, 8.7 mmol), 6-chloropurine ribonucleoside (1.7 g, 6 mmol), and triethylamine (0.88 g, 8.7 mmol), in ethanol (150 mL), was refluxed for 18 h. Upon cooling, a solid was obtained which was filtered, washed with ethanol, and dried at 45 °C in vacuum to give 0.85 g (36%) of *N*-[(1,2,3,4-tetrahydro-1-naphthalenyl)methyl]adenosine (**46**): mp 126–131 °C; ^{1}H NMR (DMSO) δ 1.6–2.0 (m, 4 H), 2.65 (m, 2 H), 3.15 (m, 1 H), 3.5–3.7 (m, 4 H), 3.95 (d, 1 H, J = 3 Hz), 4.1 (m, 1 H), 4.5–4.7 (m, 1 H), 5.2 (d, 1 H, J = 4.5 Hz), 5.3 (m, 1 H), 5.4 (d, 1 H, J = 5.7 Hz), 5.8 (d, 1 H, J = 6.3 Hz), 7.0–7.3 (m, 4 H), 8.1 (br s, 1 H), 8.24 (s, 1 H), 8.36 (s, 1 H). Anal. C, H, N.

***N*-[(3,4-Dihydro-1-naphthalenyl)methyl]adenosine (45).** A reaction mixture of 6-chloropurine ribonucleoside (1.1 g, 4 mmol), 3,4-dihydro-1-naphthalenemethanamine hydrochloride (1.2 g, 6.1 mmol), and triethylamine (1.2 g, 12 mmol) was refluxed in ethanol (150 mL) for 72 h. Upon cooling, solid material was obtained which was filtered, washed with ethanol, and dried to give 1.5 g (94%) of *N*-[(3,4-dihydro-1-naphthalenyl)methyl]-adenosine (**45**) having a melting point of 131–134 °C: ^{1}H NMR (DMSO) δ 2.18–2.25 (m, 2 H), 2.67–2.72 (t, J = 8.4 Hz, 2 H), 3.56–3.6 (m, 1 H), 3.65–3.71 (m, 1 H), 4.0 (q, 1 H), 4.15 (q, 1 H), 4.54 (br, 2 H), 4.64 (q, 1 H), 5.21 (d, J = 4.7 Hz, 1 H), 5.42 (m, 1 H), 5.46 (d, J = 6.1 Hz, 1 H), 5.9 (d, J = 6.2 Hz, 1 H), 5.97 (s, 1 H), 7.17 (s, 1 H), 7.34 (m, 1 H), 8.08 (s, 1 H), 8.24 (s, 1 H), 8.37 (s, 1 H). Anal. C, H, N.

***N*-[(1,2,3,4-Tetrahydro-1-hydroxy-1-naphthalenyl)-methyl]adenosine (44).** A reaction mixture of 6-chloropurine ribonucleoside (1.7 g, 6 mmol), 1-(aminomethyl)-1,2,3,4-tetrahydro-1-naphthalenol (1.7 g, 10 mmol), and triethylamine (2.0 g, 20 mmol) was refluxed in ethanol (100 mL) for 18 h. Upon cooling, the volatiles were removed under reduced pressure and the residue was washed with water (2 × 100 mL) followed by coevaporation with methanol (4 × 100 mL) to give 2.2 g (85%) of *N*-[(1,2,3,4-tetrahydro-1-hydroxy-1-naphthalenyl)methyl]-adenosine (**44**) having a melting point of 110–115 °C: ^{1}H NMR (DMSO) δ 1.6–2.1 (m, 4 H), 2.7–2.8 (m, 2 H), 3.5–3.7 (m, 2 H), 3.7–4.1 (m, 2 H), 3.9–4.0 (m, 1 H), 4.05–4.15 (m, 1 H), 4.6 (q, 1 H), 5.15 (d, 1 H), 5.3 (m, 1 H), 5.45 (d, 1 H), 5.66 (br, 1 H), 5.9 (d, 1 H), 7.0–7.2 (m, 3 H), 7.4 (br, 1 H), 7.5–7.7 (m, 1 H), 8.2 (s, 1 H), 8.3 (s, 1 H). Anal. C, H, N.

The requisite amines were prepared as follows.

1-(Aminomethyl)-1,2,3,4-tetrahydro-1-naphthalenol. To a reaction mixture of 3,4-dihydro-1(2*H*)-naphthalenone (7.3 g, 50 mmol) and $AlCl_3$ (5 mg) was added TMSCN (5.5 g, 55 mmol) under nitrogen and it was stirred at room temperature for 18 h. The reaction mixture was then dissolved in THF (25 mL) and slowly added to a suspension of $LiAlH_4$ (2.3 g, 60 mmol) in THF (40 mL) at a rate that maintained gentle reflux. It was refluxed for an additional 3 h, cooled to room temperature, and quenched with slow addition of water (40 mL) followed by 1 N NaOH (3 mL). The precipitate was filtered and THF was removed from the filtrate by evaporation under reduced pressure. The remaining aqueous layer was extracted with ether (2 × 50 mL). The organic extract was dried over anhydrous $MgSO_4$ and filtered and solvent was removed to yield 6.4 g (73%) of 1-(aminomethyl)-1,2,3,4-tetrahydro-1-naphthalenol having a melting point of 58–61 °C.

3,4-Dihydro-1-naphthalenemethanamine Hydrochloride. 1-(Aminomethyl)-1,2,3,4-tetrahydro-1-naphthalenol (1.6 g, 9 mmol) was heated at 60 °C in ethanol saturated with HCl (100 mL) and water (20 mL). The solution was cooled and evaporated to dryness. The residue was treated with ether (200 mL). The solid thus obtained was filtered and dried to afford 1.3 g (72%) of 3,4-dihydro-1-naphthalenemethanamine hydrochloride, mp 189–191 °C.

1,2,3,4-Tetrahydro-1-naphthalenemethanamine. 1-(Aminomethyl)-1,2,3,4-tetrahydro-1-naphthalenol (2.6 g, 15 mmol) was subjected to hydrogenolysis over 10% Pd/C in methanol saturated with HCl. Following completion of the reaction, the catalyst was filtered off, and the volatiles were removed from the filtrate. The residue was partitioned between 5% NaOH and chloroform (100 mL). The aqueous layer was extracted with chloroform (2 × 100 mL), and the combined organic extracts were washed with water (100 mL), dried over $MgSO_4$, filtered, and evaporated to dryness to yield 1.95 g (85%) of 1,2,3,4-tetrahydro-1-naphthalenemethanamine as an oil.

Preparation of Pure Diastereomers (5 and 6) of *N*-(2,3-Dihydro-5-methoxy-1*H*-inden-1-yl)adenosine. Resolution of 2,3-Dihydro-5-methoxy-1*H*-inden-1-amine. A solution of

(2,3-dihydro-5-methoxy-1*H*-inden-1-amine (9.9 g, 61 mmol) in absolute methanol (25 mL) was added to a hot solution of (*R*)-*N*-acetyl-(3,4-dimethoxyphenyl)alanine (18.1 g, 68 mmol) in absolute methanol (150 mL). The solution was kept at room temperature for 18 h. The crystalline solid obtained was filtered and dried, affording 6.0 g of salt A. The filtrate was concentrated to a total volume of 100 mL and, upon standing, gave 6.25 g of salt B. The filtrate was further concentrated to a volume of 50 mL and, upon standing, 6.5 g of salt C was obtained. Evaporation of solvent from the filtrate afforded 7.4 g of salt D.

Salt A (1.8 g, 42 mmol) was dissolved in 10% NaOH solution (15 mL) and extracted with chloroform (3 × 50 mL). The chloroform extract was washed with water, dried over $MgSO_4$, filtered, and evaporated to yield liquid amine. It was dissolved in 25 mL of ethanol and to this solution, 6-chloropurine ribonucleoside (0.9 g, 32 mmol) and triethylamine (0.48 g, 48 mmol) were added, and the reaction mixture was refluxed for 20 h. Evaporation of ethanol gave solid material which was treated with cold water. The solid was filtered, dried, and taken into methanol (20 mL). The solid material thus obtained was filtered and dried to afford 0.75 g of diastereomer **5** having a melting point of 152–155 °C; $[\alpha]_D$ = −140.2° (*c* = 1.13% DMF). Anal. C, H, N.

Similarly, diastereomer **6** [mp 170–173 °C; $[\alpha]_D$ = −6.73° (*c* = 1.01% DMF)] was obtained from the salt D. Anal. C, H, N.

Preparation of Pure Diastereomers (16 and 17) of *N*-(5-Butoxy-2,3-dihydro-1*H*-inden-1-yl)adenosine. Resolution of 5-butoxy-1-indanylamine. 5-Butoxy-2,3-dihydro-1*H*-inden-1-amine was resolved with commercially available dibenzoyl-L-tartaric acid monohydrate. To a solution of dibenzoyl-L-tartaric acid (9.5 g, 25 mmol) in methanol (100 mL) was added a solution of 5-butoxy-2,3-dihydro-1*H*-inden-1-amine (5.2 g, 25 mmol) on a steam bath. The solution upon cooling gave 7.2 g of sale E. This salt was further recrystallized twice from methanol–water to afford 2.2 g of salt G. The filtrate from the first crystallization was concentrated gradually (five times) to a volume of 50 mL and at each concentration, the salt that crystallized was filtered off. Following final concentration, the filtrate was evaporated to dryness to afford 6.8 g of salt H. In this experiment, the salts were analyzed by HPLC for their diastereomeric purity. Both salts G and H were >97% pure as determined by HPLC.

Salt G was dissolved in 10% NaOH and extracted with chloroform (3 × 75 mL). The extract was dried over $MgSO_4$, filtered, and evaporated, yielding the liquid amine (0.65 g, 31 mmol). It was dissolved in ethanol (20 mL) and to this solution, 6-chloropurine ribonucleoside (0.81 g, 28 mmol) and triethylamine (0.31 g, 31 mmol) were added. The reaction was refluxed for 20 h. The volatiles were removed under reduced pressure, and the residue was treated with cold water. The solid obtained was filtered, dried, and purified by flash column chromatography on silica gel using 5% methanol–chloroform as an eluent. Evaporation of solvent from the pure fractions gave solid material which was crystallized from chloroform–hexane to afford 0.89 g of the diastereomer **16** having a melting point of 126–128 °C: $[\alpha]_D$ = −120.6° (*c* = 1.12% DMF). Anal. C, H, N.

Similarly, diastereomer **17** [mp 148–151 °C, $[\alpha]_D$ = +11.7° (*c* = 1.1% DMF)] was obtained from salt H. Anal. C, H, N.

Pharmacological Method. Compounds were evaluated for effects on blood pressure and heart rate in conscious male, spontaneously hypertensive rats (20–24 weeks old, Charles River Laboratories). Indwelling aortic catheters were implanted for continuous direct measurement of aortic blood pressure and heart rate in free-moving unanesthetized rat as previously described.[17] Rats were housed in individual cages and allowed to recover from surgery for 24–48 h before dosing. Compounds were suspended in 4% gum acacia and administered by gavage in a 2 mL/kg volume. All doses are expressed as the free base.

Blood pressure and heart rate were measured continuously for up to 10 h postdose with a computer-based data-acquisition system. Predrug control values were obtained by averaging the two 30-min intervals obtained before dosing.

Acknowledgment. We thank Mr. J. Fergus and Ms. G. Lu for receptor binding data and Ms. B. Olszewski for pharmacological results.

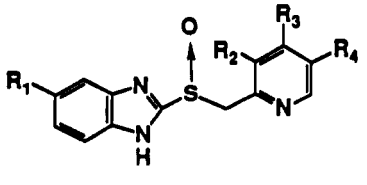
Studies on (H^+-K^+)-ATPase Inhibitors of Gastric Acid Secretion. Prodrugs of 2-[(2-Pyridinylmethyl)sulfinyl]benzimidazole Proton-Pump Inhibitors

John C. Sih,* Wha Bin Im, André Robert, David R. Graber, and David P. Blakeman

Research Laboratories, The Upjohn Company, Kalamazoo, Michigan 49001. *Received February 22, 1990*

The synthesis of N-substituted benzimidazole (H^+-K^+)-ATPase or proton-pump inhibitors is described. These compounds were prepared to function as prodrugs of the parent N–H compound and evaluated for their ability to inhibit gastric (H^+-K^+)-ATPase and gastric acid secretion. The prodrugs reported rely on either in vivo esterase hydrolysis for liberation of the parent compound (type I and type II) or require an acid environment for release of the active drug (type III and type IV). The *N*-(acyloxy)alkyl-substituted benzimidazoles **9**, **11**, and **24** showed improved chemical stability in the solid state and in aqueous solutions when compared to their parent N–H compounds. When given orally, **24** was found to be twice as potent as omeprazole in both the Shay rat and inactivation of gastric (H^+-K^+)-ATPase in the rat. The *N*-ethoxy-1-ethyl-substituted benzimidazoles **48–50** were found equally as effective as the N–H compound for inhibition of rat (H^+-K^+)-ATPase activity. In the Shay rat **48** at 10 mg/kg was approximately twice as active as parent timoprazole.

2-[(2-Pyridinylmethyl)sulfinyl]-1*H*-benzimidazole (**1**, timoprazole) is a substituted benzimidazole analogue and the prototype of a new class of antisecretory agents which



1, Timoprazole (R_1=R_2=R_3=R_4=H)

2, Omeprazole (R_1=R_3=OCH_3; R_2=R_4=CH_3)

inhibit gastric ATPase.[1] The proton pump, located in the apical membrane of the parietal cell, is responsible for the secretion of acid in the stomach when stimulated by the enzyme adenosine triphosphatase (ATPase). The substituted benzimidazoles are thought to reduce acid secretion in vivo by selectively blocking the hydrogen/potassium-adenosine triphosphatase enzyme.[2] Omeprazole

(1) (a) Olbe, L.; Berglindh, T.; Elander, B.; Helander, H.; Fellenius, E.; Sjöstrand, S. E.; Sundell, G.; Wallmark, B. *Scand. J. Gastroenterol.* **1979**, *Suppl. 14* (*55*), 131. (b) Fellenius, E.; Berglindh, T.; Sachs, G.; Olbe, L.; Elander, B.; Sjöstrand, S. E.; Wallmark, B. *Nature* (*London*) **1981**, *290*, 159. (c) Brändström, A.; Lindberg, P.; Junggren, U. *J. Gastroenterol.* **1985**, *Suppl. 20* (*108*), 15. (d) Brändström, A.; Lindberg, P.; Junggren, U.; Wallmark, B. *Scand. J. Gastroenterol.* **1986**, *Suppl. 21* (*118*), 54.

0022-2623/91/1834-1049$02.50/0