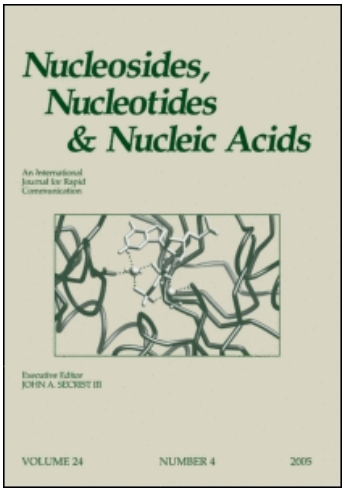

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CORONARY VASOACTIVITY OF NOVEL N^6-SUBSTITUTED ADENOSINES

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Abstract. Previous studies of the structure-coronary vasoactivity relationships of adenosines substituted at N-6 identified certain simple alkyl and aralkyl groups that can promote activity and others that reduce activity. The present study shows that such alkyl and aryl moieties, when combined in an N-6 substituent, can contribute additively to coronary vasoactivity.

Certain substituents at N-6 enhance the coronary vasoactivity of adenosine.[1] Structure-activity correlations aimed at accounting for the substantial, stereoselective coronary vasoactivity of N^6-(*1R*-methyl-2-phenethyl)adenosine, **2**, characterizes an N-6 region of specialized structure that interacts with the N-6 substituent of this nucleoside.[2,3] Such studies identified several simple alkyl and aryl substituents at N-6 that increased coronary vasoactivity by as much as four- to five-fold. Examples of the alkyl substituents include *2S*-butyl and 3-pentyl, and examples of aryl substituents include 2-(2-thienyl)ethyl and 2-(3,4,5-trimethoxyphenyl)ethyl. A hypothetical model of the N-6 region of the receptor (Figure 1) consists of five subregions, each structurally and chemically complementary to the components of the exocyclic N-6 substituent that confers coronary vasoactivity on **2**. In accordance with the structure of **2**, the regions are called aryl, N-6 and alkyl S-1, S-2 and S-3.[2,4] The present study emphasizes three subregions, namely, the aryl subregion that lies under the asymmetric carbon and which accommodates

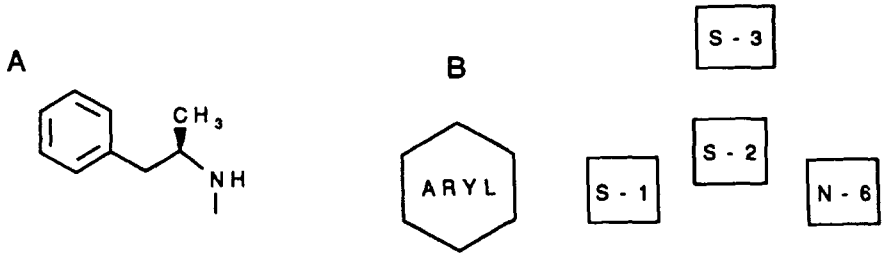


Figure 1. *A.* Structure of the N-6 Substituent of **2**. *B.* Model of the N-6 region of the coronary artery adenosine receptor. See text for details.

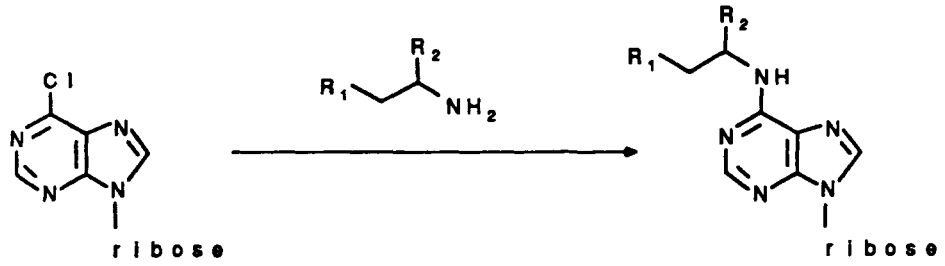
residues as large as naphthalene, the S-2 subregion, which has limited bulk tolerance that confers stereoselectivity, and the S-3 subregion, which is hydrophobic and large enough to accommodate an ethyl group.

Work by others has confirmed the usefulness of this model and has provided important additional details about the structure of the N-6 region of the A_2 adenosine receptor. The synthesis[5] of a large number of adenosines substituted at N-6 and their assay by the inhibition of radioligand binding to the A_2 receptor in rat brain striatum[6] has provided the information needed to apply the molecular modeling approach[7,8] to describing the topography of the N-6 region of the receptor. The discovery of a second aryl subregion is one achievement of that approach.

Here we test the hypothesis that the alkyl and aryl moieties in an N-6 substituent contribute additively to the coronary vasoactivity of adenosine. the analogues used to test this hypothesis contain some N-6 substituents that the receptor model predicts will increase activity as well as others that the model predicts will decrease activity.

Chemistry. The reaction of 6-chloropurine riboside, **1**, with a primary or secondary amine in the presence of an acid scavenger such as triethylamine[9] is a general route to adenosines substituted at N-6 (Scheme 1). An asymmetric synthesis[10] yielded the *R* and *S* enantioners of 1-phenyl-2-butylamine, 1-phenyl-2-pentylamine and 1-(3,4,5-trimethoxyphenyl)-2-butylamine as well as the *R* enantiomers of 1-(2-methoxyphenyl)-2-propylamine and 1-(4-

Scheme 1



1 **2-15**

methoxyphenyl)-2-propylamine, all of which are known. *R* and *S* amphetamine, *2S*-amino-3-phenyl-1-propanol and *2S*-amino-3-phenylpropionamide are commercially available. The reduction of 1-(2-thienyl)-2-nitro-1-butene with $LiAlH_4$ yielded racemic 1-(2-thienyl)-2-butylamine,[11] which was resolved by means of fractional crystallization of the tartarate salts. One goal, the synthesis of N^6-(3-amino-1-phenyl-*2S*-propyl)adenosine, proved unattainable. The reaction of **1** with *2S*-amino-3-phenylpropionamide proceeded smoothly to yield N^6-*1S*-carbamoyl-2-phenethyl)adenosine, **9**, but attempts to reduce the amide by $LIAlH_4$, by BH_3-tetrahydrofuran or by H_2 and Pd/C yielded only starting material. Table 1 reports the analytical and physical data of novel nucleosides **4-15**.

Coronary Vasoactivity. Table 2 summarizes assays of the coronary vasoactivity of **4-15** and, for purposes of comparison, includes previously reported measurements of the vasoactivity of N^6-2-phenethyladenosine and of the *S* diastereomer of N^6-(1-methyl-2-phenethyl) adenosine, **3**. Note that all the analogues are N^6-2-(aryl)ethyladenosines; the substituents at N-6 differ according to either (*a*) the substituent on ethyl C-1 (analogues **2-9**) or (*b*) the aryl group on ethyl C-2 (analogues **10 - 15**).

Analogues **2-9** probe two attributes of the S-3 receptor subregion, namely, its size and hydrophobicity. The activity of **2** is nearly twice that of N^6-2-phenethyladenosine; this is evidence that the S-3 subregion exists. The

Table 1. Physical and Analytical Data of Analogues 4-15

No.	R_1[a]	R_2	Anal[b]	Yield	Purific[c]	mp	$[\alpha]_D^{20}$	UV $\lambda_{max}(\epsilon)$
4	Ph	*R*-Et	C,H,N	82	A,B	135-136	- 120	271 (18,100)
5	Ph	*S*-Et	C,H,N	87	A	96-100	+ 7	271 (18,000)
6	Ph	*R*-Pr	C,H,N	75	C	173-175	- 110	270 (18,100)
7	Ph	*S*-Pr	C,H,N	70	C	137-139	- 20	271 (18,100)
8	Ph	*S*-CH_2OH	C,H,N	43	D	95-100	—	270 (18,500)
9	Ph	*S*-$CONH_2$	C,H,N	81	D	113-115	- 29	270 (18,000)
10	2-thienyl	*R*-Et	C,H,N,S	71	E	139-140	- 83	272 (18,100)
11	2-thienyl	*S*-Et	C,H,N,S	60	E	—	- 42	272 (18,100)
12	3,4,5-$(OMe)_3$Ph	*R*-Et	C,H,N	78	E	154-156	- 110	272 (18,000)
13	3,4,5-$(OMe)_3$Ph	*S*-Et	C,H,N	55	E	90-92	- 42	272 (18,000)
14	2-(OMe)Ph	*R*-Me	C,H,N	70	E	147-148	- 106	272 (18,000)
15	4-(OMe)Ph	*R*-Me	C,H,N	62	E	162-163	- 111	272 (18,100)

[a] Abbreviations are: Ph, phenyl; Me, methyl; Et, ethyl; Pr, propyl. [b] Analyses agreed with calculated composition within ± 0.4%. [c] Methods included A, flash chromatography on 40-60 μm silica gel eluted with $CHCl_3/C_2H_5OH$, 10:1; B, crystallization from ethyl acetate/hexane after chromatography; C, reverse phase HPLC on C-18 silica, gradient elution with 50→70% CH_3OH/H_2O; D, same as C except 30→50% CH_3OH/H_2O gradient; E, reverse phase HPLC on C-18 silica, isocratic elution with 50% CH_3OH/H_2O.

Table 2. Coronary Vasoactivity of N^6-Substituted Adenosines

No	N^6-Substituent	MPR *vs* Adenosine [a]	k' [b]
-	2-phenethyl	2.0±	
2	*1R*-methyl-2-phenethyl	3.7 ± 0.26	3.13
3	*1S*-methyl-2-phenethyl	0.41 ± 0.07	3.15
4	*1R*-ethyl-2-phenethyl	9.0 ± 0.34	5.22
5	*1S*-ethyl-2-phenethyl	0.36 ± 0.24	5.19
6	*1R*-propyl-2-phenethyl	2.0 ± 0.70	6.55
7	*1S*-propyl-2-phenethyl	0.48 ± 0.13	6.59
8	*1S*-hydroxymethyl-2-phenethyl	1.6 ± 0.08	1.63
9	*1S*-carbamoyl-2-phenethyl	0.47 ± 0.08	1.06
10	*1R*-ethyl-2-(2-thienyl)ethyl	25 ± 5.0	—
11	*1S*-ethyl-2-(2-thienyl)ethyl	13 ± 3.1	—
12	*1R*-ethyl-2-(3,4,5-trimethoxyphenyl)ethyl	11 ± 3.3	—
13	*1S*-ethyl-2-(3,4,5-trimethoxyphenyl)ethyl	0.18 ± 0.01	—
14	*1R*-methyl-2-(2-methoxyphenyl)ethyl	0.16 ± 0.06	—
15	*1R*-methyl-2-(4-methoxyphenyl)ethyl	0.63 ± 0.10	—

[a] Molar potency ratio, calculated as described in EXPERIMENTAL SECTION. The EC_{50} of adenosine for the 65 assays of analogues **2** and **4-15** averaged 0.98 ± 0.01 μ*M*.

[b] Hydrophobicity index, calculated as described in EXPERIMENTAL SECTION

activity of **4** is twice that of **2** and over four times that of **6**, evidence that the S-3 subregion is large enough to accommodate the 1-ethyl group of **4** but not the 1-propyl group of **6**. Although **6** is more hydrophobic than **4** and accordingly is more tightly bound to plasma proteins (and thus penetrates to tissue receptors less readily),[2] the difference in the hydrophobicity indices of the two nucleosides (k') is too small to account for the difference in vasoactivity. Analogue **8** is an oxygen isostere of **4**. It is substantially more polar and less active than **4**, additional evidence that the S-3 subregion is hydrophobic. Analogue **9** is even more polar and less active than **8**, further evidence that the S-3 subregion is hydrophobic.

Analogues **10-15** examine the effect on coronary vasoactivity of modifying the aryl moiety. The coronary vasoactivity of N^6-2-(2-thienyl)ethyladenosine is twice that of the phenethyl congener,[3] a result that predicts the potency of **10**, which is over twice that of **4**. However, N^6-(3,4,5-trimethoxyphenyl)ethyladenosine is nearly three times as active as the unsubstituted phenethyl congener, but the vasoactivity of **12** is essentially the same as that of **4**.

The introduction of a methoxy group into the phenyl ring reduces the coronary vasoactivity of N^6-2-phenethyladenosine,[3] an observation that predicts that similar modification will reduce the vasoactivity of **2**. The activities of **14** and **15** are substantially less than that of **2**, bearing out the prediction.

Stereoselectivity. In each of the analogues studied here the carbon atom adjacent to N-6 is a chiral center. Stereoselective discrimination between agonists such as **2** and **3** is thought to be an important difference between the two major types of adenosine receptors. A_1 receptors are highly stereoselective but the A_2 receptors that mediate coronary vasodilation [12] are not. Typically, **2** is 50- to 100- fold more potent than **3** at A_1 receptors, but at A_2 receptors the potency ratio is 10 or less. [13-15] In the present study, the activity ratio **2/3** is low, as expected from the literature. However, the activity ratio **4/5** is 25, much higher than expected for an A_2 receptor and the activity ratio **12/13** is still higher, 63. Further, with the exception of **11**, the activity of the *S* diastereomers is low and relatively constant, the MPRs being 0.4 or less. As a consequence, stereoselectivity varies directly with the activity of the *R* diastereomer. The present observations suggest that what previously appeared to be low stereoselectivity of the A_2 receptor may simply reflect the fact that none of the *R* diastereomers tested were very active.

The absolute configurations of the enantiomers of 1-(2-thienyl)-2-butylamine are unknown, but the difference in the activities of the adenosines derived from them provides indirect evidence about their configurations. The present work and a previous study [2] of the stereoselectivity of analogues of **2** show that the diastereomer having the same absolute configuration as **2** is the more active of the pair. Invoking this rule and taking into account the optical

rotation measurements indicating that the optical purity of (+)-1-(2-thienyl)-butylamine is lower than the (-) enantiomer, we interpret the potencies of **10** and **11** as evidence that **10** is N^6-[*1R*-ethyl-2-(2-thienyl)ethyl]adenosine and **11** is the *S* diastereomer, significantly contaminated with **10**.

EXPERIMENTAL

Starting materials were from Aldrich Chemical Co., Milwaukee, WI. and Sigma Chemical Co., St. Louis, MO. Optical rotations were determined on a Perkin-Elmer 241 MC polarimeter, and melting points (uncorrected) on a Thomas-Hoover apparatus. The retention times of the nucleosides on an 0.45 X 15 cm column of octadecylsilyl silica (Ultrasphere ODS ™, Rainin Instrument Co., Waltham, MA.) eluted at 2000 p.s.i. with 0.01 *M* $NaHPO_4$, pH 7.0: methanol, 40:60, v/v, served for the calculation of k', an index of hydrophobicity by the formula $k'=(t-t_o)/t_o$, where t is the retention time of the nucleoside and t_o is the transit time of the solvent.

N^6-(*1R*-ethyl-2-phenethyl)adenosine. 4 . The synthesis of 4 is a typical example of the syntheses of **5-15**. A mixture of 1.5 g (5.2 mmole) 6-chloropurine riboside, 0.85 g (5.7 mmole) *R*(-)-1-phenyl-2-butylamine and 2.2 ml (15.6 mmole) triethylamine in 50 ml absolute ethanol were heated for 24 hours at reflux. The residue was evaporated to give a syrup. Table I lists methods of purification and yields of this and all the other nucleosides.

2′,3′,5′-triacetyl-N^6-1-acetoxy-3-phenyl-2-S-propyl)adenosine. To circumvent the diffuclty of obtaining a satisfactory elemental analysis on **7**,which is extremely hygroscopic, the nucleoside was acetylated to reduce hygroscopicity and facilitate purification. A mixture of 2 g (7 mmole) **7**, 10 mL acetic anhydride and 40 mL dry pyridine was stirred 18 hours at room temperature and evaporated. A solution of the residue in $CHCl_3$ was washed with 4 X 75 ml water, dried over $MgSO_4$ and evaporated. The resulting syrup was dissolved in methanol, decolorized with charcoal and purified by LPLC. The mobile phase was a linear methanol gradient formed by pumping absolute methanol into a mixing chamber containing methanol: water, 3:7 v/v. Fractions containing product were combined and evaporated to give 2.1 g (74%) of the peracylated nucleoside which, like **7**, did not crystallize. Elemental analysis for

$C_{27}H_{31}N_5O_9$ (569.57) calculated: C, 56.93; H, 5.49 and N, 12.30. Found C, 57.01; H, 5.52 and N 11.99.

(–)-1-(2-Thienyl)-2-butylamine. A solution of 8.4 g (54 mmole) (±)-1-(2-thienyl)-2-butylamine and 8.3 g (55 mmole) 2*R*,3*R*-(+)-tartaric acid in 100 ml boiling 2-propanol was filtered by gravity, slowly cooled and the crystals filtered off. Recrystallization (5 times) to constant rotation yielded 2 g of tartarate having an $[\alpha]_D^{20}$ of + 10.6° (c = 1,95% EtOH). The amine liberated by alkali had an $[\alpha]_{365}^{20}$ of – 18.1° (c = 1, 95% EtOH).

(+)-1-(2-Thienyl)-2-butylamine. Eight crystallizations of the salt formed from *2S,3S*-(-)-tartaric acid (19.2g, 128 mmole) and (±)-1-(2-thienyl)-2-butylamine (19.9g, 128 mmole) yielded 5 g of product of $[\alpha]_D^{20}$ of – 33.8°(c= 1, 95% EtOH). The amine liberated by alkali had an $[\alpha]_{365}^{20}$ of + 10.0° (c = 1, 95% EtOH).

Assay of Coronary Vasoactivity.[12,16] Induction with Na thiamylal (18 mg/kg i.v.) followed by ventilation with O_2-enriched room air containing 0.5-1% fluothane maintained dogs in surgical anesthesia for the implantation of an electromagnetic flowmeter and pneumatic occluded on either the circumflex or anterior descending branch of the left coronary artery. A plastic catheter inserted into the coronary artery distal to the occluder served for nucleoside administration and a catheter advanced into the aortic root *via* the left carotid artery served for monitoring coronary perfusion pressure. We used coronary conductance, the ratio of coronary blood flow rate (ml/min) divided by perfusion pressure (mm Hg), as an index of coronary vasodilation. Each assay consisted of a series of adenosine infusions at increasing rates followed, after a washout period, by a series of infusions of an adenosine analogue. The experimental observations consisted of measurements of coronary flow and perfusion pressure during the steady state response to the infusion of adenosine or an analogue. The infusate concentration, rate of infusion, coronary flow rate and hematocrit yielded an estimate of the concentration of nucleoside in coronary plasma (neglecting binding to plasma proteins and penetration of nucleoside into cells). The regression of logit (conductance) on log [nucleoside] yielded an estimate of EC_{50}, the nucleoside concentration causing half-maximum coronary

vasodilation. To minimize between-animal variability in responsiveness, activity is expressed as a molar potency ratio (MPR), the quotient of the EC_{50} of adenosine divided by the EC_{50} of the test analogue. Data are reported as the mean ± SEM of assays in 5 dogs.

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