Highly Selective Adenosine A_2 Receptor Agonists in a Series of N-Alkylated 2-Aminoadenosines

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A wide variety of 2-substituted aminoadenosines were prepared for comparison with the moderately A_2 receptor selective adenosine agonist 2-anilinoadenosine (CV-1808). High selectivity combined with significant affinity at the A_2 receptor in rat membranes was observed for those amines bearing a two-carbon chain to which was attached an aryl, heteroaryl, or alicyclic moiety. 2-(2-Phenethylamino)adenosine (**3d**), a 14-fold A_2 selective compound, was modified by introduction of a variety of substituents in the benzene ring and the side chain. Some of these changes led to improved A_2 affinity and increased selectivity. Replacement of the phenyl moiety by cyclohexenyl produced a 210-fold selective agonist **3ag** (CGS 22989) whereas the cyclohexanyl analogue **3af** (CGS 22492) was 530-fold selective at the A_2 site. These compounds showed hypotensive activity in rat models over a range of doses without the bradycardia observed with less selective agonists.

Introduction

Adenosine (**1a**) was reported to have potent hypotensive and bradycardic activity by Drury and Szent-Gyorgyi in 1929.[1] In the subsequent 55 years, the literature on molecular modifications of this structure illustrated that cardiovascular activity appeared limited largely to analogues with the purine ring and β-ribofuranosyl moiety intact. Monosubstitution of the 6-amino group led to compounds reported to have hypotensive, bradycardic, coronary and peripheral vasodilating, and platelet-aggregation-inhibiting activity.[2,3] From this research, the important standards N^6-cyclohexyladenosine (CHA, **1b**), N^6-cyclopentyladenosine (CPA, **1c**), and N^6-(phenylisopropyl)adenosine (**1d**) (D-(*R*)-PIA and L-(*S*)-PIA) emerged. Uronic acid ethyl ester **2a**[4,5] is claimed to increase coronary flow in dogs[6] and *N'*-ethyl carboxamide **2b** (NECA)[7] is reported to be a potent coronary dilator[6,8] and hypotensive.[6] Certain 2-substituted adenosines were found to have vasodepressor activity,[9-12] including 2-CADO (**3a**).[13] Among the 2-amino analogues, 2-anilinoadenosine (**3b**, CV-1808) appeared particularly interesting as a long-acting coronary dilator[14] with platelet antiaggregating properties.[15]

Similarly, determination of the adenylate cyclase stimulating effects in human fibroblast cells of more than 100 adenosine analogues and antagonists indicated that those few compounds classified as full agonists or high-efficacy partial agonists had the basic purine heterocycle and most of the features of the ribosyl moiety.[16] This information guided our choice of modified adenosine structures as likely cardiovascular agents.

In the late 1970s, the recognition of purinergic receptors[17] in peripheral cell membranes, particularly the A_1 and A_2 receptors,[18,19] stimulated a new burst of activity in

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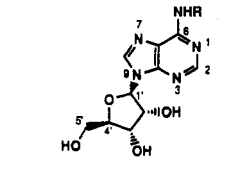
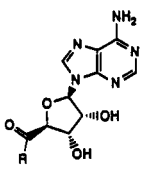
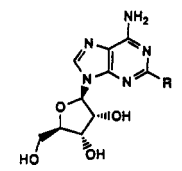


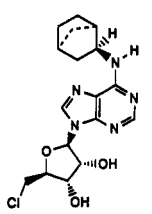
1a : R = H
1b : R = cyclohexyl
1c : R = cyclopentyl
1d : R = phenylisopropyl



2a : R = OCH_2CH_3
2b : R = $NHCH_2CH_3$



3a : R = Cl
3b : R = NH-Phenyl



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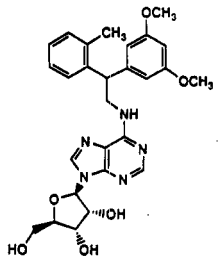
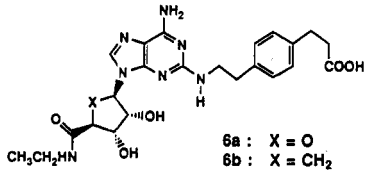


5: (-) Isomer



6a : X = O
6b : X = CH_2

adenosine research.[20] The negative dromo-, chrono-, and inotropic effects of adenosine are thought to be A_1 mediated[21] whereas the vasodilatory effects are A_2 mediated.[22] In order to design a new type of antihypertensive agent, we searched for a compound with potent A_2 receptor binding with minimal A_1 effects.

In the last decade, extensive exploration of N^6-substituted adenosines[23] led to highly potent and selective A_1 agonists. This is not surprising, since **1b** and **1c** were found to be potent and selective for the A_1 receptor.[22b] The most striking example reported was 4, with a K_i of 0.24 nM at the A_1 receptor and 16 000-fold selectivity in rat brain striatal membranes.[24] Less predictably, a 40-fold selective A_2 agonist **5** was discovered among these N^6-substituted compounds with a K_i at the A_2 site of 3.1 nM,[25] though binding affinity at the A_1 site was still substantial (118 nM).

From the moderately A_2-selective agonist **3b** and from NECA, which binds well to the A_2 receptor,[19] 2-substituted adenosines incorporating the NECA side chain[26] and 2-substituted compounds containing both a NECA side chain and the cyclopentane moiety in place of the tetrahydrofuran ring of ribose (carbocyclic adenosines)[27,28] were designed. These efforts led to structure **6a**,[29] showing a K_i of 22 nM at the A_2 site with 140-fold selectivity,[29] and **6b**, with a K_i of 43 nM at A_2 and 400-fold selectivity.[28] The high selectivity of **6a** coupled with only micromolar affinity at A_1 sites led to its use in tritiated form as a receptor ligand in our A_2 binding assay instead of [^{3}H]NECA.[30]

Potential ease of synthesis led us to prepare substituted 2-aminoadenosines. Many analogues of **3b**, i.e., substituted 2-(phenylamino)- and 2-(pyridylamino)adenosines, had been prepared and tested as coronary dilators in anaesthetized dogs and found to be significantly active[31] though no binding data were reported. A few 2-(alkylamino)- and 2-(aralkylamino)adenosines also were tested and found to be much less active than **3b**.[11]

We now wish to describe the synthesis and rat brain binding SAR of a series of adenosines substituted only at the 2-position by aryl-, cycloalkyl- and heterocycle-substituted alkylamino groups along with the effects of selected compounds in anaesthetized normotensive rats and conscious spontaneously hypertensive rats (SHR).

Chemistry

Compounds **3c–3aq** shown in Table I were prepared by reaction of commercially available 2-CADO (**3a**) with an aliphatic primary or secondary amine at 130–140 °C over sufficient time to cause disappearance of starting material, as indicated by thin-layer chromatography. Conditions used were excess (2–5 mol) amine without solvent (method A) or a 10–100% molar excess of amine with diisopropylethylamine (at least 1 mol to react with the hydrogen chloride generated) in isoamyl alcohol (method B). Typical examples are described in the Experimental Section.

A_1/A_2 Binding Results

A_1 and A_2 binding data in rat striatal membranes for the 2-aminoadenosines are shown in Table I. Replacement of the anilino group of **3b** by benzylamino[11] (**3c**) resulted in a pronounced loss of binding affinity. However, the addition of one more carbon in the side chain (**3d**)[11] caused an increase in selectivity and affinity at the A_2 site slightly

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superior to that of the standard **3b**. Further lengthening of the side chain by one or two atoms (**3e,f**) produced compounds with markedly decreased affinity for the A_1 site and some loss of affinity at A_2. Introduction of a heteroatom into the side chain (**3i**) was unpromising and not further exemplified. N-Methylation of **3d** increased the selectivity markedly (**3g**), but modification of the phenethyl moiety into a 2-dihydroindanyl side chain (**3h**) reduced binding affinity and selectivity. Methyl and hydroxyl groups in the β-position of the side chain were tolerated either separately (**3j–l**) or together (**3m,n**) although β,β-dimethyl (**3o**) or the bulky β-phenyl group (**3p**) decreased A_1 and A_2 binding affinity markedly. In the mono-β-substituted products the *S* antipode appeared to be the most potent one[32] and the methyl substituent conferred more selectivity than the hydroxyl group. Together, the (*S*)-β-hydroxy-β-methyl substitution product **3n** was more selective (62-fold) than any other compound with a phenethyl side chain unsubstituted in the benzene ring. Lengthening of the side chain through ring substitution was helpful. Para substitution of carboxylic ester groups or a phosphonate (**3u**) separated from the ring by zero to two carbon atoms (**3r–t**) produced compounds with increased A_2 selectivity and much reduced affinity for the A_1 site. Extended lipophilic side chains led to a wide variation in selectivity (**3v–x**). The amino acid ribosyl analogue of **6a** and **6b** (structure **3y**) was less potent than **6a**, but 170-fold A_2 selective. A change from para substitution to meta substitution (**3z**) still resulted in an A_2 selective compound, but this variation augured no improvement. Heteroaromatic groups and bulkier bicyclic groups could also replace the phenyl (**3aa–ae**).

An important variation was the replacement of phenyl by an alicyclic moiety. Compound **3af** (Table III), 2-[(2-cyclohexylethyl)amino]adenosine (CGS 22492), showed an IC_{50} of 22 nM binding affinity at the A_2 site, with 530-fold selectivity. In compound **3ag** (CGS 22989), the cyclohexenyl group could be considered intermediate in structure between the flat electron-rich phenyl and the bulky lipophilic cyclohexane. This analogue showed binding affinity at the A_2 site comparable to that of **3af** with selectivity intermediate between those of **3d** and **3af**. Analogues with a wide variety of alicyclic groups (Examples **3ah–ak**) were found which were superior to **3d**. The adamantylethyl analogue **3ai** was the best of the examples chosen, as it showed an IC_{50} of 27 nM binding affinity for the A_2 site and 360-fold selectivity. Replacement of the phenyl by 4-tetrahydropyranyl (**3am**) or morpholino (**3al**) greatly decreased binding affinity at either site, indicating that the electron-rich oxygen atom strongly interferes with binding. Compound **3af** was not improved by N-methylation (**3an**) or extension of the side chain (**3ao,ap**). The lack of significant binding affinity of **3aq**, the open chain analogue related to **3af**, **3ah**, and **3am**, illustrates that a lipophilic side chain alone is not sufficient to produce strong binding affinity. *The ring attached to the chain plays an important role in the binding.*

The compounds of most interest were the readily accessible compounds **3ag** and **3af**, which show low nanomolar binding affinity at the A_2 receptor coupled with 210- and 530-fold selectivity, respectively.

(32) Early in the project, the two β-methyl-β-phenethyl isomers were compared by using the original literature method for determining A_2 binding.[38] Compound **3j**, derived from (*S*)-(−)-β-methylphenethylamine[42] had IC_{50} values at A_1 and A_2 sites of 1208 and 23 nM, respectively (n = 1) whereas the compound derived from the (*R*)-(+)-amine showed IC_{50} values of 1810 and 726 nM, respectively (n = 1).

Anaesthetized Normotensive Rat Studies

The effect on blood pressure and heart rate in anaesthetized normotensive rats was measured for 16 2-aminoadenosines following intravenous administration, including the standard **3b** (Table II). In addition, effects were measured on five standards, four of which were more potent in binding at the A_1 receptor than at A_2. These standards, spanning an A_1 selectivity of unity to 1160-fold, showed bradycardic activity with ED_{25} values near the ED_{25} values for blood pressure lowering. In marked contrast, all 2-aminoadenosines showed tachycardia. In fact, the data reflects that in all but three examples, tachycardia was still observed at *10 times* the ED_{25} dose for hypotensive activity, even though the binding ratios span a 10–530-fold A_2 selectivity range. These compounds behaved like the standard peripheral vasodilator hydralazine.[33]

The absolute A_2 binding values did not correlate with the ED_{25} values for blood pressure lowering after iv administration. Notably, **3s** was weakly active and two of the most promising leads based on binding affinity and selectivity, **3v** and **3ag**, did not even reach an ED_{25} for blood pressure lowering at the highest doses that could be tested. The novel 2-aminoadenosines were then tested in naive rats with two successive doses, the second dose being given when blood pressure and heart rate had returned to baseline. This test revealed that **3v** caused tachyphylaxis, i.e., a 17% blood pressure drop on the first dose and only 3% on the second dose. The other analogues did not show tachyphylaxis with the second dose. Compound **3v** was the only example tested having an extended lipophilic side chain lacking polar moieties in the chain, and it is tempting to speculate that this feature of the molecule is responsible for the tachyphylaxis.

Spontaneously Hypertensive Rat Studies

Eighteen 2-aminoadenosines including **3b** were tested po in the spontaneously hypertensive rat model (Table III). All caused significant hypotension at doses of 10 mg/kg or less. In all but one example (**3k**), no bradycardia was observed. In 10 compounds, statistically significant tachycardia was observed in terms of maximum response when compared to a vehicle-treated group. Compounds **3b**, **3d**, **3af**, and **3ag**, tested at multiple doses, displayed dose-related blood pressure lowering. The standard **3b**, a 10-fold selective A_2 agonist, caused the least effect on heart rate over a 30-fold dose range, though a downward trend was observed with increasing doses after the initial weakly active dose. The 14-fold selective **3d** showed tachycardia at all doses with significance reached at the low and high doses only. The 7.8-fold selective agonist **3k** elicited significant tachycardia at the low dose, whereas significant bradycardia was evident at the high dose. Reductions in blood pressure and heart rate at higher doses suggested that some A_1-mediated response was occurring. The A_1-selective agent CPA (**1c**), as expected, caused pronounced hypotension with severe bradycardia at low doses. The 2-fold A_1-selective standard NECA (**2b**) caused hypotension and slight tachycardia at the low dose. At only a 3-fold higher dose, hypotension was accompanied by severe bradycardia, a strong indication of A_1 activity. The highly A_2-selective agents **3af** and **3ag** showed only hypotension and tachycardia over a 10-fold dose range.

The 530-fold A_2-selective agent **3af** was tested over 4 days orally in spontaneously hypertensive rats at 1 mg/kg

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per day (Table IV). A 50–63-mm drop in blood pressure was accompanied by tachycardia (48–81 beats/min) each day. Peak hypotensive effects occurred between 1 and 6 h each day. Decreased behavioral and locomotor effects in rodents have been observed after treatment with adenosine agonists.[34] Studies in monkeys with the standard agonists suggest that such effects are A_2 mediated.[35] However, visual inspection of the rats showed no signs of decreased motor activity nor any other overt changes during the entire 4-day period.

Discussion

A series of adenosine derivatives, readily prepared from 2-chloroadenosine, showed binding affinity in rat brain at the A_2 receptor site in the low nanomolar range and spanned a breadth of 2–530-fold selectivity for the A_2 site. These compounds complement the substantial list of A_1-selective adenosine agonists discovered by previous investigators. A clearer understanding of the physiological consequences of stimulating A_1 and A_2 receptors and the definition of receptor subtypes in various tissues should be more readily achieved with this enlarged set of biological tools.

Modification of the side chain of 2-aminoadenosines showed that a two-carbon chain appended with a wide variety of aromatic, heteroaromatic, and alicyclic groups produced compounds with IC_{50}'s for A_2 binding affinity in the low nanomolar range with moderate to very high selectivity. A wide variety of substituents, particularly in the para position of the benzene ring of 2-(phenethylamino)adenosine, were tolerated by the A_2 receptor and, in many examples, A_2 binding affinity and selectivity were improved significantly by ring substitution.

Hypotension without bradycardia over a wide range of doses was observed during tests with analogues that showed an A_2 selectivity of ca. 10 or greater. It was shown recently that the hypotensive activity of **3b** and **6a** is attenuated by pretreatment with 8-(*p*-sulfophenyl)theophylline, an adenosine antagonist, indicating that the hypotensive activity is caused by activation of extracellular adenosine receptors.[33,36] The tachycardia observed in the rat is typical for peripheral vasodilators which lack direct negative chronotropic and inotropic activity.[33] Furthermore, it was shown recently that the tachycardia produced by the A_2-selective agents **3b**, **6a**, or (*dl*)-**5** is attenuated strongly by β-blockade (metoprolol) without loss of hypotensive activity.[33,36] This indicates that the tachycardia is largely mediated by reflex activation of the sympathetic nervous system. The conclusion is supported by the finding that **6a** has no direct positive chronotropic effects on heart rate in the isolated working rat heart.[29]

Stimulation of A_1 receptors causes bradycardia, whereas activation of A_2 receptors produces peripheral vasodilation, leading to a reflex increase in heart rate. Hypothetically, the net effect on heart rate of an adenosine agonist with a "balanced" A_1/A_2 ratio should be minimal. The bradycardic effects caused by A_1 receptor stimulation apparently cannot be "built in" to the molecule to counteract the tachycardia accompanying A_2 receptor agonism, as illustrated by results obtained from studies of **2b**, **3b**, and **3k** and the 7-fold A_2 selective compound (±)-*trans*-3-(6-amino-9*H*-purin-9-yl)-*trans*-5-(*N*-ethylcarbamoyl)-*cis*-1,2-cyclopentanediol (C-NECA).[27] The present series of compounds, though it spans a wide range of A_1/A_2 ratios, is not exhaustive. In particular, agonists with A_1/A_2 ratios of less than 5 coupled with high affinity for both receptor subtypes have not been studied extensively in this investigation. Nonetheless, studies following intravenous[36] as well as oral administration show that tachycardia predominates until a critical (as yet undefined) threshold is reached for A_1 receptor activation leading to bradycardia. The threshold for activation is dependent upon both the affinity of the agonist for the specific receptor subtype and the affinity ratio for the two subtypes. Therefore, in selecting an adenosine agonist for development as an antihypertensive agent, it seems more plausible to choose one with low affinity for A_1 receptors to avoid the risk of compromising cardiac function since any reflex tachycardia resulting from A_2 agonism may be attenuated by concurrent β-adrenergic blockade.

Favorable antihypertensive effects shown by the most selective analogue, **3af**, combined with the lack of tolerance and lack of noticeable side effects in a 4-day study in the SHR, suggest an opportunity for the development of a vasodilator with a mechanism of action not presently found in currently marketed drugs.

Experimental Section

Biochemical Test Methods. Binding Studies. Evaluation of compounds for their ability to bind to rat brain A_1 receptors was based on previously published methodology. A_1 binding was measured in adenosine deaminase (ADA) pretreated rat cortical membranes using [^{3}H]-CHA (**1b**; specific activity 25 Ci/mmol) in the presence of 10 μM R-PIA (**1d**) to define specific binding.[37] Assays were run at 23 °C for 2 h using 100–200 μg of protein of ADA-treated tissue in a final volume of 1 mL of 50 mM Tris-HCl buffer, pH 7.4; [^{3}H]CHA was included at a final concentration of 1 nM. Bound radioactivity was isolated by vacuum filtration over Whatman GF/B filters and unbound radioactivity removed with 2 × 5 mL washes with ice-cold buffer. After equilibration in 4 mL of scintillation cocktail, radioactivity was determined by conventional liquid scintillation spectrometry at an efficiency of 50%.

Binding at A_2 receptors was measured in ADA-pretreated rat striatal membranes using [^{3}H]-**6a** (specific activity 30–80 Ci/mmol)[30] by modification of the method previously described for [^{3}H]NECA.[38] Rat striatum was homogenized with a Brinkmann polytron (setting 6 for 20 s) in 20 volumes of ice-cold 50 mM Tris-HCl, pH 7.4, containing 10 mM $MgCl_2$. This membrane homogenate was then centrifuged at 48000*g* for 10 min at 4 °C. The resulting pellet was resuspended in buffer containing 2 IU/mL ADA (Boehringer-Mannheim) to 20 mg/mL original tissue weight and incubated at 37 °C for 30 min to inactivate endogenous

(34) (a) Snyder, S. H.; Katims, J. J.; Annau, Z.; Bruns, R. F.; Daly, J. W. Adenosine receptors and behavioral actions of methylxanthines. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78* (5), 3260–3264. (b) Barraco, R. A.; Coffin, V. L.; Altman, H. J.; Phillis, J. W. Central effects of adenosine analogs on locomotor activity in mice and antagonism of caffeine. *Brain Res.* **1983**, *272*, 392–395. (c) Logan, L.; Carney, J. M. Antagonism of the behavioral effects of L-phenylisopropyladenosine (L-PIA) by caffeine and its metabolites. *Pharmacol. Biochem. Behav.* **1984**, *21*, 375–379.

(35) (a) Spealman, R. D.; Coffin, V. L. Behavioral effects of adenosine analogs in squirrel monkeys: relation to adenosine A_2 receptors. *Psychopharmacology* **1986**, *90*, 419–421. (b) Coffin, V. L.; Spealman, R. D. Behavioral and cardiovascular effects of analogs of adenosine in cynomolgus monkeys. *J. Pharmacol. Exp. Ther.* **1987**, *241* (*1*), 76–83.

(36) Webb, R. L.; Barclay, B. W.; Graybill, S. C. Cardiovascular Effects of A_2-Adenosine Agonists in the Conscious Spontaneously Hypertensive Rat: A Comparative Study with Three Structurally Distinct Agonists. *J. Pharmacol. Exp. Ther.*, in press.

(37) Bruns, R. F.; Daly, J. W.; Snyder, S. Adenosine receptors in brain membranes: binding of N^6-cyclohexyl[^{3}H]adenosine and 1,3-diethyl-8-[^{3}H]phenylxanthine. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5547–5551.

(38) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. Characterization of the A_2 adenosine receptor labeled by [^{3}H]NECA in rat striatal membranes. *Mol. Pharmacol.* **1986**, *29*, 331–346.

Table I. 2-Substituted Adenosines

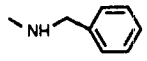
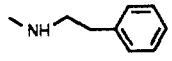
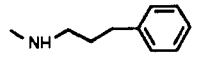
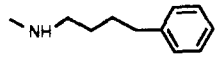
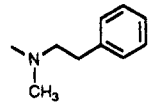
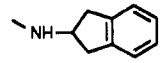
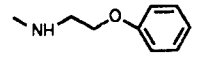
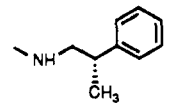
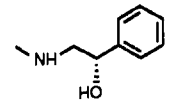
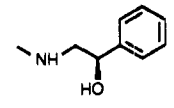
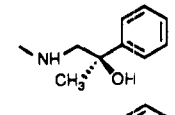
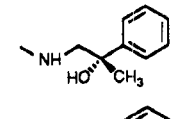
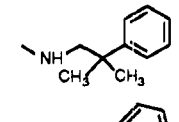
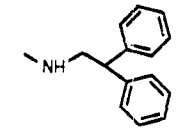
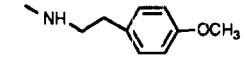
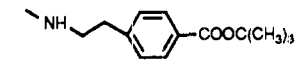
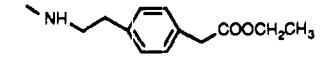
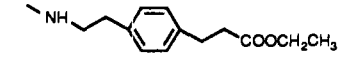
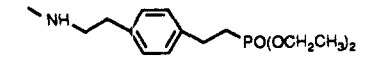
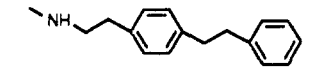
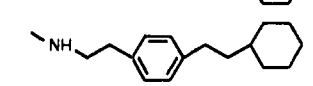
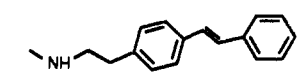
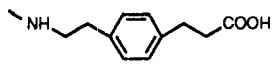
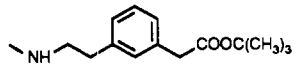
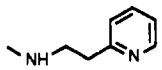
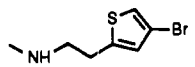
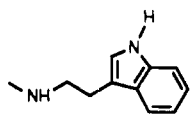
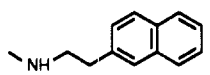
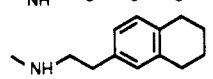
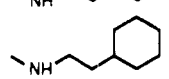
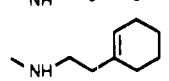
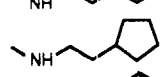
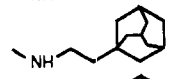
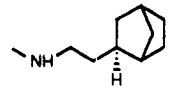
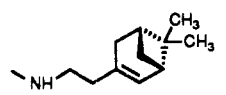
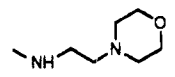
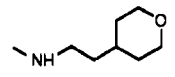
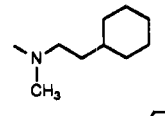
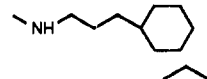
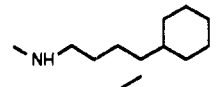
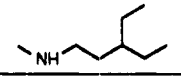
no.	2-substituent	synthetic method	% yield[b]	mp, °C	formula[a]	A_1 IC_{50}, nM ± SEM	A_2 IC_{50}, nM ± SEM	A_1/A_2
3c		B	32	126–130[c]	$C_{17}H_{20}N_6O_4$	10471 ± 963	5888 ± 1110	1.8
3d		A	18	144–146[d]	$C_{18}H_{22}N_6O_4$	977 ± 13	68 ± 13	14
3e		A	19	123–126	$C_{19}H_{24}N_6O_4$	2570 ± 437	257 ± 18	10.0
3f		B	49	137–140	$C_{20}H_{26}N_6O_4$	6918 ± 923	112 ● 4	62
3g		A	43	82–94	$C_{19}H_{24}N_6O_4$	5623 ± 310	145 ● 26	39
3h		B	12	150–153[e]	$C_{19}H_{22}N_6O_4$	2951 ± 903	288 ● 31	10.3
3i		B	40	158–160	$C_{18}H_{22}N_6O_5$	3802 ± 551	537 ± 40	7.1
3j		A	48	111–115	$C_{19}H_{24}N_6O_4$	1660 ± 145	78 ± 8	21
3k		A	50	152–156	$C_{18}H_{22}N_6O_5$	186 ± 20	24 ± 3	7.8
3l		A	15	135–139[j]	$C_{18}H_{22}N_6O_5$	2291 ± 211	794 ± 46	2.9
3m		B	7	105–115	$C_{19}H_{24}N_6O_5$[g]	1259 ± 133	589 ± 77	2.1
3n		B	13	125–135	$C_{19}H_{24}N_6O_5$	1862 ± 103	30 ± 9	62
3o		B	5	180–185[f]	$C_{20}H_{26}N_6O_4$	>10000	603 ± 57	>16.6
3p		A	13	124–125	$C_{24}H_{26}N_6O_4$[g]	14791 ± 2858	6607 ± 821	2.2
3q		A	45	133–136	$C_{19}H_{24}N_6O_5$	912 ± 80	23 ± 6	40
3r		B	68	155–160	$C_{22}H_{30}N_6O_6$	7244 ± 2166	49 ± 10	148
3s		B[h]	34	102–112	$C_{22}H_{28}N_6O_6$	2290 ± 148	62 ± 15	37
3t		A[h]	15	110–118	$C_{23}H_{30}N_6O_6$	3631 ● 384	44 ± 1	83
3u		A	6	140–145[i]	$C_{24}H_{35}N_6O_7P$	3890 ● 653	42 ± 3	93
3v		A	29	148–150[f]	$C_{26}H_{30}N_6O_4$	3890 ± 358	174 ± 44	22
3w		A	28	154–160[f]	$C_{26}H_{36}N_6O_4$	427 ± 32	120 ± 29	3.6
3x		A	21	165–169[f]	$C_{26}H_{28}N_6O_4$	7586 ± 384	87 ± 18	87

Table I (Continued)

no.	2-substituent	synthetic method	% yield[b]	mp, °C	formula[a]	A_1 IC_{50}, nM ± SEM	A_2 IC_{50}, nM ± SEM	A_1/A_2
3y		A[j]	50	135–139	$C_{21}H_{26}N_6O_6$[k]	12589 ● 1882	74 ± 22	170
3z		B	16	143–146	$C_{24}H_{32}N_6O_6$	1380 ± 146	89 ± 18	15.5
3aa		A	71	180–182	$C_{17}H_{21}N_7O_4$	3981 ± 476	209 ± 27	19
3ab		A	27	136–144	$C_{16}H_{19}BrN_6O_4S$	2344 ± 340	68 ● 13	34
3ac		A	45	129–141	$C_{20}H_{23}N_7O_4$	724 ± 150	51 ● 19	14
3ad		A	6	160–163[f]	$C_{22}H_{24}N_6O_4$	676 ± 47	35 ± 5	19
3ae		B	12	140–143[f]	$C_{22}H_{28}N_6O_4$	692 ● 91	17 ± 5	41
3af		A	71	136–141	$C_{18}H_{28}N_6O_4$	11748 ± 4216	22 ● 4	530
3ag		A	68	116–122	$C_{18}H_{26}N_6O_4$	2692 ± 526	13 ± 4	210
3ah		A	32	124–131	$C_{17}H_{26}N_6O_4$	13182 ± 1000	132 ● 21	100
3ai		A	58	164–166	$C_{22}H_{32}N_6O_4$	9772 ● 787	27 ± 3	360
3aj		A	17	128–130	$C_{19}H_{28}N_6O_4$	7586 ± 750	42 ● 10	180
3ak		A	20	140–142	$C_{21}H_{30}N_6O_4$	12022 ± 802	138 ± 16	87
3al		A	20	193–195[k]	$C_{16}H_{25}N_7O_5$	7762 ● 1535	2399 ± 883	3.2
3am		A	23	120–130	$C_{17}H_{26}N_6O_5$[l]	>10000	11220 ± 1265	>1
3an		A	17	85–95	$C_{19}H_{30}N_6O_4$	5129 ● 1262	309 ● 121	16.6
3ao		A	40	124–127	$C_{19}H_{30}N_6O_4$	8318 ± 574	132 ± 18	63
3ap		A	49	188–192	$C_{20}H_{32}N_6O_4$	5248 ● 640	93 ± 24	56
3aq		A	8	128–136[f]	$C_{17}H_{28}N_6O_4$	5888 ● 785	603 ● 249	9.8

[a] All new compounds had satisfactory C, H, and N microanalytical data within ±0.4 with the following exceptions: **3c** (N: calcd, 22.57; found, 22.10), **3m** (C: calcd, 52.53; found, 53.09), and **3y** (C: calcd, 55.01; found, 54.53). NMR and IR spectra were in agreement with the structural assignments. [b] Purified yields from **3a**. [c] Literature[12a] mp 100–105 °C (monohydrate). [d] Literature[12a] 125–128 °C. [e] Recrystallized from methanol. [f] Recrystallized from acetonitrile. [g] Hydrate. [h] Obtained from the acid: See the Experimental Section. [i] Recrystallized from ethyl acetate-ether. [j] Obtained from the tert-butyl ester as hydrochloride, for which melting point and yield are shown. Microanalysis done on free amino acid, mp 184–188 °C; see the Experimental Section. [k] Recrystallized from ethanol. [l] Hydrochloride salt.

adenosine. The membrane homogenate was recentrifuged and the final pellet was frozen at −70 °C until time of assay.

Routine assays were carried out in triplicate in 12 × 75 mm polypropylene test tubes containing an aliquot of striatal membranes (100–200 μg of protein/mL) in incubation buffer (50 mM Tris-HCl and 10 mM $MgCl_2$, pH 7.4) with ca. 5 nM [^{3}H]-**6a** and/or inhibitor in a final volume of 1 mL. All assays were conducted at 23 °C for 90 min. Nonspecific binding was defined in the presence of 20 μM 2-CADO. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure with a Brandel Cell Harvester (Gaithersburg, MD). Filters were washed twice with ice-cold buffer (5 mL) and placed in scintillation vials, and bound radioactivity was determined by using conventional liquid scintillation spectroscopy techniques at an efficiency of 40–50%.

For the competition studies, 7–10 concentrations of inhibitor were included in the incubation buffer. All data represented the geometric mean ± SEM for a minimum of three separate ob-

Table II. Blood Pressure Effects in Normotensive Anaesthetized Rats

no.	IC_{50}, nM A_1	IC_{50}, nM A_2	A_1/A_2	BP ED_{25},[c] mg/kg iv	HR at dose[d] % Δ	HR at dose[d] mg/kg iv	dose/ BP/BP[e]
1b	5[a]	790[a]	0.006	0.004	−25	0.002	
1c	1[a]	1160[a]	0.001	0.004	−25	0.002	
1d (*R*)	5[a]	530[a]	0.009	0.005	−25	0.005	
2b	16[a]	15[a]	1.1	0.00075	−25	0.002	
3a	37[a]	160[a]	0.23	0.015	−25	0.017	
3b	1380[b]	145[b]	9.5	0.029	+10.5	0.29	
3d	977	68	14	0.003	+23	0.03	0.003/−25/−25
3g	5623	145	39	0.03	+14	0.3	0.03/−27/−25
3h	2951	288	10.3	0.028	+5	0.3	0.03/−23/−23
3j	1660	78	21	0.007	+13	0.07	0.007/−25/−25
3s	2290	62	37	0.15	+24	1.0	0.15/−25/−25
3u	3890	42	93	0.034	+17	0.3	0.034/−25/−25
3v	3890	174	22	>0.3 (−19%)[f]	+18	0.3[f]	0.1/−15/−3
3y	12589	74	170	0.007	+14	0.07	0.007/−25/−25
3aa	3981	209	19	0.028	+21	0.3	0.028/−25/−25
3ab	2344	68	34	0.026	+19	0.3	0.026/−23/−23
3af	11748	22	530	0.009	+22	0.1	0.009/−27/−27
3ag	2692	13	210	>1.0 (−13%)[f]	+16	0.3[f]	0.01/−17/−17
3ah	13182	132	100	0.011	+25	1.0	0.011/−25/−25
3ai	9772	27	360	0.004	+8	0.04	0.004/−23/−25

[a] Data on A_1 and A_2 binding from ref 30, expressed as IC_{50} values. [b] A_1: 1380 ± 92 A_2: 145 ± 11. [c] Dose that reduces blood pressure 25%. [d] Heart rate change (tachycardia or bradycardia) is expressed in percent at the indicated dose. [e] Dose/BP drop/BP drop after second dose. [f] ED_{25} not reached at 1.0 mg/kg iv.

servations. IC_{50} values were determined by using a nonlinear least squares analysis program.[39]

Pharmacological Test Procedures. Normotensive Anaesthetized Rat Studies. Adult male Sprague–Dawley rats, Tac:N(SD)fBR (300–400 mg), were anaesthetized with inactin (100 mg/kg, ip). A femoral artery and contralateral saphenous vein were cannulated for direct blood pressure (BP) measurement and iv drug administration, respectively. Animals were allowed a 15-min equilibration period before testing. For each drug, three to six rats (average of four) were used. Vehicle (0.1% DMSO in 1 N saline, 1.0 mL/kg, iv) was administered over a 30-s period followed by a 0.3-mL saline flush administered over a 30-s period. Changes in diastolic BP were recorded with a polygraph while heart rate (HR) was recorded as a derivative of the BP pulse. The vehicle showed no effect. The test drug dissolved in the vehicle was administered in the same manner and a dose–response curve was established. Percentage changes in BP and HR were recorded and 25% changes (ED_{25}) for BP and HR calculated.

Compounds were tested for tachyphylaxis in naive animals surgically prepared as above. Where possible, the iv dosage was the ED_{25} dose, otherwise the dose giving the highest BP response (usually the highest administered dose) was administered. When BP and HR returned to predrug levels, the animals were then challenged with the same dose. If BP and HR levelled off within 10% of the predrug levels, the second dose was administered within 15–30 min. A compound was considered to show tachyphylaxis if the second BP response was attenuated.

Conscious Spontaneously Hypertensive Rat Studies. Male spontaneously hypertensive rats (SHR) (Tac:N(SHR)fBR; 275–325 g, Taconic Farms, Germantown, NY) were anaesthetized with methoxyflurane and the femoral artery was catheterized for determination of mean arterial BP and HR.[29] Animals were given 1–2 days to recover from surgery prior to receiving any drugs. BP and HR parameters were continuously recorded in conscious unrestrained rats for 30 min before drug administration (baseline, time 0) and for 6 h following oral dosage. Separate groups of animals were used to evaluate each dose of each compound. The maximum change in mean arterial pressure or HR was determined for each group of animals during the 6-h observation period and the group mean ± standard error was calculated. All values were compared to a vehicle-treated (3% cornstarch) group of animals using ANOVA followed by a Dunnett's test to determine differences which were considered significant if $P < 0.05$.

To determine the BP and HR effects with repeated administration, rats were dosed orally by gavage once daily for 4 consecutive days. BP and HR were monitored in conscious SHR each day. Hemodynamic parameters were recorded continuously on each day for a 30-min period immediately preceding dosing and for 6 h postdrug administration.

Chemistry. All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected and expressed in degrees centigrade. All proton and ^{13}C NMR spectra were recorded on either a Varian XL-300 or a Bruker AM-300 spectrometer and chemical shifts are expressed in ppm relative to tetramethylsilane as internal standard.

Reactions were carried out under a nitrogen atmosphere and monitored on silica gel 60 TLC plates (from EM Science). For flash chromatography, silica gel 60 (230–400 mesh) from E. Merck was used under nitrogen pressure. In some examples, products were obtained by evaporation and trituration of the residue with solvent but usually they were purified by normal-phase silica gel chromatography and obtained as amorphous solids after trituration with a solvent. In some cases, recrystallization was used, usually with severe losses of material since yield optimization through modification of reaction conditions and purification was not done. The reaction temperature was critical. Displacement of the halogen proceeded sluggishly at lower temperatures but excessive decomposition occurred at higher temperatures. Amines were purchased or prepared as described in the literature or by standard literature procedures. The ethanol used was anhydrous, denatured with 0.5% toluene. Other solvents were analytical grade. Methods used to prepare unpublished amines are outlined below. 2-Chloroadenosine (**3a**) was purchased from Sigma Chemical Co.

IR spectra were taken in Nujol mulls and recorded on a Nicolet 5SX FTIR spectrometer. Mass spectra were taken with a Hewlett-Packard 5985B mass spectrometer either in the CI or EI mode. C, H, and N analyses in the Experimental Section and in Table I are within ±0.04 units unless otherwise indicated.

2-[(2-Cyclohexylethyl)amino]adenosine (3af) (Method A). A mixture of 2-chloroadenosine (**3a**, 2.1 g, 7 mmol) and 2-cyclohexylethylamine (4.5 g, 35 mmol) was stirred in an oil bath at 140 °C for 4 h. TLC in 9:1 methylene chloride–methanol saturated with ammonia showed a major spot at $R_f = 0.3$ and no starting material ($R_f = 0.2$). The solution was cooled to room temperature, diluted with ethanol (100 mL), and treated overnight under stirring with propylene oxide (25 mL). The precipitated solid was collected, washed with a little ethanol, then ether, and dried at 100 °C under vacuum (ca. 0.1 mm) for 15 h to afford a white solid: 1.95 g, 71%; mp 136–141 °C; $[\alpha]^{25}_D = -28.7°$ ($c = 0.97$, DMSO); IR 3371, 3164, 1668, 1598, 1541, 1376, 1116 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.79–0.96 (m, 2 H, ring, ortho, equatorial), 1.01–1.23 (m, 3 H, ring, meta + para, equatorial), 1.29 (m, C*H* in ring connected to side chain), 1.39 (q, $CHCH_2CH_2NH$), 1.52–1.76 (m, 5 H, axial protons in ring), 3.24 (q, $-CH_2NH-$), 3.57 (m, CH_2OH),

(39) RS/1, Bolt, Beranek & Newman, Boston MA.

Table III. Oral Antihypertensive Effects in Conscious Spontaneously Hypertensive Rats[a]

compd	dose, mg/kg po	N	mean arterial pressure		heart rate	
			baseline	change	baseline	change
vehicle	1 mL/kg	10	177 ± 7	−3 ± 4	341 ± 11	41 ± 15
1c	0.3	6	159 ± 7	−19 ± 5*	352 ± 10	−53 ± 33*
	1.0	6	156 ± 4	−71 ± 6*	334 ± 13	−189 ± 13*
2b	0.1	6	168 ± 7	−27 ± 8*	377 ± 7	53 ± 32
	0.3	6	168 ± 4	−42 ± 4*	419 ± 8	−76 ± 36*
3b	0.3	10	165 ± 4	−33 ± 4*	409 ± 6	25 ± 9
	1.0	7	180 ± 5	−45 ± 7*	391 ± 19	66 ± 17
	3.0	10	170 ± 4	−43 ± 6*	391 ± 9	42 ± 16
	10.0	13	163 ± 3	−50 ± 5*	409 ± 8	38 ± 14
3d	1.0	10	158 ± 3	−25 ± 6*	343 ± 8	139 ± 8*
	3.0	6	175 ± 4	−47 ± 6*	390 ± 8	71 ± 11
	10.0	6	182 ± 8	−68 ± 6*	406 ± 8	85 ± 12*
3g	10.0	5	179 ± 6	−34 ± 3*	343 ± 15	95 ± 13*
3j	10.0	5	177 ± 5	−43 ± 9*	342 ± 13	123 ± 19*
3k	3.0	9	157 ± 6	−28 ± 7*	301 ± 6	143 ± 20*
	10.0	9	181 ± 5	−78 ± 6*	394 ± 13	−76 ± 35*
3n	1.0	9	163 ± 5	−38 ± 9*	387 ± 15	12 ± 19
3s	1.0	7	165 ± 7	−22 ± 10	375 ± 12	29 ± 12
	3.0	9	183 ± 5	−36 ± 7*	393 ± 12	32 ± 19
3t	3.0	5	169 ± 4	−31 ± 10*	333 ± 4	98 ± 18*
3x	3.0	9	183 ± 6	−44 ± 11*	330 ± 11	96 ± 7*
3y	10.0	6	162 ± 6	−44 ± 10*	348 ± 8	74 ± 16*
3aa	3.0	10	162 ± 3	−33 ± 11*	379 ± 9	22 ± 14
3ab	10.0	9	167 ± 5	−37 ± 9*	411 ± 10	6 ± 9
3ad	3.0	9	165 ± 4	−39 ± 7*	350 ± 14	113 ± 14*
3ae	3.0	8	174 ± 6	−30 ± 9*	372 ± 8	70 ± 25
3af	0.3	11	160 ± 5	−14 ± 5	362 ± 5	64 ± 11
	1.0	9	166 ± 2	−43 ± 8*	387 ± 9	39 ± 16
	3.0	10	177 ± 6	−69 ± 5*	374 ± 10	102 ± 14*
3ag	1.0	8	157 ± 3	−28 ± 5*	368 ± 12	88 ± 22*
	3.0	8	160 ± 5	−40 ± 10*	352 ± 17	131 ± 23*
	10.0	8	174 ± 6	−60 ± 7*	378 ± 12	94 ± 17*
3ah	3.0	9	159 ± 5	−26 ± 6*	379 ± 9	85 ± 12*
3ai	3.0	7	160 ± 4	−30 ± 4*	372 ± 17	67 ± 21

[a] All values represent the mean ± standard error of the mean for each group of animals. Drugs were administered by oral gavage in a 3% cornstarch vehicle. Mean arterial pressure (mmHg) and heart rate (beats per min) were continuously recorded prior to drug administration (baseline) and for 6 h following oral dosing. The maximum group changes in mean arterial pressure and heart rate are depicted above. The asterisk (*) denotes a significant difference ($P < 0.05$) compared to the vehicle control using a one-way ANOVA followed by Dunnett's multiple comparison test. N represents the number of animals per group.

Table IV. Effects of 4-Day Repeated Oral Administration of **3af** on Blood Pressure and Heart Rate in Conscious Spontaneously Hypertensive Rats[a]

	day 1		day 2		day 3		day 4	
	BP	HR	BP	HR	BP	HR	BP	HR
vehicle (3% cornstarch) (N = 11)	−15 ± 3	8 ± 7	−18 ± 4	36 ± 12	−18 ± 4	8 ± 12	−18 ± 6	34 ± 22
3af (1 mg/kg/day) (N = 12)	−54 ± 7*	48 ± 14*	−63 ± 8*	70 ± 24*	−54 ± 10*	60 ± 16*	−50 ± 12*	81 ± 11*

[a] Abbreviations: BP, HR: Maximum change in mean arterial blood pressure (mmHg) and heart rate (beats/min) ± mean standard error for the group. Blood pressure and heart rate were monitored over the first 6 h of each day. Data was analysed by ANOVA followed by Dunnett's test to determine differences from vehicle group where * represents statistical significance, $p < 0.05$.

3.89 (ddd, 4′-*H*), 4.12 (ddd, 3′-*H*), 4.59 (dd, 2′-*H*), 5.11 (d, 2 H, 3′- and 5′-O*H*), 5.35 (d, 2′-O*H*), 5.71 (d, 1′-*H*, $J_{H1'-H2'}$ = 6.7 Hz), 6.08 (t, N*H*CH_2), 6.68 (s, NH_2), 7.89 (s, 8-*H*); ^{13}C NMR (DMSO-d_6) δ 25.78 (para ring C), 26.14 (2 meta ring C), 32.80 (2 ortho ring C), 34.75 (ring C connected to side chain), 36.74 (CHCH_2CH_2), 38.74 (CH_2CH_2NH), 113.41 (C-5), 136.39 (C-8), 151.46 (C-4), 155.71 (C-6), 159.07 (C-2); MS m/e 393 (M + 1).

2-[[2-[2-(5,6,7,8-Tetrahydronaphthyl)]ethyl]amino]-adenosine (2ae) (Method B). A mixture of **3a** (1.05 g, 3.5 mmol), 2-[2-(5,6,7,8-tetrahydronaphthyl)]ethylamine (1.25 g, 7 mmol), *N,N*-diisopropylethylamine (4.5 g, 35 mmol), and isoamyl alcohol (15 mL) was stirred in an oil bath at 140 °C for 48 h. The mixture was concentrated at reduced pressure and the residue taken up in ethyl acetate, washed with dilute sodium bicarbonate solution, dried over sodium sulfate, and concentrated to dryness. The residue was flash chromatographed through a 37 × 190 mm column of silica with 15:1 methylene chloride–ammonia-saturated methanol. Thirty-milliliter fractions were collected and monitored by TLC. Fractions containing the desired product were collected, concentrated to dryness, and triturated with ether. A light brown solid was obtained (0.5 g, 33%) and recrystallized from acetonitrile to afford the pure product as an off-white solid: 180 mg; mp 140–143 °C; $[\alpha]^{25}_D$ = −30.4° (c = 0.71, DMSO); MS m/e 441 (M + 1).

The starting amine was prepared as follows: **Methyl 2-(5,6,7,8-Tetrahydronaphthoate)** was prepared from cyclohexanone by the literature procedure,[40] reduced with lithium aluminum hydride in ether in an ice bath over 1.5 h, and then oxidized with a 12-fold molar amount of active manganese dioxide in methylene chloride at room temperature overnight to afford the aldehyde in 93% yield. An ice-cooled solution of the aldehyde and nitromethane (5% molar excess) was treated dropwise with a 10 N aqueous sodium hydroxide (10% molar excess) and allowed to stir at ambient temperature over 2 h. The thick mixture was quenched in ice-cold 6 N hydrochloric acid and extracted with ethyl acetate, and the acetate layer washed with water, then brine,

(40) (a) Boger, D. L.; Mullican, M. D. Inverse Electron Demand Diels–Alder Reaction of 3-Carbomethoxy-2-pyrones with 1,1-Dimethoxyethylene. *Tetrahedron Lett.* **1982**, *23*, 4551–4554. (b) Inverse Electron Demand Diels–Alder Reactions of 3-Carbomethoxy-2-pyrones. *Tetrahedron Lett.* **1983**, *24*, 4939–4942.

and dried over sodium sulfate. The crude nitro olefin obtained as a colorless oil (98%) was reacted with a 110% molar excess of lithium aluminum hydride in ether at room temperature overnight. Standard aqueous alkaline workup yielded an oil (39%) containing some α-(aminomethyl)[2-(5,6,7,8-tetrahydronaphthyl)]methanol (detected by NMR analysis). The whole was dissolved in methylene chloride and treated with 6.5 N HCl in ether followed by thionyl chloride in ether and reacted overnight at room temperature. The mixture was concentrated to dryness, dissolved in ethanol, and hydrogenated at 50 psi with 10% palladium on charcoal over 6 h. The residue was suspended in ethyl acetate, neutralized with bicarbonate solution, dried, and concentrated at reduced pressure to afford **2-[2-(5,6,7,8-tetrahydronaphthyl)]ethylamine** as a colorless oil (30% yield from the nitro olefin), which was pure according to TLC and NMR: ^{1}H NMR ($CDCl_3$) δ 1.76–1.83 (m, 2 CH_2), 1.97 (s, NH_2), 2.62–2.71 (dd, CH_2), 2.73–2.87 (m, 2 CH_2), 2.88–2.98 (dd, CH_2), 6.9–7.1 (m, 3 H, aromatic).

2-[4-(*tert*-Butoxycarbonyl)phenyl]ethylamine. The acid chloride of 4-bromobenzoic acid was converted to the *tert*-butyl ester by addition of an ether solution to butyllithium (2.5 M in hexane) in excess *tert*-butanol at room temperature overnight. The crude ester, obtained as a yellow oil in 86% yield was used in the following reaction: A mixture of the ester (5.2 g, 0.02 mol), *N*-vinylphthalimide (3.65 g, 0.02 mol), palladium acetate (104 mg, 0.46 mmol), tri-*o*-tolylphosphine (468 mg, 1.5 mmol), *N,N*-diisopropylethylamine (4.7 mL, 0.027 mol), and acetonitrile (7.5 mL) was heated overnight at 90 °C. The mixture was quenched in cold water and the greenish solid collected and dried under vacuum. This was suspended in methylene chloride, treated with a little silica, evaporated to dryness, and flash chromatographed, first with 3:1 hexane–ether and later with 1:1 hexane–ether. The desired material was collected and dried to a yellow powder (5 g). This was dissolved in ethanol (170 mL) and tetrahydrofuran (120 mL) and hydrogenated with 10% palladium on charcoal (1.9 g) at 50 psi overnight. The mixture was filtered through diatomaceous earth and the solvent removed to afford a white solid (5 g). This was dissolved in ethanol (50 mL), hydrazine hydrate (5 mL) was added, and the mixture was stirred 1.5 h at 80 °C and cooled and the phthalhydrazide filtered off and washed with ethanol. The filtrate and washes were concentrated to dryness, extracted with ether, washed with dilute potassium hydroxide, then with brine, dried (Mg_2SO_4), concentrated to dryness, and flash chromatographed with 9:1 methylene chloride–methanol to remove nonpolar material followed by 19:1 methylene chloride–ammonia-saturated methanol to elute the product, obtained as a pale yellow oil (1.8 g, 41%) after evaporation of solvent: ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 3 CH_3), 2.65–2.75 (m, CH_2), 2.9–3.0 (m, CH_2), 3.5 (s, CH_2), 7.1–7.2 (m, 4 H, aromatic).

2-[4-(2-Phenylethenyl)phenyl]ethylamine. A mixture of 2-(4-bromophenyl)ethylamine (30 g, 0.15 mol), phthalic anhydride (22.2 g, 0.15 mol), and acetic acid (300 mL) was heated at reflux for 18 h, then concentrated to dryness at reduced pressure and stirred for 0.5 h in ethanol (150 mL). The precipitate was collected and vacuum oven dried to afford 4-bromo-(2-phthalimidoethyl)benzene (47.1 g, 95%). A portion (23.1 g, 70 mmol) was mixed with styrene (9.5 g, 90 mmol), palladium acetate (0.16 g, 0.7 mmol), tri-*o*-tolylphosphine (0.85 g, 2.8 mmol), and triethylamine (46.5 g, 0.456 mol) and heated at reflux for 18 h. The mixture was treated with cold dilute HCl and extracted three times with ethyl acetate. The extract was washed with water, then brine, and dried over sodium sulfate. The dried extract contained 5.0 g of yellow solid. The aqueous phase was then extracted with methylene chloride three times. This washed, dried extract yielded 24.7 g of white solid. The material was recrystallized from 2-methoxyethanol to afford pure 4-(2-phthalimidoethyl)stilbene: 21.3 g, 86%; mp 212–215 °C. Anal. ($C_{24}H_{19}NO_2$) C, H, N. This intermediate (5.65 g, 16 mmol) was refluxed in ethanol (100 mL) containing hydrazine hydrate (1.6 mL, 32 mmol) for 18 h. The material was concentrated to dryness at reduced pressure, stirred with ice-cold 2 N potassium hydroxide solution, and extracted with ethyl acetate. The organic extract was washed with water, then with brine, dried (Na_2SO_4), and concentrated to the desired amine, obtained as a colorless oil (3.5 g, 97%): ^{1}H NMR ($CDCl_3$) δ 1.28 (s, NH_2), 2.7–2.8 (dd, CH_2), 2.92–3.0 (dd, CH_2), 7.05–7.55 (m, 11 H, aromatic + vinyl). This was used to prepare **3x**.

2-[4-(2-Phenylethyl)phenyl]ethylamine. 2-[4-(2-phenylethenyl)phenyl]ethylamine (2.23 g, 10 mmol) was hydrogenated in ethanol (100 mL) containing 1 N HCl (20 mL) with 10% palladium on carbon (0.25 g) at 50 psi for 3 h. The mixture was filtered and the filtrate was concentrated to dryness at reduced pressure, treated with excess 2 N sodium hydroxide, and extracted with ethyl acetate. This extract was washed with water, then with brine, dried (Na_2SO_4), and concentrated to a colorless oil (2.0 g, 91%): ^{1}H NMR ($CDCl_3$) δ 1.15 (s, NH_2), 2.7–2.78 (dd, CH_2), 2.9 (s, CH_2CH_2), 2.92–3.0 (dd, CH_2), 7.1–7.4 (m, 9 H, aromatic). This amine, prepared previously by a different route,[41] was used directly to synthesize **3v**.

2-[4-(2-Cyclohexylethyl)phenyl]ethylamine. 2-(4-Bromophenyl)ethylamine was converted in two steps to *N*-[2-[4-(2-cyclohexylethenyl)phenyl]ethyl]phthalimide as described above for the stilbene derivative except that vinylcyclohexane was used. The product, mp 138–140 °C from ethanol, was obtained in 46% yield. Anal. ($C_{24}H_{25}NO_2$) C, H, N. It was hydrogenated in ethyl acetate over 10% palladium on carbon in 6 h at 50 psi and recrystallized from ethanol to afford pure *N*-[2-[4-(2-cyclohexylethyl)phenyl]ethyl]phthalimide (mp 135–138 °C) in 80% yield. Anal. ($C_{24}H_{27}NO_2$) C, H, N. Reaction with ethanolic hydrazine hydrate as described previously yielded the desired amine as a colorless oil in 81% yield: 1H NMR ($CDCl_3$) δ 0.85–1.0 (m, CH_2), 1.1–1.3 (m, 3 CH_2), 1.42–1.52 (m, CH_2), 1.6–1.8 (m, 5 H, CH + CH_2 + NH_2), 2.55–2.62 (m, CH_2), 2.68–2.74 (dd, CH_2), 2.92–2.98 (dd, CH_2), 7.05–7.18 (m, 4 H, aromatic). This was used directly to prepare **3w**.

Diethyl [2-[4-(2-Aminoethyl)phenyl]ethyl]phosphonate. Coupling of 4-bromobenzyl cyanide with diethyl vinylphosphonate as described above followed by hydrogenation twice with 10% palladium on carbon in ethanol at 50 psi over 6 h gave diethyl [2-(4-cyanomethylphenyl)ethyl]phosphonate as an oil in 64% yield. The nitrile (4.5 g, 16 mmol) was dissolved in a mixture of methanol (45 mL) and tetrahydrofuran (90 mL). Cobalt chloride hexahydrate (14.86 g, 62.5 mmol) in water (90 mL) was added, and after 5 min, sodium borohydride (2.84 g, 74.6 mmol) was added gradually. The black mixture was stirred 10 min, then filtered through diatomaceous earth. The solvent was evaporated and the residue chromatographed with 19:1 methylene chloride–ammonia-saturated methanol to give a yellow oil (3.2 g, 70%) that was used directly to prepare **3u**: ^{1}H NMR ($CDCl_3$) δ 1.28–1.34 (2 t, 2 CH_3CH_2), 1.7 (s, NH_2), 1.97–2.1 (m, CH_2), 2.68–2.74 (m, CH_2), 2.83–2.98 (m, 2 CH_2), 4.02–4.13 (2 q, 2 CH_2CH_3), 7.07–7.27 (m, 4 H, aromatic).

2-[[2-[4-(2-Carboxyethyl)phenyl]ethyl]amino]adenosine (3y). A mixture of *tert*-butyl 3-[4-(2-aminoethyl)phenyl]-propionate[26] (9.4 g, 37.7 mmol) and **3a** (3.5 g, 11.6 mmol) was stirred at 130 °C for 6 h. This was taken up in ethyl acetate, washed with sodium bicarbonate solution, and dried (Mg_2SO_4). The concentrated extract was dissolved in methanol, treated with a little silica, evaporated to dryness, and chromatographed on silica with 9:1 methylene chloride–ammonia-saturated methanol as eluent. Concentration of the fractions containing the desired material and trituration with ethyl acetate afforded the *tert*-butyl ester as an off-white solid (3.5 g, 59%). This was suspended in a mixture of tetrahydrofuran (13.5 mL), methanol (26.9 mL) and 10% sodium hydroxide (32.3 mL), stirred 1.5 h at ambient temperature, and concentrated to dryness. The residue was taken up in water, washed with ether, and acidified with cold 1 N HCl. The resulting precipitate was washed with water, then with ether and air-dried to afford the hydrochloride salt of **3y** (2.88 g, 85%; mp 135–139 °C). A portion (0.5 g) was treated with propylene oxide (2 mL) in methanol (10 mL) for 2 h. The solution was concentrated to dryness and triturated with ether to give **3y** (56%). Recrystallization from acetonitrile with drying overnight at 100 °C (0.1 mm) gave the analytical sample, mp 184–188 °C.

2-[[2-[4-(2-Carbethoxyethyl)phenyl]ethyl]amino]-adenosine (3t). A mixture of **3y**·HCl (100 mg, 0.2 mmol) in

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dimethylformamide (1 mL) was treated with 50% sodium hydride in oil (20 mg) for 20 min, and then with ethyl iodide (0.02 mL) for 20 min longer. The mixture was concentrated to dryness at 0.1 mm, taken up in ethyl acetate, washed with sodium bicarbonate solution, dried (Mg_2SO_4), concentrated to dryness, and triturated with ether to afford the ester as an off-white solid, mp 110–118 °C (30 mg, 31%). Similarly, **3s** was prepared from **3a** through the use of *tert*-butyl 4-(2-aminoethyl)phenylacetate.[26]

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Synthesis and Evaluation of Stilbene and Dihydrostilbene Derivatives as Potential Anticancer Agents That Inhibit Tubulin Polymerization

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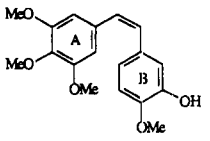
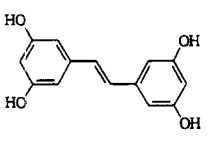
An array of *cis*-, *trans*-, and dihydrostilbenes and some *N*-arylbenzylamines were synthesized and evaluated for their cytotoxicity in the five cancer cell cultures A-549 lung carcinoma, MCF-7 breast carcinoma, HT-29 colon adenocarcinoma, SKMEL-5 melanoma, and MLM melanoma. Several *cis*-stilbenes, structurally similar to combretastatins, were highly cytotoxic in all five cell lines and these were also found to be active as inhibitors of tubulin polymerization. The most active compounds also inhibited the binding of colchicine to tubulin. The most potent of the new compounds, both as a tubulin polymerization inhibitor and as a cytotoxic agent, was (*Z*)-1-(4-methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)ethene (**5a**). This substance was almost as potent as combretastatin A-4 (**1a**), the most active of the combretastatins, as a tubulin polymerization inhibitor. Compound **5a** was found to be approximately 140 times more cytotoxic against HT-29 colon adenocarcinoma cells and about 10 times more cytotoxic against MCF-7 breast carcinoma cells than combretastatin A-4. However, **5a** was found to be about 20 times less cytotoxic against A-549 lung carcinoma cells, 30 times less cytotoxic against SKMEL-5 melanoma cells, and 7 times less cytotoxic against MLM melanoma cells than combretastatin A-4. The relative potencies **5a** > **8a** > **6a** for the cis, dihydro, and trans compounds, respectively, as inhibitors of tubulin polymerization are in agreement with the relative potencies previously observed for combretastatin A-4 (**1a**), dihydrocombretastatin A-4 (**1c**), and *trans*-combretastatin A-4 (**1b**). The relative potencies **5a** > **8a** > **6a** were also reflected in the results of the cytotoxicity assays. Structure–activity relationships of this group of compounds are also discussed.

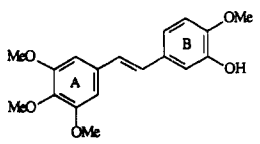
Interest in the synthesis and evaluation of polymethoxylated stilbenes and dihydrostilbenes as potential anticancer agents stems from the discovery of many such natural products as antimitotic and antileukemic agents.[1-12] This includes isolation of many stilbene derivatives, termed combretastatins, from the South African tree *Combretum caffrum*. Many of these combretastatins were found to be cytotoxic, with combretastatin A-4 (**1a**) the most potent.[7]



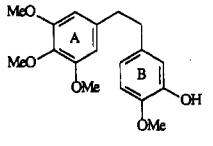
Combretastatin A-4 (1a)



Piceatannol (2)



trans-Combretastatin A-4 (1b)



Dihydrocombretastatin A-4 (1c)

This compound was found to cause mitotic arrest in L1210 murine leukemia cells, inhibit tubulin polymerization, and competitively inhibit the binding of radiolabeled colchicine to tubulin.[7] It is presently being investigated under the sponsorship of the Cancer Research Campaign Clinical Trails scheme.[13] A recent investigation of combretastatins revealed that combretastatin A-4 (**1a**) was active against multidrug resistant (MDR) cancer cell lines.[13] Combretastatin A-4 (**1a**) as well as its trans isomer **1b**, its dihydro derivative **1c**, and a number of related substances have been found to cause mitotic arrest in cells in culture at cytotoxic concentrations.[1,2,4-9,13-17] Several other *trans*-stilbenes and 1,4-diarylalkanes were also reported to be

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