2-Aralkoxyadenosines: Potent and Selective Agonists at the Coronary Artery A_2 Adenosine Receptor

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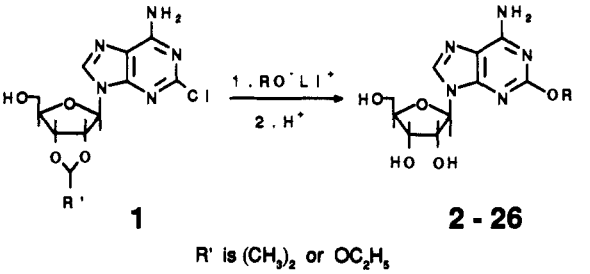
A Langendorff guinea pig heart preparation served for the assay of agonist potency of a series of 26 2-aralkoxyadenosines at the A_1 and A_2 receptors of, respectively, the atrioventricular node (conduction block) and coronary arteries (vasodilation). All of the analogues are weak agonists at the A_1 receptor, requiring concentrations >9 μM to cause second degree heart block. At the A_2 receptor 2-phenethoxyadenosine is the most potent of the 2-phenylalkyladenosines. The activity of ring-substituted (F, Cl, CH_3, and OCH_3) 2-phenethoxyadenosines increases ortho < meta < para. The EC_{50}s of coronary vasoactivity of several para-substituted analogues are in the subnanomolar range. The most potent analogue, 2-[2-(4-methylphenyl)ethoxy]adenosine 19, has an EC_{50} for coronary vasodilation of 190 pM and an A_1/A_2 selectivity ratio of 44000. Aryl groups such as thienyl, indoloyl, or naphthyl also support A_2 agonist activity. Although 2-oxoadenosine is 3 times more vasoactive than 2-aminoadenosine, the activites of the phenyl derivatives are markedly different; 2-phenoxyadenosine is 23 times weaker than 2-(phenylamino)adenosine (CV-1808).

A previous report[1] describes the synthesis of a series of 2-alkoxyadenosines and bioassays of their agonist potencies at the A_1 and A_2 adenosine receptors (A_1AR and A_2AR) in the AV node and coronary arteries, respectively, of guinea pig hearts. Analysis of the structure–activity relationships (SAR) of these 2-alkoxyadenosines yielded provisional models of the C-2 regions of the A_1AR and A_2AR (Figure 1). Features common to both receptors include (a) an X subregion that accommodates the oxygen atom that links the alkyl substituent to purine C-2 and (b) an alkyl subregion of very limited bulk tolerance that seems to interact negatively with short *n*-alkyl substituents and even more poorly with wide *sec*-alkyl groups. A prominent feature of the A_2AR is a hydrophobic subregion that accommodates cycloalkyl and bicycloalkyl groups. The interaction of such groups with the hydrophobic subregion overcomes the negative influence of interaction with the alkyl subregion and can promote strong agonist activity. By contrast, such substituents do not promote the agonist activity of adenosine at the A_1AR. Such a result implies that either the A_1AR lacks a hydrophobic subregion or, alternatively, that binding to such a subregion does not promote activity.

The present study extends the mapping of the C-2 regions of the A_1AR and A_2AR by examining the SAR of several types of 2-aralkoxyadenosines. The only previous study of 2-(ar)alkoxyadenosines is that of Marumoto et al.,[2] who report the synthesis of 2-phenoxyadenosine, three of its ring-substituted derivatives, and 2-phenoxyethoxyadenosine. In the open-chest-dog preparation used for bioassays, all were weaker coronary vasodilators than adenosine. The same authors also evaluated the 2-benzyl, 2-benzylamino, 2-phenethylamino and 2-benzylthio derivatives of adenosine and found that these analogues, too, were moderately to markedly less active than adenosine. Because this investigation antedated the discovery of A_1AR and A_2AR,[3,4] the authors did not evaluate selectivity. Subsequent comparisons of the activity of 2-(phenylamino)adenosine (CV-1808) showed that this analogue is 5–20-fold selective for the A_2AR.[5,6] Recent work shows that the 2-phenethylamino derivative of *N*-ethyladenosin-5′-uronamide (NECA) and several of its ring-substituted analogues are both potent and selective agonists at the A_2AR.[7] Although the number of analogues included in that report is not large, their high potency and selectivity urges further studies of the C-2 region of the A_2AR such as those described below.

Scheme I



Results and Discussion

Chemistry. The reaction of the 2′,3′-*O*-isopropylidene or the 2′,3′-*O*-ethoxymethylidine ketal of 2-chloroadenosine (1) with either sodium phenolate or an lithium alkoxide[1,2] furnished all the analogues described below (Scheme I). The alcohols were either commercially available or were obtained through the reduction of the corresponding acyl chloride with $LiAlH_4$. For most analogues, workup consisted of the removal of unreacted alcohol by flash chromatography, hydrolysis of the blocking group, and purification by preparative reverse-phase HPLC. Analogues **12** and **13**, the 3- and 4-chloro derivatives of 2-phenethoxyadenosine, are exceptions, crystallizing spontaneously after the deblocking step. Owing to the susceptibility to acid hydrolysis of the glycosylic bond of the 2-(ar)alkoxyadenosines,[1] substantial losses of product occurred during the deblocking step. The discovery near the end of this project of a satisfactory procedure for deblocking, namely, refluxing in a solution of 1–2% formic acid and 50% acetic acid in water *with close monitoring by HPLC*, improved yields.

We previously found that alkoxides containing a basic nitrogen atom did not displace the chlorine atom from blocked 2-chloroadenosine.[1] Such was also the case with the pyridyl ethoxides, which contain a basic nitrogen. The nitrogen of indole is not basic and, accordingly, the lithium

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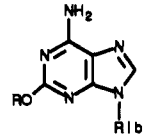
Table I. Physical and Analytical Data for Analogues 1–26



no.	R	formula	anal.	mp, °C	purific[a]	% yield	UV; λ_{max} (ϵ)
2	C_6H_5	$C_{16}H_{17}N_5O_5$	C, H, N	124	A	22	267 (13 200), 276 sh (9 600)
3	$C_6H_5CH_2$	$C_{17}H_{19}N_5O_5$	C, H, N	172–175	A	42	252 sh (9 900), 267 (12 600)
4	$C_6H_5(CH_2)_2$	$C_{18}H_{21}N_5O_5$	C, H, N	95–97	B, 60	65	254 sh (8 000), 267 (10 000)
5	$C_6H_5(CH_2)_3$	$C_{19}H_{23}N_5O_5$	C, H, N	100–102	C, 50/60	20	254 sh (9 300), 268 (11 700)
6	$C_6H_5(CH_2)_4$	$C_{20}H_{25}N_5O_5$	C, H, N	93–96	C, 50/60	23	254 sh (9 600), 268 (12 000)
7	$C_6H_5(CH_2)_5$	$C_{21}H_{27}N_5O_5$	C, H, N	102–104	C, 60/70	19	254 sh (10 000), 268 (12 700)
8	$2\text{-}FC_6H_5(CH_2)_2$	$C_{18}H_{20}FN_5O_5$	C, H, N, F	124–126	C, 30/50	29	257 sh (10 000), 267 (12 300)
9	$3\text{-}FC_6H_4(CH_2)_2$	$C_{18}H_{20}FN_5O_5$	C, H, N, F	149–154	C, 50/80	6	254 sh (10 200), 267 (12 600)
10	$4\text{-}FC_6H_4(CH_2)_2$	$C_{18}H_{20}FN_5O_5$	C, H, N, F	148–150	C, 50/70	36	253 sh (11 300), 267 (14 600)
11	$2\text{-}ClC_6H_4(CH_2)_2$	$C_{18}H_{20}ClN_5O_5$	C, H, N, Cl	98–100	C, 45/65	34	255 sh (7 900), 267 (9 700)
12	$3\text{-}ClC_6H_4(CH_2)_2$	$C_{18}H_{20}ClN_5O_5$	C, H, N, Cl	118–120	D	25	255 sh (9 200), 268 (11 600)
13	$4\text{-}ClC_6H_4(CH_2)_2$	$C_{18}H_{20}ClN_5O_5$	C, H, N, Cl	159	D	16	254 sh (9 400), 267 (12 000)
14	$2\text{-}CH_3OC_6H_4(CH_2)_2$	$C_{19}H_{23}N_5O_6$	C, H, N	126–130	C, 40/70	38	255 sh (11 300), 269 (15 100)
15	$3\text{-}CH_3OC_6H_4(CH_2)_2$	$C_{19}H_{23}N_5O_6$	C, H, N	103–105	C, 40/65	37	255 sh (11 100), 269 (15 000)
16	$4\text{-}CH_3OC_6H_4(CH_2)_2$	$C_{19}H_{23}N_5O_6$	C, H, N	145–146	C, 40/70	48	255 sh (11 200), 268 (14 800)
17	$2\text{-}CH_3C_6H_4(CH_2)_2$	$C_{19}H_{23}N_5O_5$	C, H, N	166–168	C, 40/70	20	254 sh (10 900), 268 (13 800)
18	$3\text{-}CH_3C_6H_4(CH_2)_2$	$C_{19}H_{23}N_5O_5$	C, H, N	111–113	C, 45/65	30	255 sh (9 400), 268 (11 800)
19	$4\text{-}CH_3C_6H_4(CH_2)_2$	$C_{19}H_{23}N_5O_5$	C, H, N	97–100	C, 50/70	38	256 sh (8 600), 267 (10 700)
20	$2\text{-thienyl-}(CH_2)_2$	$C_{16}H_{19}N_5O_5S$	C, H, N, S	104–106	C, 35/50	23	236 sh (12 600), 267 (11 300)
21	$3\text{-thienyl-}(CH_2)_2$	$C_{16}H_{19}N_5O_5S$	C, H, N, S	99–102	C, 35/50	22	241 sh (11 400), 268 (12 100)
22	$3\text{-indolyl-}(CH_2)_2$	$C_{20}H_{22}N_6O_5$	C, H, N	138–140	C, 40/70	37	255 sh (11 000), 268 (14 500)
23	$1\text{-naphthyl-}(CH_2)_2$	$C_{22}H_{23}N_5O_5$	C, H, N	125–130	C, 50/80	7	271 (15 200), 291 sh (4 500)
24	$2\text{-naphthyl-}(CH_2)_2$	$C_{22}H_{23}N_5O_5$	C, H, N	104–108	B, 40	30	254 sh (11 000), 268 (14 500)
25	$3,4\text{-}(CH_3O)_2C_6H_3(CH_2)_2$	$C_{20}H_{25}N_5O_7$	C, H, N	202–204	C, 30/50	37	255 sh (8 800), 269 (12 200)
26	$3,4,5\text{-}(CH_3O)_3C_6H_2(CH_2)_2$	$C_{21}H_{27}N_5O_8$	C, H, N	110–112	C, 30/50	5	255 sh (9 200), 269 (11 900)

[a] Purification methods included A, flash chromotagraphy on silica gel eluted with $CH_3OH/CHCl_3$, 15:85 v/v; B, reverse-phase HPLC, isocratic elution with indicated percent of CH_3OH in H_2O; C, reverse-phase HPLC, gradient elution at initial/final percent of CH_3OH in H_2O; and D, crystallization from CH_3OH–H_2O.

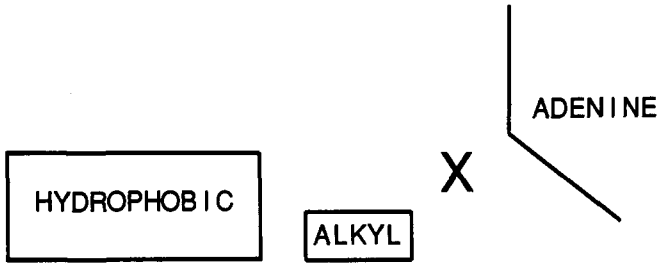


Figure 1. Diagrammatic representation of the C-2 region of adenosine receptors.

salt of 2-indol-3-ylethanol reacted satisfactorily. The thienyl ethoxides, too, gave useful yields of product.

Table I lists the properties of novel analogues.

Cardiovascular Activity. In the assay system used here,[1] prolongation of the stimulus–QRS interval of the electrocardiogram of a paced guinea pig heart reflects potency at the atrioventricular node[8] and coronary vasodilation potency at the A_2AR in coronary artery,[9] respectively. Table II lists the results of such assays and also measurements of k', a hydrophobicity index. All the analogues appear to be full agonists at both receptors, causing second degree heart block and maximum coronary vasodilation.

Analogues 2–7, 2-phenoxyadenosine and its lower alkyl 2-phenylalkoxy congeners, probe the importance of the length of the alkyl chain on activity. At the A_1AR the activities of 2–7 are low, the EC_{50}s of stimulus–QRS interval prolongation ranging between 4.6 and 47 μM. Activity is unrelated to alkyl chain length. At the A_2AR mediating coronary vasodilation, the activity of 2 is low; the EC_{50} is 0.42 μM, 8 times higher than that of adenosine and 23 times higher than that of 2-(phenylamino)adenosine (CV-1808), its nitrogen isostere.[1] Such a result is baffling. The coronary vasoactivity of 2-oxoadenosine (isoguanosine) is 3 times higher than that of 2-aminoadenosine, yet the potency ranking of the phenyl derivatives is reversed. The activity of 2-(benzyloxy)adenosine (3) is the same as that of 2, but further increasing the length of the alkyl chain by one more methylene residue greatly increases activity. Phenethoxy analogue 4 is the most potent member of this series; the EC_{50} of coronary vasoactivity is 2.8 nM, an increase in potency of nearly 150 times over that of 2 or 3. The EC_{50}s of 5–7 are somewhat lower than that of 3 but still in the low nanomolar range.

Analogues 8–19 explore the effects of ring substituents on the activity of 3. The substituents are fluorine (8–10), chlorine (11–13), methoxy (14–16), and methyl (17–19). At the A_1AR, ring substitution has a negligible effect on activity, the EC_{50}s of stimulus–QRS interval prolongation differing from that of 4 by 2.5-fold or less. At the A_2AR, by contrast, the ring position and hydrophobicity of a substituent strongly influences activity. With each kind of substituent, activity increases ortho < meta < para. At each ring position a methoxy group contributes least and a methyl group the most to activity; among the para substituted analogues the activity ranking, $OCH_3 < F < Cl < CH_3$, parallels the hydrophobicity of each substituent as reflected in the hydrophobicity index, k'. Thus, hydrophobicity appears to explain the potency ranking of the para substituted 2-phenethoxyadenosines. Analogues 10, 13, and 19 are active in the subnanomolar range. The most active of the four, 2-[2-(4-methylphenyl)ethoxy]adenosine (19), has an EC_{50} for coronary vasodilation of 190 pM. Owing to the relatively constant activity of 8–19 at the

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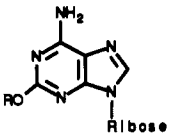
Table II. Cardiac and Coronary Activity of 2-Aralkoxyadenosines



no.	substit	N[a]	-log EC_{50}, M: stim–QRS[b]	-log EC_{50}, M: coronary[c]	A_1/A_2[d]	k'
	adenosine	14	5.47 ± 0.07	7.29 ± 0.06	80 ± 11	
			$C_6H_5(CH_2)_n$			
	R =					
2	C_6H_5	4	4.33 ± 0.09	6.38 ± 0.06	130 ± 40	0.31
3	$C_6H_5CH_2$	4	5.20 ± 0.06	6.38 ± 0.09	17 ± 4.3	0.61
4	$C_6H_5(CH_2)_2$	6	4.70 ± 0.09	8.55 ± 0.04	8200 ± 2200	0.90
5	$C_6H_5(CH_2)_3$	4	4.70 ± 0.04	7.21 ± 0.08	330 ± 33	1.36
6	$C_6H_5(CH_2)_4$	4	5.15 ± 0.03	8.02 ± 0.04	760 ± 85	2.20
7	$C_6H_5(CH_2)_5$	4	5.34 ± 0.07	8.20 ± 0.05	730 ± 61	3.63
			R = $XC_6H_4(CH_2)_2$			
	X =					
8	2-F	4	4.45 ± 0.05	8.58 ± 0.03	14000 ± 2200	0.94
9	3-F	4	4.46 ± 0.09	8.72 ± 0.10	18000 ± 2300	0.83
10	4-F	6	4.59 ± 0.10	9.06 ± 0.08	38000 ± 12000	0.86
11	2-Cl	4	4.66 ± 0.03	8.26 ± 0.07	4000 ± 500	1.55
12	3-Cl	4	4.79 ± 0.10	8.52 ± 0.07	6400 ± 1600	1.63
13	4-Cl	4	4.84 ± 0.04	9.41 ± 0.04	39000 ± 6500	1.64
14	2-CH_3O	4	4.45 ± 0.03	7.50 ± 0.05	1100 ± 150	1.04
15	3-CH_3O	4	4.79 ± 0.10	8.59 ± 0.10	8200 ± 3300	0.87
16	4-CH_3O	4	4.71 ± 0.08	8.85 ± 0.06	14000 ± 1300	0.82
17	2-CH_3	4	4.60 ± 0.11	8.42 ± 0.14	9100 ± 4400	1.39
18	3-CH_3	4	4.89 ± 0.07	8.80 ± 0.06	8400 ± 1200	1.48
19	4-CH_3	4	5.13 ± 0.04	9.72 ± 0.11	44000 ± 9700	1.56
			R = $Ar(CH_2)_2$			
	Ar =					
20	2-thienyl	4	4.93 ± 0.10	8.43 ± 0.03	3500 ± 750	0.67
21	3-thienyl	4	4.73 ± 0.06	8.47 ± 0.06	5700 ± 700	0.67
22	3-indolyl	4	4.85 ± 0.09	8.01 ± 0.03	1600 ± 480	0.68
23	1-naphthyl	4	5.08 ± 0.05	8.30 ± 0.05	1700 ± 290	2.45
24	2-naphthyl	4	4.94 ± 0.07	9.30 ± 0.05	23000 ± 3200	2.45
25	3,4-$(CH_3O)_2C_6H_3$	5	4.37 ± 0.02	8.31 ± 0.10	7400 ± 1200	0.53
26	3,4,5-$(CH_3O)_3C_6H_2$	4	4.33 ± 0.08	7.66 ± 0.06	2300 ± 520	0.51

[a] Number of assays. [b] Prolongation of the stimulus–QRS interval, an index of agonist activity at the A_1AR. [c] Coronary vasodilation, an index of agonist activity at the A_2AR. [d] Selectivity ratio, stim–QRS ÷ coronary. [e] In the series of analogues 2–7, n increases consecutively 0, 1, 2...5.

A_1AR, the A_1/A_2 activity ratio varies directly with activity at the A_2AR. The A_1/A_2 activity ratio of **19** is 44000, that of **10** is 38000, that of **13** is 39000, and that of **16** is 14000. The potencies and selectivities of these analogues compare favorably with those of *N*-ethyl-2-[[2-[4-(2-carboxyethyl)phenyl]ethyl]amino]adenosine-5′-uronamide (CGS 21,680), currently the standard A_2AR agonist.[10] In the same guinea pig heart Langendorff preparation as used in the present study, CGS 21,680 exhibits an EC_{50} for coronary vasodilation of 0.74 ± 0.08 nM and an A_1/A_2 activity ratio of 33000 ± 2300.[1]

Analogues **20–24** are congeners of **4** that contain aryl groups other than benzene. All are very poor A_1AR agonists. As A_2AR agonists the two thiophene congeners, **20** and **21**, are essentially equipotent with **4** and the larger but more polar 3-indolylethyl analogue **22** is only 3 times less potent. The rather high vasoactivity of **24** is evidence that the hydrophobic subregion is substantially larger than a cyclohexane or benzene ring. The two naphthyl congeners, **23** and **24**, differ in A_2AR agonist activity by a factor of 10. The more potent 2-naphthyl isomer, **24**, has an EC_{50} for coronary vasoactivity of 0.50 nM. Studies with molecular models suggest that bulk tolerance might be somewhat limited in the part of the receptor occupied by ortho and, to a lesser extent, meta substituents on the benzene ring of **4**.

3,4-Dimethoxyphenyl and 3,4,5-trimethoxyphenyl analogues **25** and **26** together with monomethoxy derivative **16** explore the effects of polarity and steric bulk on activity at the A_2AR. The potency ranking **16** > **25** > **26** parallels the hydrophobicity indices. Studies of molecular models show that it is possible to superimpose the methoxy groups of **25** on the distal ring of the naphthyl group of **23**. Accordingly, hydrophobicity, rather than steric hinderance, seems a likely explanation for the difference in activity between **16** and **26**. It is possible that the second meta substituent of **26** could be sterically hindering, but the rather mild loss of activity suggests that the greater polarity of this analogue is probably a better explanation.

Antagonism by Alkylxanthines

Antagonism by alkylxanthines such as theophylline[11] is one criterion used to show that adenosine acts through adenosine receptors. The unselective antagonist 8-(p-sulfophenyl)theophylline[12] (8-PST) at a dose of 50 μM competitively antagonized the activity of **4** and **10** at both

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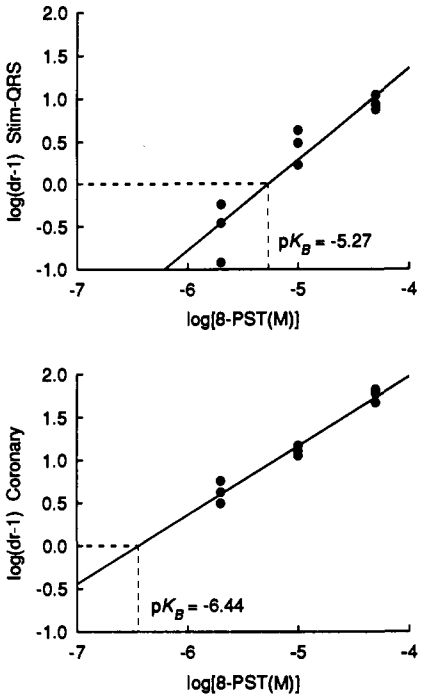

Figure 2. Schild plots characterizing the antagonism by 8-(*p*-sulfophenyl)theophylline of the actions of **19** at the A_1AR of AV node (above) and at the A_2AR of coronary artery (below). Abbreviations: dr, dose ratio, the quotient of the EC_{50} of agonist in the presence of antagonist divided by the EC_{50} in the absence of antagonist; Stim–QRS, stimulus–QRS interval, an index of activity at the A_1AR of AV node; coronary flow, an index of activity at the A_2AR in coronary artery.

receptors. At the A_1AR, 8-PST shifted the dose–response curves of **4** and **10** rightward 3.2- and 3.1-fold, respectively; at the A_2AR the corresponding rightward shifts were 225- and 40-fold. In addition to these studies at a single concentration of agonist, we estimated the pK_B of 8-PST vs **19** (Figure 2). Such estimates of pK_B, 5.27 at the A_1AR and 6.45 at the A_2AR, are similar to estimates in the literature.[8,13]

Receptor Models. The uniformly low activity of the 2-aralkoxyadenosines at the A_1AR and the lack of correlations between structure and activity support a major conclusion reached by analysis of the SAR of the 2-aralkoxyadenosines, namely, that the A_1AR lacks a hydrophobic subregion or, alternatively, that the interaction of 2-substituents with this subregion does not contribute to activity.[1] That study also showed that the 2-isoalkoxyadenosines and 2-(cyclohexylalkoxy)adenosines exhibit A_1AR activity that parallels the number of methylene residues in the alkyl chain. It was pointed out that such a result was not necessarily evidence for interaction with the C-2 region. Instead, it could reflect the interaction of such long-chain alkyl groups with the N-6 region of the A_1AR. Indeed, branched-chain and cycloalkyl substituents at N-6 greatly enhance the potency of adenosine at the A_1AR[14,15] but aromatic groups in such substituents will lower activity.[14,16,17] The A_1AR potency of **2–7** did not correlate with alkyl chain length, possibly a reflection of the negative influence of the phenyl group on interactions of these C-2 substituents with the N-6 region.

The activities of the 2-aralkoxyadenosines at the A_2AR confirm and refine the model of the A_2AR developed from the SAR of the 2-alkoxyadenosines. At the coronary receptor, the activities of the para-substituted 2-phenethoxyadenosines are inversely proportional to the polarity of the nucleosides, a result consistent with the existence of a hydrophobic subregion in the coronary A_2AR. The high activity of 2-[2-(2-naphthyl)ethoxy]adenosine (**24**) is evidence that the hydrophobic subregion is larger than indicated by the previously reported[1] activity of 2-[2-(cyclohexyl)ethoxy]adenosine. Additionally, the 10-fold difference in the activities of the 1- and 2-naphthyl isomers suggests that there may be local areas of limited bulk tolerance within the hydrophobic subregion. The low activity of 2-(benzyloxy)adenosine supports the notion that the alkyl region adversely affects activity.

In summary, this study of the agonist activities of 2-aralkoxyadenosines confirms the provisional models of the C-2 regions of the A_1AR and A_2AR described previously. Several of these analogues are very potent and highly selective A_2AR agonists.

Experimental Section

Flash chromatography on 60-μm silica gel eluted with 2% CH_3OH in $CHCl_3$ purified the blocked nucleosides. Deblocking entailed boiling in a solution of 2% formic acid and 50% acetic acid with close monitoring by HPLC. The purification of **2–26** employed a Rainin Autoprep fitted with a 1 × 25 cm column of C-18 silica, eluted with CH_3OH–water in either the isocratic or gradient mode. Melting points are uncorrected. A Varian EM 360L spectrometer yielded proton NMR spectra of nucleoside solutions in DMSO-d_6, which were consistent with the assigned structures. The ultraviolet spectra were determined on a Beckman DU64 spectrophotometer. MHW Laboratories, Tucson, AZ, performed the elemental analysis, which differed from the calculated composition by <0.4%. Product accounted for >99% of the UV-absorbing material in samples submitted for bioassay. The retention time of a nucleoside on a reverse-phase HPLC column served for the calculation[18] of a hydrophobicity index, k', by the formula $k' = (t - t_o)/t_o$, where t is the retention time of the solute and t_o is the transit time of the solvent. In the present experiments the mobile phase consisted of a mixture containing 35% 10 mM $NaHPO_4$, pH 7.0, and 65% CH_3OH.

Preparation of 2-Arylalkoxyadenosines from Primary Alcohols. 2-[2-(4-Methylphenyl)ethoxy]adenosine [6-Amino-2-[2-(4-methylphenyl)ethoxy]-9-β-D-ribofuranosyl-9*H*-purine]. A solution of 2-(4-methylphenyl)ethanol (4.6 g, 33.6 mmol) in 70 mL of dry 1,2-dimethoxyethane was cooled to 10 °C in an ice bath. To this solution was added 1.6 M *n*-butyllithium (19.9 mL, 31.9 mmol), the solution was stirred for 15 min, and then dry 2-chloro-2′,3′-*O*-(ethoxymethylidene)adenosine (3.0 g, 8.4 mmol) was added in one portion. After 5 days at reflux, HPLC showed that <5% of the starting material was present. The solvents were removed in vacuo and a solution of the residue in 70 mL water was extracted with ethyl acetate (4 × 50 mL). The combined extracts were dried over $MgSO_4$ and evaporated in vacuo to a dark syrup for purification by flash chromatography. Fractions containing product were concentrated and dissolved in acetic acid (25 mL), water (25 mL), and 98% formic acid (1 mL). After boiling of the solution for 2 h, HPLC showed that the blocked nucleoside had disappeared. Solid sodium bicarbonate was carefully added until the pH was basic. Removing the solvents in vacuo and the purifying product by preparative reverse-phase HPLC, as described in Table I, yielded 1.23 g (38%) of a white solid.

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Bioassay. A Langendorff guinea pig heart preparation paced at 260 beats/min via the left atrium served for assays of A_1AR and A_2AR agonist activity. The perfusion buffer consisted of (mM) NaCl (120), $NaHCO_3$ (27), KCl (3.7), KH_2PO (1.3), $MgSO_4$ (0.64), $CaCl_2$ (1.3), pyruvate (2), and glucose (5). The buffer was saturated with 95% O_2–5% CO_2, equilibrated at 37 °C in a heat exchanger and delivered at a pressure equivalent to 55 mmHg. Continuous drainage of the left ventricle by means of a catheter inserted across the mitral valve insured that this cardiac chamber did no external work. An electrode in the right ventricle monitored the electrocardiogram. Time collections of cardiac effluent in a graduated cylinder during the steady-state phase of the flow responses to analogue administration measured total coronary flow, which was also monitored by an in-line electromagnetic flowmeter in the aortic perfusion cannula. The rate of the nucleoside infusion was increased stepwise until the appearance of second degree heart block. The quotient of the ratio of nucleoside influsion (mol/min) divided by coronary flow rate (L/min) equals agonist concentration in the perfusate. The EC_{50} of prolongation of the stimulus–QRS interval, the concentration of agonist needed to prolong the interval by 50% of the maximum response,[8] reflects activity at the A_1AR. Logit transformation of the coronary flow data and solution of the regression of logit (coronary flow) on log [analogue] for logit = 0 yielded an estimate of EC_{50} of coronary vasodilation, an index of A_2AR activity. Table II reports the mean ± SEM of the -log EC_{50} values from assays in four or more hearts. The quotient of the EC_{50} of stimulus–QRS prolongation divided by the EC_{50} of coronary vasodilation provided an index of selectivity. Values of the index <1 indicate selectivity for the A_1AR and values >1 selectivity for the A_2AR. Table II reports the mean ± SEM of the A_1/A_2 activity ratios of individual experiments.

Registry No. 1 (R′ = Me_2), 24639-06-3; 1 (R′ = OEt), 56720-43-5; **2**, 50257-82-4; **3**, 131865-78-6; **4**, 131865-79-7; **5**, 131865-80-0; **6**, 131865-81-1; **7**, 131865-82-2; **8**, 131865-83-3; **9**, 131865-84-4; **10**, 131865-85-5; **11**, 131865-86-6; **12**, 131865-87-7; **13**, 131865-88-8; **14**, 131865-89-9; **15**, 131865-90-2; **16**, 131865-91-3; **17**, 131865-92-4; **18**, 131865-93-5; **19**, 131865-94-6; **20**, 131865-95-7; **21**, 131865-96-8; **22**, 131865-97-9; **23**, 131865-98-0; **24**, 131865-99-1; **25**, 131866-00-7; **26**, 131866-01-8.

Inhibition of Human Placental Aromatase by Novel Homologated 19-Oxiranyl and 19-Thiiranyl Steroids

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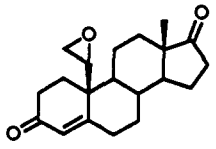
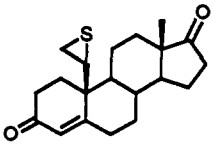
Novel homologated 19-oxiranyl- and 9-thiiranylandrost-4-ene-3,17-diones (**8a,b** and **9a,b**, respectively) have been synthesized. The configuration and conformation of compound **8a** have been established by X-ray crystallographic analysis. All four compounds have been shown to be competitive inhibitors of human placental aromatase. The thiiranes were more potent inhibitors than the corresponding oxiranes, and the 2′*S* isomers (**8b** and **9b**) were better inhibitors than the 2′*R* (**8a** and **9a**) diastereomers in each series. Spectroscopic studies with purified human placental aromatase suggest that the oxiranyl oxygen and thiiranyl sulfur of 2′*S* compounds **8b** and **9b** coordinate to the enzyme's heme iron.

Aromatase is a cytochrome P-450 enzyme complex which is responsible for the important transformation of androgens (1) to estrogens (2) (Scheme I). Inhibitors of aromatase may be valuable as therapeutic agents in the treatment of estrogen-sensitive breast tumors and as possible antifertility agents, and a number of competitive inhibitors has been reported in recent years.[1,2]

Previous work from our laboratories showed that 10β-oxiranyl- and 10β-thiiranylestr-4-ene-3,17-diones (**3** and **4**, respectively) were potent competitive inhibitors of human placental aromatase.[3-5] Furthermore, stereoselectivity was observed, with the 19*R* diastereomers (**3a**, **4a**)



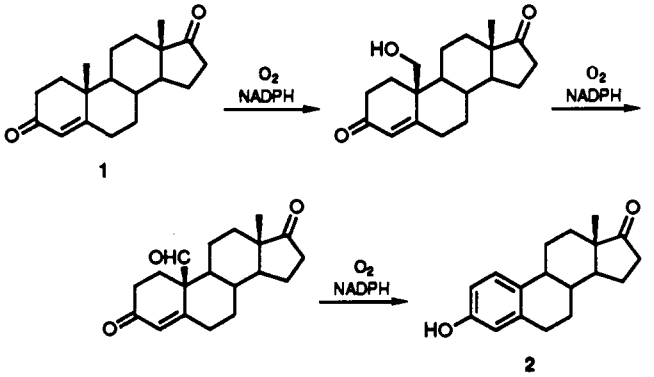
3a (19*R*, K_i = 7 nM)
3b (19*S*, K_i = 75 nM)



4a (19*R*, K_i = 1 nM)
4b (19*S*, K_i = 75 nM)

being 10–75 times more potent inhibitors than the 19*S* isomers (**3b**, **4b**). Spectroscopic studies with purified enzyme demonstrated that in the case of the 19*R* isomers, the oxiranyl and thiiranyl heteroatom coordinated with the enzyme's heme iron. This coordination, combined with the inherent binding selectivity of the steroid nucleus, endowed these inhibitors with high specificity.

Scheme I



X-ray crystallographic analysis of the above 10β-oxiranes showed[3] that the oxirane heteroatom in the more potent

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