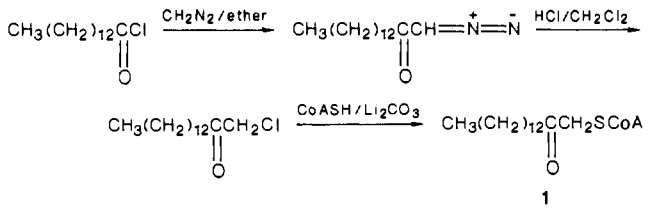
Scheme I



1

Department of Chemistry, The Ohio State University.

Registry No. 1 (free acid), 121124-66-1; 1·4Li, 121124-67-2; CoA, 85-61-0; NMT, 110071-61-9; $CH_3(CH_2)_{12}COCl$, 112-64-1; $N_2{=}CHCO(CH_2)_{12}CH_3$, 90670-23-8; $ClCH_2CO(CH_2)_{12}CH_3$, 121097-11-8.

† Purdue University.
‡ Ohio State University.
§ Present address: Schering Research, 86 Orange Street, Bloomfield, NJ 07003.

Lisa A. Paige,† Guo-qiang Zheng,‡ Shawn A. DeFrees†,§ John M. Cassady,‡ Robert L. Geahlen*,†

Department of Medicinal Chemistry and Pharmacognosy School of Pharmacy and Pharmacal Sciences Purdue University West Lafayette, Indiana 47907 and College of Pharmacy Ohio State University Columbus, Ohio 43210

Received March 2, 1989

Articles

C2,N^6-Disubstituted Adenosines: Synthesis and Structure–Activity Relationships

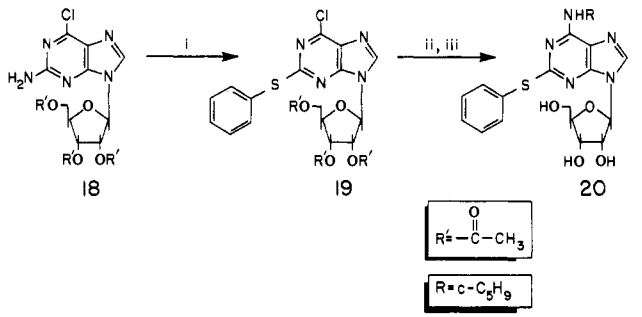
Bharat K. Trivedi*,† and Robert F. Bruns‡

Departments of Chemistry and Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105. *Received August 5, 1988*

Extracellular adenosine receptors have been divided into two major subtypes, called A_1 and A_2. Substitution of the adenosine molecule with appropriate groups at C2 or N^6 is known to impart selectivity for the A_2 receptor over the A_1 receptor. In the present study, we investigated whether substitution at both C2 and N^6 would have additive effects on the A_2/A_1 affinity ratio, thereby providing compounds with greater A_2 selectivity than presently available agents. Disappointingly, additivity appeared to hold only when an A_1-selective group was present at N^6. For instance, 2-(phenylamino) substitution of the A_1-selective agonist *N*^{6}-cyclopentyladenosine resulted in a 70-fold shift in selectivity in favor of the A_2 receptor, but the same substitution applied to the A_2-selective agonist *N*^{6}-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]adenosine resulted in a 100-fold loss of affinity with no change in A_2 selectivity.

Adenosine causes a variety of physiological responses, which are mediated by two subtypes of extracellular receptors, called A_1 and A_2. These two receptor subtypes can be distinguished on the basis of structure–activity relationships,[1-3] and specific receptor binding assays exist for both subtypes.[4,5] Considerable effort has been devoted to the search for adenosine agonists with improved selectivity for A_1 or A_2 receptors. Although agonists with 1000-fold or greater selectivity for the A1 receptor are known,[6] until recently the most A_2-selective agonist was 2-(phenylamino)adenosine (CV-1808, compound **7** in Table II),[7] which shows only 5-fold A_2 selectivity.[5] Very recently, *N*^{6}-[2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethyl]-adenosine (compound **50** in Table IV) was shown to possess about a 30-fold selectivity for the A_2 receptor.[8] Because **7** is substituted at C2, whereas **50** is substituted at N^6 we became interested in the possibility that the functional groups responsible for conferring selectivity on these two compounds might interact with independent sites on the adenosine receptor, thereby allowing additive enhancement of selectivity by combining structural modifications at both positions. Because many other C2 and N^6 groups with widely differing effects on A_1 and A_2 affinity have been reported (see Tables II and IV),[7,9-11] we also tested representative combinations of these groups for their effects on adenosine-receptor selectivity. Our results indicate that the effects of C2 and N^6 substitution are only

Scheme I[a]



18 19 20

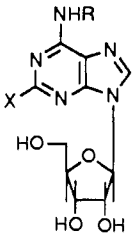
[a] (i) PhSSPh, isoamyl nitrite, CH_3CN, Δ; (ii) RNH_2, DME, Et_3N, room temperature; (iii) MeOH–NH_3, room temperature.

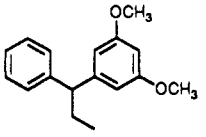
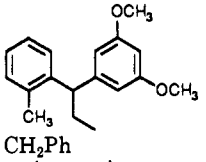
† Department of Chemistry.
‡ Department of Pharmacology. Present address: Eli Lilly and Company, Indianapolis, IN 46285.

(1) van Calker, D.; Muller, M.; Hamprecht, B. *J. Neurochem.* **1979**, *33*, 999–1005.
(2) Londos, C.; Cooper, D. M. F.; Wolff, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 2551–2554.
(3) Hamprecht, B.; van Calker, D. *Trends Pharmacol. Sci.* **1985**, *6*, 153–154.
(4) Yeung, S. M. H.; Green, R. D. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1984**, *325*, 218–225.
(5) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331–346.
(6) Daly, J. W.; Padgett, W.; Thompson, R. D.; Kusachi, S.; Bugni, W. J.; Olsson, R. A. *Biochem. Pharmacol.* **1986**, *35*, 2467–2481.

0022-2623/89/1832-1667$01.50/0

Table I. Physical Properties of Novel Adenosine Agonists



example	R	X	mp, °C	formula analysis
13	cyclopentyl	NH_2	210–211	$C_{15}H_{22}N_6O_4$
14	cyclohexyl	NH_2	214–215	$C_{16}H_{24}N_6O_4$
15	(*R*)-1-methyl-2-phenylethyl	NH_2	108–110	$C_{19}H_{24}N_6O_4$
16	2,2-diphenylethyl	NH_2	134–137	$C_{24}H_{26}N_6O_4 \cdot 0.5H_2O$
17	9-fluorenylmethyl	NH_2	154–158	$C_{24}H_{24}N_6O_4 \cdot 0.5CH_3OH$
20	cyclopentyl	SPh	105–110	$C_{21}H_{25}N_5O_4S$
21	cyclohexyl	SPh	103–108	$C_{22}H_{27}N_5O_4S$
23	2,2-diphenylethyl	SPh	116–119	$C_{30}H_{29}N_5O_4S$
24	1-naphthylmethyl	SPh	115–120	$C_{27}H_{25}N_5O_4S$
27	cyclopentyl	SO_2Ph	72–75	$C_{21}H_{25}N_5O_6S$
28	cyclohexyl	SO_2Ph	87–93	$C_{22}H_{27}N_5O_6S \cdot 0.5C_2H_5OH$
29	1-naphthylmethyl	SO_2Ph	117–122	$C_{27}H_{25}N_5O_6S$
36	1-naphthylmethyl	F	131–135	$C_{21}H_{20}N_5O_4F$
37	1-naphthylmethyl	cyclohexylamine	137–140	$C_{24}H_{32}N_6O_4 \cdot 0.25H_2O$
38	1-naphthylmethyl	$NHCH_2Ph$	205–208	$C_{28}H_{28}N_6O_4$
44	1-naphthylmethyl	NHPh	205–210	$C_{27}H_{28}N_6O_4$
45	2,2-diphenylethyl	NHPh	116–121	$C_{30}H_{30}N_6O_4 \cdot 0.4H_2O$
47	9-fluorenylmethyl	NHPh	220–222	$C_{30}H_{28}N_6O_4$
49		NHPh	118–121	$C_{32}H_{34}N_6O_6$
51		NHPh	126–130	$C_{33}H_{36}N_6O_6$
53	CH_2Ph	NHPh	222–224	$C_{23}H_{24}N_6O_4$
55	cyclopropyl	NHPh	200–202	$C_{19}H_{22}N_6O_4$
30	cyclopentyl	NHPh	172–175	$C_{21}H_{26}N_6O_4 \cdot 0.4H_2O$
56	cyclohexyl	NHPh	229–231	$C_{22}H_{28}N_6O_4$
58	2-*endo*-norbornyl	NHPh	210–212	$C_{23}H_{28}N_6O_4$
60	(*S*)-2-hydroxypropyl	NHPh	120–123	$C_{19}H_{24}N_6O_5 \cdot 0.25H_2O$
62[a]	1-pyrrolidinyl	NHPh	222–224	$C_{20}H_{24}N_6O_4$

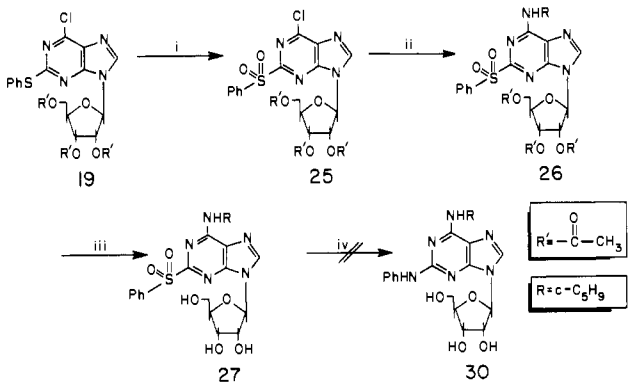
[a] Compound **62** is 2-(phenylamino)-6-(1-pyrrolidinyl)-9-β-D-ribofuranosyl-9*H*-purine

partially additive, with the least additivity unfortunately being seen with the most A_2-selective parent groups.

Chemistry

The 2-amino analogues (examples **13–17**; Table I) were synthesized in a standard fashion by reacting 2-amino-6-chloropurine ribonucleoside with an appropriate amine in the presence of a base in refluxing ethanol. Synthesis of 2-phenylthio derivatives (Tables II and III) was achieved by utilizing a recently reported method[12] shown in Scheme I. The corresponding phenylsulfone derivatives (examples **27–29**) were synthesized as follows (Scheme II): the in-

Scheme II[a]



[a] (i) $KMnO_4$, AcOH, 0–10 °C, 3 h; (ii) RNH_2, Et_3N, DME, room temperature, 2.5 h; (iii) MeOH–NH_3, 4 h; (iv) $PhNH_2$, DMF, Δ.

(7) (a) Marumoto, R.; Yoshikoto, Y.; Miyashita, O.; Shima, S.; Imai, K.; Kawazoe, K.; Honjo, M. *Chem. Pharm. Bull.* **1975**, *23*, 759. (b) Omura, K.; Marumoto, R.; Furukawa, Y. *Chem. Pharm. Bull.* **1981**, *29*, 1870.

(8) Bridges, A. J.; Bruns, R. F.; Ortwine, D. F.; Priebe, S. R.; Szotek, D. L.; Trivedi, B. K. *J. Med. Chem.* **1988**, *31*, 1282–1285.

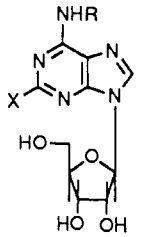
(9) Bridges, A. J.; Moos, W. H.; Szotek, D. L.; Trivedi, B. K.; Bristol, J. A.; Heffner, T. G.; Bruns, R. F.; Downs, D. A. *J. Med. Chem.* **1987**, *30*, 1709–1711.

(10) Trivedi, B. K.; Bristol, J. A.; Bruns, R. F.; Haleen, S. J.; Steffen, R. P. *J. Med. Chem.* **1988**, *31*, 271–273.

(11) Trivedi, B. K.; Bridges, A. J.; Patt, W. C.; Priebe, S. R.; Bruns, R. F. *J. Med. Chem.* **1989**, *32*, 8–10.

(12) Trivedi, B. K. *Nucleosides Nucleotides* **1988**, *7*, 393–402.

Table II. Affinities of C2-Modified Adenosines and Reference Agents in A_1 and A_2 Receptor Binding Assays

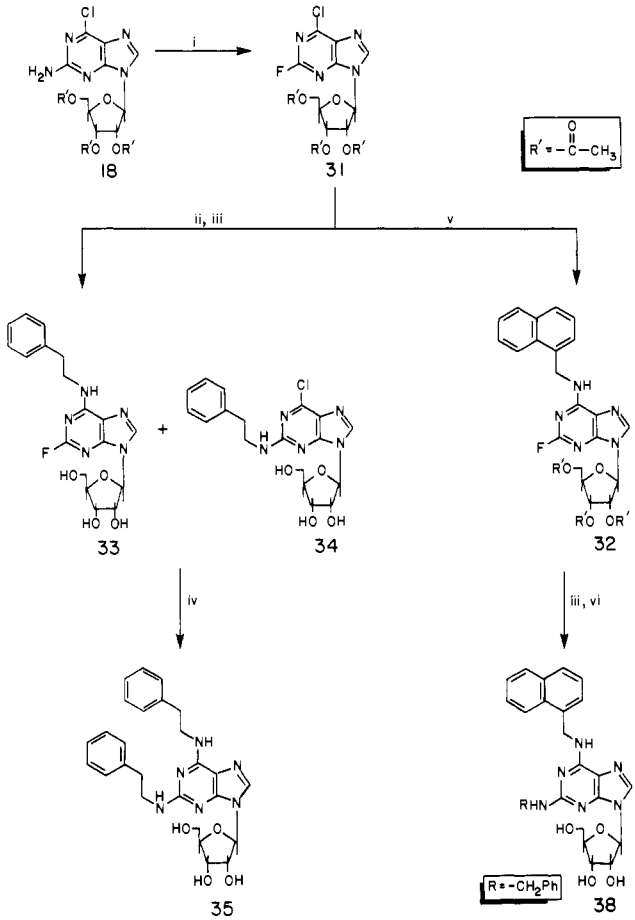


example	X	R	K_i, nM[a] A_1	A_2	A_2/A_1
1	H	H	12.8[b]	37.0	2.9
2	Cl	H	9.3	63	6.8
3	OH	H	94	330	3.5
4	SPh	H	2200	2000	0.90
5	SO_2Ph	H	2600	1230	0.46
6	4-OMePh	H	1320	600	0.45
7	NHPh	H	600	116	0.190
8	H	cyclopentyl	0.59	460	780
9	H	cyclohexyl	1.42	610	430
10	H	(*R*)-1-methyl-2-phenylethyl	1.17	124	106
11	H	2,2-diphenylethyl	6.8	25	3.6
12	H	1-naphthylmethyl	24	9.4	0.38

[a] A_1 affinities were determined in [^{3}H]-N^6-cyclohexyladenosine binding to rat whole-brain membranes, and A_2 affinities were determined in [^{3}H]NECA binding to rat striatal membranes.[5] Values for compounds **2**, **3**, **6**, and **7** are from ref 5. All values are means of three or more independent determinations. [b] The affinity of adenosine cannot be determined directly because of the necessity for adenosine deaminase in the binding assays.[5] The values given are derived from Free–Wilson analysis of mono- and disubstituted adenosine analogues.[5]

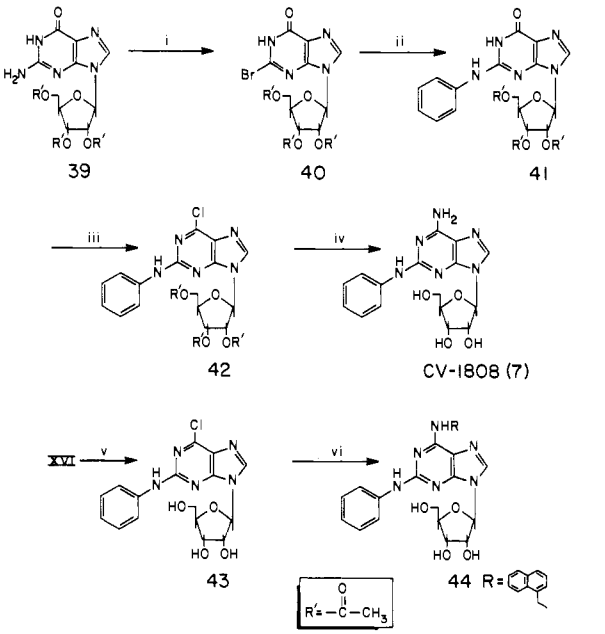
termediate 6-chloro-2-(phenylthio)purine ribonucleoside triacetate (**19**) was first oxidized with $KMnO_4$ in acetic acid to the sulfone derivative **25**, followed by treatment with an appropriate amine to afford the adenosine derivative **26**. The deprotection was carried out in methanolic ammonia at room temperature to yield the target phenyl sulfone adenosines **27–29**. Attempts to convert these sulfone derivatives to the corresponding CV-1808 analogues by direct displacement with aniline under various conditions failed, mainly due to the poor nucleophilicity of aniline. Alternatively (Scheme III), the 2-amino-6-chloropurine ribonucleoside triacetate (**18**) upon treatment with HF–pyridine complex at low temperature[13] afforded the 2-fluoro-6-chloro compound **31** in a good yield. Reaction of **31** with (1-naphthylmethyl)amine yielded the 2-fluoroadenosine derivative **32**. However, the specificity seen in the above reaction did not generalize to all nucleophiles. Attempts to displace chlorine selectively in a similar fashion with 2-phenethylamine failed, yielding a 1:1 mixture of regioisomeric products **33** and **34** following deprotection. This was confirmed by proton NMR spectra in which the sugar protons as well as the C8 proton showed separate chemical shifts. This mixture upon further treatment with 2-phenethylamine in refluxing ethanol gave *N*-(2-phenylethyl)-2-[(2-phenylethyl)amino]adenosine (**35**). Similarly, reaction of **31** with (2,2-diphenylethyl)amine gave a 2:1 ratio of corresponding regioisomeric products. Thus we surmise that, to some extent, the regiospecific displacement seen with (1-naphthylmethyl)amine is due to a steric factor. Nevertheless, compound **32**, following deprotection, can be reacted with a variety of primary

Scheme III[a]



[a] (i) HF–pyridine, *tert*-butyl nitrite, −55 to −30 °C; (ii) Ph-$(CH_2)_2NH_2$, DME, Et_3N, room temperature; (iii) MeOH–NH_3, room temperature; (iv) $Ph(CH_2)_2NH_2$ (xs) EtOH, Δ; (v) (1-naphthylmethyl)amine Et_3N, DME, room temperature; (vi) RNH_2, EtOH, Et_3N, Δ.

Scheme IV[a]

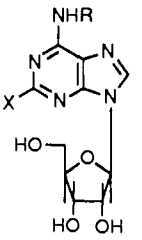


[a] (i) $CHBr_3$, *n*-amyl nitrite, Δ; (ii) $PhNH_2$, MeOH, Δ; (iii) $POCl_3$, *N*,*N*-dimethylaniline, Et_4NCl, CH_3CN, Δ; (iv) NH_3–MeOH, Δ; (v) MeOH–NH_3, room temperature; (vi) RNH_2, Et_3N, EtOH, Δ.

amines to afford analogues **37–38**. Once again, attempts to incorporate a phenylamino group at the C2 position

(13) Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2608–2611.

Table III. Affinities of C2,N^6-Disubstituted Adenosines in A_1 and A_2 Receptor Binding Assays



example	R	X	K_i, nM[a] A_1	A_2	A_2/A_1
13	cyclopentyl	NH_2	8.3	6100	730
14	cyclohexyl	NH_2	19.3	3500	181
15	(*R*)-1-methyl-2-phenylethyl	NH_2	19.2	1530	80
16	2,2-diphenylethyl	NH_2	61	135	2.2
17	9-fluorenylmethyl	NH_2	18.5	22	1.20
20	cyclopentyl	SPh	37	4000	107
21	cyclohexyl	SPh	160	6700	42
22	(*R*)-1-methyl-2-phenylethyl	SPh	210	1000	4.7
23	2,2-diphenylethyl	SPh	840	800	0.95
24	1-naphthylmethyl	SPh	1470	610	0.42
27	cyclopentyl	SO_2Ph	96	2300	24
28	cyclohexyl	SO_2Ph	420	5100	12.3
29	1-naphthylmethyl	SO_2Ph	2000	270	0.133
35	$(CH_2)_2Ph$	$NH(CH_2)_2Ph$	1620	2000	1.26
36	1-naphthylmethyl	F	18.1	14.9	0.82
37	1-naphthylmethyl	cyclohexylamine	45000	10300	0.23
38	1-naphthylmethyl	$NHCH_2Ph$	530	350	0.66

[a] A_1 affinities were determined in [^{3}H]-*N*^{6}-cyclohexyladenosine binding to rat whole-brain membranes, and A_2 affinities were determined in [^{3}H]NECA binding to rat striatal membranes.[5] K_i values for compounds **16** and **29** are means of three and two independent experiments, respectively. Other results are from single determinations.

under various conditions failed.

Finally, we resorted to an alternate route in which the aniline function is incorporated in the beginning of the synthesis. Such a synthetic methodology[12] is shown in Scheme IV. Using this efficient scheme, we prepared several 2-(phenylamino)adenosine analogues (Table IV) and evaluated these compounds in the A_1 and A_2 receptor binding assays.

Receptor Binding and Structure–Activity Relationships. Incorporation of NH_2 at the C2 position had a deleterious effect on both A_1 and A_2-receptor affinities (examples **13–17**; Table III). Interestingly, although the phenylthio group is similar to a phenylamino group in size and shape, the phenylthio analogues (**4**, **20–24**, Tables II and III) also showed weak binding at both receptors; in contrast, 2-(phenylamino)adenosine (**7**) had an A_2 affinity of 116 nM and a 5-fold A_2 selectivity. The 2-(phenylthio) derivatives retained similar A_2/A_1 affinity ratios as compared to the parent N^6 derivatives (Tables II and III). The corresponding sulfone derivatives (**27–29**) began to show good A_2 selectivity as represented by 2-(phenylsulfonyl)-*N*^{6}-(1-naphthylmethyl)adenosine (**29**). This doubly modified adenosine analogue had an A_2-binding affinity similar to that of CV-1808 (**7**), but was slightly more selective. The improvement in selectivity compared to that of the parent N^6 derivative **25** is primarily due to greater loss of affinity at the A_1 receptor than at the A_2 receptor. This indicates that there is a more significant tolerance at the 2-position domain of the A_2 receptor than at the A_1 receptor. The loss of A_1 affinity may be due to either steric bulk or unfavorable charge interactions from the sulfone moiety.

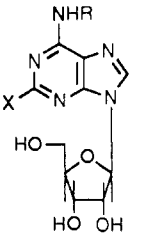
The 2-fluoro derivative (**36**) served as a precursor to various C2-substituted adenosines (**37** and **38**). Interestingly, the 2-(cyclohexylamino) derivative **37**, although A_2 selective, had a very weak affinity at both receptors, especially compared to the corresponding 2-(phenylamino) derivative **44**. The major difference between the two analogues is that the saturated six-membered ring in the former occupies a larger space than the planar aromatic ring present in the latter compound. This suggests that a limited pocket favorable for hydrophobic interactions may exist near the C2 position of adenosine at the A_2 receptor.

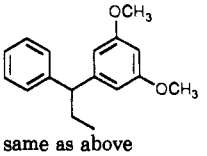
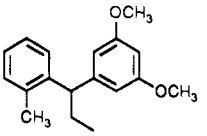
The compounds discussed so far were synthesized for SAR studies preliminary to the synthesis of the corresponding 2-(phenylamino) analogues. Our primary interest was to generate a series of CV-1808 analogues having a wide variety of N^6 substituents. We first synthesized analogues in which we incorporated the N^6 side chains of highly A_2-selective agonists[8] as represented by compounds **47**, **49**, and **51**. The receptor-binding results were discouraging (Table IV), since the anticipated additivity was not observed in these molecules. However, lack of additivity has also been reported with N^6, 5′ doubly modified agonists.[8,14] Compound **47** loses significant affinity at both receptors, yet gains some selectivity for the A_2 receptor. Similarly, compounds **49** and **51**, although highly selective, lose potency significantly at both the receptors. Compound **47** shows the highest loss of affinity at the A_2 receptor (400-fold) when compared to its parent, **46**.

Several 2-(phenylamino) derivatives of A_1-selective N^6-modified adenosines were synthesized in order to determine whether the lack of additivity seen with A_2-selective N^6 derivatives would also pertain to A_1-selective compounds. Most of the resultant compounds (**30**, **55**, **56**, **58**, and **60**) showed increased A_2 affinity and, although still A_1-selective, were much less so than the parent N^6 derivatives. These results indicate that 2-(phenylamino) sub-

(14) Olsson, R. A.; Kusachi, S.; Thompson, R. D.; Ukena, D.; Padgett, W.; Daly, J. W. *J. Med. Chem.* **1986**, *29*, 1683–1689.
(15) Moos, W. H.; Szotek, D. S.; Bruns, R. F. *J. Med. Chem.* **1985**, *28*, 1383–1384.
(16) Hamilton, H. W.; Taylor, M. D.; Steffen, R. P.; Haleen, S. J.; Bruns, R. F. *Life Sci.* **1987**, *41*, 2295–2302.

Table IV. Effects of 2-(Phenylamino) Substitution on A_1 and A_2 Affinities of N^6-Modified Adenosines



example	R	X	K_i, nM[a] A_1	K_i, nM[a] A_2	A_2/A_1
7	H	NHPh	600	116	0.190
12	1-naphthylmethyl	H	24	9.4	0.38
44	1-naphthylmethyl	NHPh	560	230	0.41
11	2,2-diphenylethyl	H	6.8	25	3.6
45	2,2-diphenylethyl	NHPh	2700	650	0.24
46	9-fluorenylmethyl	H	5.2	4.9	0.94
47	9-fluorenylmethyl	NHPh	8900	2100	0.24
48		H	30	6.1	0.20
49	same as above	NHPh	9000	470	0.052
50		H	142	4.4	0.031
51	same as above	NHPh	10300	340	0.034
52	CH_2Ph	H	120	280	2.4
53	CH_2Ph	NHPh	1630	7100	4.4
54	cyclopropyl	H	3.2	1240	390
55	cyclopropyl	NHPh	68	960	14.1
8	cyclopentyl	H	0.59	460	780
30	cyclopentyl	NHPh	12.4	144	11.6
9	cyclohexyl	H	1.42	610	430
56	cyclohexyl	NHPh	54	450	8.4
57	2-*endo*-norbornyl	H	0.42	770	1850
58	2-*endo*-norbornyl	NHPh	7.7	472	61
59	(*S*)-2-hydroxypropyl	H	5.0	9300	1870
60	(*S*)-2-hydroxypropyl	NHPh	50	1700	34
10	(*R*)-1-methyl-2-phenylethyl	H	1.17	124	106
61	(*R*)-1-methyl-2-phenylethyl	NHPh	152	240	1.56
62[b]	1-pyrrolidinyl	NHPh	18400	58000	3.2

[a] A_1 affinities were determined in [^{3}H]-N^6-cyclohexyladenosine binding to rat whole-brain membranes, and A_2 affinities were determined in [^{3}H]-NECA binding to rat striatal membranes.[5] Results for compounds **7** and **52** are from ref 5; those for **48** and **50** are from ref 8; that of **11** is from ref 9; those for **12** and **46** are from ref 10; that of **58** is from ref 11; those for **8** and **9** are from ref 15; that of **59** is from ref 16. A_1 and A_2 affinities for the above compounds and A_2 affinities for compounds **44**, **47**, **51**, **58**, and **61** are means of three separate experiments. Other values are single determinations. [b] Compound **62** is 2-(phenylamino)-6-(1-pyrrolidinyl)-9-β-D-ribofuranosyl-9*H*-purine.

stitution can improve A_2 affinity as well as reduce A_1 affinity, implying that the 2-position domain of the A_2 receptor may contain a hydrophobic binding region that is absent in the A_1 receptor. An interesting exception to the rule that A_1-selective N^6 derivatives show additive effects on 2-(phenylamino) substitution is **61**, a combination between R-PIA (**10**) and CV-1808 (**7**). This compound shows a 2-fold loss of A_2 affinity compared to R-PIA (**10**), suggesting that the lack of additivity seen with A_2-selective agonists is not due to A_2 selectivity per se, but rather to structural features that the A_2-selective compounds share with R-PIA but not with the other A_1-selective agonists in Table IV. The pertinent structural feature is probably the presence of one or more aromatic rings distal to N^6. R-PIA and the A_2-selective compounds possess aralkyl side chains, whereas all of the remaining A_1-selective compounds have compact alkyl or cycloalkyl groups at N^6.

We can envision several possible explanations for the lack of additivity between C2 and N^6 modifications. The first would involve direct steric interference between the two side chains due to a partial overlap between the C2 and N^6 aryl-binding pockets. Thus, distal aryl groups at C2 and N^6 would occupy their respective pockets when present alone, but could not both occupy the overlapping portion of the two pockets when present in the same molecule. In this case, one of the two aryl groups would be forced into an unfavorable position, resulting in loss of affinity. This interpretation is consistent with present knowledge of adenosine-receptor structure–activity relationships. In particular, the N^6 side chain most likely points in the direction of N^1 rather than N^7,[11] so the more distal parts of the N^6 chain could easily wrap around into the C2 pocket. Whether the N^6 chain actually does bend in that direction is unclear, since the distal portion of the N^6 domain has not yet been mapped. However, this hypothesis does explain why additivity was seen with alkyl and cycloalkyl groups at N^6, since these groups would not extend far enough to encroach into the C2 pocket.

Alternatively, the two side chains could interfere with each other in an indirect manner. For instance, binding of the N^6 side chain could induce an allosteric change in the receptor, resulting in closure of the C2 pocket. Conversely, the presence of a side chain at C2 or N^6 might cause a shift in the position of adenosine on the receptor,

thereby displacing the other side chain to an unfavorable location. Although the present data do not distinguish between direct and indirect steric interference, the previously reported lack of additivity between N^6 and 5′ substitution[5,14] must be due to indirect interactions, since the N^6 and 5′ positions are too far apart for any direct steric interactions.

2-(Phenylamino) substitution lowered A_1 affinity in all cases, with the magnitude of the effect ranging from 10-fold for compound **60** to 1700-fold for compound **47**. These results indicate that the C2 domain of the A_1 receptor does not readily accept the phenylamino group. Interestingly, compound **60** also showed the greatest enhancement of A_2 affinity with 2-(phenylamino) substitution, while compound **47** showed the greatest deterioration. These parallelisms suggest that the A_1 receptor may also demonstrate disruptive interactions between N^6-aralkyl groups and the 2-(phenylamino) substituent.

Finally, compound **62** confirms the importance of an N^6-hydrogen for binding affinity at both the receptors, implying that the SAR of the 6-amino group itself is unaltered.

In summary, we have demonstrated that there is a hydrophobic binding site near the C2 position of adenosine that is specific for the aromatic function. Occupation of this site increases A_2 affinity and selectivity when N^6 is occupied by an alkyl or cycloalkyl group, but not when N^6 is occupied by an aralkyl side chain.

Experimental Section

Melting points are uncorrected. Analytical thin-layer chromatography (TLC) was done with precoated glass plates (EM Science silica gel 60**F**-254). Flash column chromatography was performed on silica gel 60 (230–400 mesh). ^{1}H NMR spectra were obtained on Varian EM-390 or Varian XL-200 spectrometer. Mass spectra were recorded on a Finnegan 4500 mass spectrometer with an INCOS data system or a VG 7070 E/HR mass spectrometer with an 11/250 data system. Solvents and reagents were commercially available unless otherwise noted and were used directly. Elemental analyses were determined at Parke-Davis.

General Method for the Preparation of Compounds 13–17. *N*-Cyclopentyl-2-aminoadenosine (13). A mixture of 6-chloro-2-amino-9-β-D-ribofuranosyl-9*H*-purine (2.0 g, 6.6 mmol), cyclopentylamine (0.85 g, 9.9 mmol), and Et_3N (1.17 g, 11.0 mmol) in ethanol (50 mL) was refluxed under nitrogen for 18 h. The volatiles were evaporated, and the residue was purified by flash column chromatography on silica gel with 5% methanol–chloroform as an eluant. Evaporation of solvent from pure fractions followed by crystallization from ethanol–hexane afforded 1.9 g (82.6%) of *N*-cyclopentyl-2-aminoadenosine (**13**): mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ 1.4–2.0 (m, 8 H, cyclopentane), 3.46–3.6 (m, 2 H, 2 $H_{5'}$), 3.9 (m, 1 H, C*H*-cyclopentane), 4.1 (m, 1 H, $H_{4'}$), 4.4–4.7 (m, 2 H, $H_{3'}$ and $H_{2'}$), 5.1 (d, 1 H, 3′-OH), 5.3–5.6 (m, 2 H, 5′-OH and 2′-OH), 5.75 (d, 1 H, $H_{1'}$), 5.8 (s, 2 H, N*H*$_2$), 7.15 (d, 1 H, NH), 7.95 (s, 1 H, H_8); mass spectrum, *m*/*z* 350. Anal. ($C_{15}H_{22}N_6O_4$) C, H, N.

General Method for the Preparation of the Compounds of Scheme II. 6-Chloro-2-(phenylsulfonyl)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (25). A solution of 6-chloro-2-(phenylthio)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**19**[12]) (1.7 g, 3.25 mmol) in acetic acid (30 mL) was added to a solution of potassium permanganate (1.50 g, 9.8 mmol) in water (15 mL) at 0 °C in an ice bath. The reaction mixture was stirred at 0 °C for 3 h, and then water was added until a clear yellow solution resulted. The solution was extracted with chloroform (3 × 150 mL) and washed with 5% $NaHCO_3$ (2 × 25 mL), followed by brine (1 × 50 mL). The organic phase was dried over Na_2SO_4 and filtered, and the solvent was evaporated to yield 1.5 g (82%) of a solid (**25**): ^{1}H NMR ($CDCl_3$) δ 2.1 (s, 3 H, $COCH_3$), 2.13 (S, 3 H, $COCH_3$), 2.21 (s, 3 H, $COCH_3$), 4.43–4.51 (m, 3 H, 1 $H_{4'}$, 2 $H_{5'}$), 5.6 (t, 1 H, $H_{3'}$), 5.76 (t, 1 H, $H_{2'}$), 6.28 (d, 1 H, $H_{1'}$), 7.56–7.68 (m, 3 H, phenyl), 8.20 (d, 2 H, phenyl), and 8.48 (s, 1 H, H_8); mass spectrum (FAB), *m*/*z* 552.9 (M^+).

***N*-Cyclopentyl-2-(phenylsulfonyl)adenosine 2′,3′,5′-Triacetate (26).** A solution of 6-chloro-2-(phenylsulfonyl)-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**25**) (1.09 g, 1.97 mmol) and triethylamine (0.29 g, 2.8 mmol) in DME (35 mL) was added to a solution of cyclopentylamine (0.2 g, 2.8 mmol) in DME (5 mL) and the mixture was stirred at room temperature for 3 h. The precipitated solid was filtered (EtN^+HCl^-) and washed with DME, and the volatiles were evaporated. The residue was purified by flash column chromatography on silica gel with $CHCl_3$ as an eluent. Evaporation of the solvent from the pure fractions (R_f = 0.65 in 5% MeOH–$CHCl_3$) afforded 0.96 g (81%) of *N*-cyclopentyl-2-(phenylsulfonyl)adenosine 2′,3′,5′-triacetate (**26**): mp 77–81 °C, ^{1}H NMR ($CDCl_3$) δ 1.67–2.08 (brm, 8 H, cyclopentane), 2.09 (S, 3 H, $COCH_3$), 2.13 (s, 3 H, $COCH_3$), 2.18 (s, 3 H, $COCH_3$), 4.38–4.45 (m, 4 H, 2 $H_{5'}$, $H_{4'}$, and C*H*-cyclopentane), 5.57 (t, 1 H, $H_{3'}$) 5.72 (t, 1 H, H′$_2$), 5.9 (d, 1 H, $H_{4'}$:, 6.2 (brd, 1 H, NH), 7.55–7.64 (m, 3 H, phenyl), 7.99 (s, 1 H, H_8), and 8.17 (d, 2 H, phenyl); mass spectrum (FAB), *m*/*z* 602.2. Anal. ($C_{27}H_{31}N_5O_9S$) C, H, N, S.

***N*-Cyclopentyl-2-(phenylsulfonyl)adenosine (27).** A mixture of *N*-cyclopentyl-2-(phenylsulfonyl)adenosine 2′,3′,5′-triacetate (**26**) (0.81 g, 1.3 mmol) in saturated methanolic ammonia (25 mL) was stirred at room temperature for 3 h. The volatiles were evaporated, and the residue was purified by flash column chromatography on silica gel with 4% MeOH-$CHCl_3$ as an eluent. Evaporation of solvent from the pure fraction gave 0.36 g (56%) of *N*-cyclopentyl-2-(phenylsulfonyl)adenosine (**27**): mp 72–75 °C; ^{1}H NMR (DMSO-d_6) δ 1.45–2.0 (m, 8 H, cyclopentane), 3.52–3.65 (m, 2 H, 2 $H_{5'}$), 3.95 (m, 1 H, C*H*-cyclopentane), 4.12 (m, 2 H, $H_{3'}$ and $H_{4'}$), 4.58 (q, 1 H, $H_{2'}$), 4.97 (t, 1 H, 5′-OH), 5.25 (d, 1 H, 3′-OH), 5.49 (d, 1 H, 2′-OH), 5.91 (d, 1 H, $H_{1'}$), 7.66 (m, 3 H, phenyl), 7.99 (d, 2 H, phenyl), 8.54 (d, 1 H, NH), and 8.58 (s, 1 H, H_8); mass spectrum (FAB), *m*/*z* 476. Anal. ($C_{21}H_{25}N_5O_6S$) C, H, N, S.

General Methods for the Preparation of the Compounds of Scheme III. *N*-(1-Naphthylmethyl)-2-fluoroadenosine 2′,3′,5′-Triacetate (32). A mixture of 6-chloro-2-fluoro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**31**)[13] (2.0 g, 4.6 mmol), (1-naphthylmethyl)amine (0.8 g, 5.0 mmol), and Et_3N (0.52 g 5.0 mmol) in dimethoxyethane (25 mL) was stirred at room temperature for 20 h. The precipitated solid ($Et_3NH^+Cl^-$) was filtered and washed with DME, and the volatiles were evaporated. The residue was purified by flash column chromatography on silica gel with $CHCl_3$ as an eluent. Evaporation of solvent from pure fractions afforded 1.5 g (58.6%) of *N*-(1-naphthylmethyl)-2-fluoroadenosine 2′,3′,5′-triacetate (**32**): mp 76–80 °C; ^{1}H NMR ($CDCl_3$) δ 2.08 (s, 3 H, $COCH_3$), 2.13 (s, 3 H, $COCH_3$), 2.14 (s, 3 H, $COCH_3$), 4.4 (m, 3 H, 2 $H_{5'}$), 5.8 (t, 1 H, $H_{2'}$), 6.1 (d, 1 H, $H_{1'}$), 6.4 (brs, 1 H, NH), and 7.27–8.08 (m, 8 H, phenyl and H_8); mass spectrum, *m*/*z* (relative intensity) 551 (50), 293 (60), 256 (60), 139 (100). Anal. ($C_{27}H_{26}N_5O_7F$) C, H, N, F.

***N*-(1-Naphthylmethyl)-2-fluoroadenosine (36).** A solution of *N*-(1-naphthylmethyl)-2-fluoroadenosine 2′,3′,5′-triacetate (**32**) (1.2 g, 2.18 mmol) in saturated methanolic ammonia (20 mL) was stirred at room temperature for 2.5 h. The volatiles were evaporated, and the residue upon treatment with $CHCl_3$–hexane afforded a precipitate, which was filtered and dried, resulting in 0.89 g (96%) of *N*-(1-naphthylmethyl)-2-fluoroadenosine (**36**): mp 131–135 °C; ^{1}H NMR (DMSO-d_6) δ 3.5–3.67 (m, 2 H, 2 $H_{5'}$) 3.95 (d, 1 H, $H_{4'}$), 4.15 (q, 1 H, $H_{3'}$), 4.55 (q, 1 H, $H_{2'}$), 5.06 (t, 1 H, 5′-OH), 5.13 (d, 2 H, CH_2 naphthyl), 5.22 (d, 1 H, 3′-OH), 5.49 (d, 1 H, 2′-OH), 5.82 (d, 1 H, $H_{1'}$), 7.5–8.2 (m, 7 H, phenyl), 8.42 (s, 1 H, 8 H), and 9.09 (brs, 1 H, NH); mass spectrum (FAB), *m*/*z* 426.1 (M^+). Anal. ($C_{21}H_{20}N_5O_4F$) C, H, N, F.

***N*-(1-Naphthylmethyl)-2-[(phenylmethyl)amino]-adenosine (38).** A mixture of *N*-(1-naphthylmethyl)-2-fluoroadenosine (**36**) (0.5 g, 1.1 mmol), benzylamine (0.25 g, 2.3 mmol), and triethylamine (0.23 g, 2.3 mmol) in ethanol (20 mL) was refluxed for 24 h. Upon cooling, crystalline material was obtained, which was filtered and dried, affording 0.35 g (58%) of *N*-(1-naphthylmethyl)-2-[(phenylmethyl)amino]adenosine (**38**): mp 205–208 °C; ^{1}H NMR (DMSO-d_6) δ 3.42–3.64 (m, 2 H, 2$H_{5'}$), 3.85 (d, 1 H, $H_{4'}$), 4.08 (q, 1 H, $H_{3'}$), 4.38 (d, 2 H, CH_2Ph), 4.52 (q, 1 H, $H_{2'}$), 5.09 (brd, 4 H, CH_2-naphthyl, 5′-OH, and 3′-OH), 5.32 (d, 1 H, 2′-OH), 5.71 (d, 1 H, H$_1$′), 6.92 (brt, 1 H, NH), 7.13–8.24 (m, 12 H, phenyl). Anal. ($C_{18}H_{28}N_6O_4$) C, H, N.

N-(2-Phenylethyl)-2-[(2-phenylethyl)amino]adenosine (35). A reaction mixture of 2-fluoro-6-chloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**31**) (1.2 g, 2.7 mmol), 2-phenylethylamine (0.37 g, 3.0 mmol), and triethylamine (0.3 g, 3.0 mmol) in DME (20 mL) was stirred at room temperature for 20 h. TLC (5% MeOH–$CHCl_3$) showed the absence of starting material. The precipitated solid ($Et_3NH^+Cl^-$) was filtered and washed with DME, and the volatiles were removed from the filtrate. The residue was then dissolved in saturated methanolic ammonia (15 mL) and stirred at room temperature for 3 h. The volatiles were removed, and the residue was treated with $CHCl_3$–MeOH–ether. The solid (1.1 g) was filtered and dried. ^1H NMR $CDCl_3$ showed a 1:1 mixture of compounds **33** and **34**. All the sugar protons were duplicate and the C8 proton from both the compounds showed two separate singlets at δ 8.37 and 8.39 in a 1:1 ratio.

This 1:1 mixture of **33** and **34** was further reacted with an excess of 2-phenylethylamine in refluxing ethanol for 20 h. The volatiles were evaporated, and the residue upon crystallization from chloroform–ether (1:4) gave a solid, which was filtered and dried, affording 0.9 g (62.5% from **31** of *N*-(2-phenylethyl)-2-[(2-phenylethyl)amino]adenosine (**35**): mp 137–142 °C; ^1H NMR (DMSO-d_6) δ 2.7–3.0 (m, 4 H, CH_2Ph), 3.42–3.65 (m, 6 H, 2 CH_2NH and 2 $H_{5'}$), 3.88 (brd, 1 H, $H_{4'}$), 4.10 (brt, 1 H, $H_{3'}$), 4.57 (t, 1 H, $H_{2'}$), 4.6–5.5 (br, 3 H, 3 OH), 5.73 (d, 1 H, $H_{1'}$), 6.34 (s, 1 H, NH), 7.22 (brs, 10 H, aromatic), 7.29 (s, 1 H, NH), and 7.90 (s, 1 H, H_8); mass spectrum (FAB), *m/z* 491.1 (M^+). Anal. ($C_{26}H_{30}N_6O_4 \cdot 1.5H_2O$) C, H, N.

Receptor Binding. Affinities of compounds for inhibition of binding of [^{3}H]-N^6-cyclohexyladenosine to A_1 receptors in rat brain membranes and for inhibition of [^{3}H]NECA binding to A_2 receptors in rat striatal membranes in the presence of 50 nM N^6-cyclopentyladenosine were determined as previously described.[5]

Acknowledgment. We thank Jim Fergus and Gina Lu for providing A_1 and A_2 binding data and Sigrid Stork for chemical syntheses.

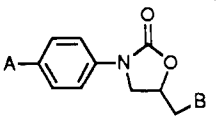
Registry No. **1**, 58-61-7; **2**, 146-77-0; **3**, 1818-71-9; **4**, 58097-85-1; **5**, 120524-27-8; **6**, 37151-17-0; **7**, 53296-10-9; **8**, 41552-82-3; **9**, 36396-99-3; **10**, 38594-96-6; **11**, 98383-40-5; **12**, 21924-65-2; **13**, 120524-28-9; **14**, 26783-44-8; **15**, 120524-29-0; **16**, 98383-43-8; **17**, 111864-02-9; **18** (R′ = H), 2004-07-1; **19**, 92123-05-2; **20**, 120524-30-3; **21**, 120524-31-4; **22**, 117325-43-6; **23**, 120524-32-5; **24**, 120524-33-6; **25**, 120524-34-7; **26**, 120524-35-8; **27**, 120524-36-9; **28**, 120524-37-0; **29**, 120524-38-1; **30**, 120524-39-2; **31**, 13276-51-2; **32**, 120524-40-5; **33**, 120524-41-6; **34**, 120524-42-7; **35**, 120524-43-8; **36**, 120524-44-9; **37**, 120524-45-0; **38**, 120524-46-1; **39**, 6979-94-8; **40**, 41623-91-0; **41**, 53296-13-2; **42**, 53296-14-3; **43**, 117325-41-4; **44**, 120524-47-2; **45**, 120524-48-3; **46**, 103450-84-6; **47**, 120524-49-4; **48**, 114675-13-7; **49**, 120524-50-7; **50**, 120442-40-2; **51**, 120524-51-8; **52**, 4294-16-0; **53**, 120524-52-9; **54**, 97374-48-6; **55**, 120524-53-0; **56**, 120524-54-1; **57**, 117773-72-5; **58**, 120524-55-2; **59**, 107656-16-6; **60**, 120524-56-3; **61**, 117325-42-5; **62**, 120524-57-4; cyclopentylamine, 1003-03-8; cyclohexylamine, 108-91-8; (*R*)-1-methyl-2-phenylethylamine, 156-34-3; 2,2-diphenylethylamine, 3963-62-0; 9-fluorenylmethylamine, 34577-90-7; 1-naphthylmethylamine, 118-31-0; benzylamine, 100-46-9; phenethylamine, 64-04-0; 2-(3,5-dimethoxyphenyl)-2-phenylethylamine, 120355-56-8; 2-(3,5-dimethoxyphenyl)-2-(2-methylphenyl)ethylamine, 120355-64-8; cyclopropylamine, 765-30-0; 2-*endo*-norbornylamine, 31002-73-0; (*S*)-2-hydroxypropylamine, 2799-17-9; pyrrolidine, 123-75-1.

Antibacterials. Synthesis and Structure–Activity Studies of 3-Aryl-2-oxooxazolidines. 1. The "B" Group

Walter A. Gregory,† David R. Brittelli,*† C.-L. J. Wang,† Mark A. Wuonola,† Ronald J. McRipley,‡ David C. Eustice,‡ Virginia S. Eberly,‡ P. T. Bartholomew,‡ Andrew M. Slee,‡ and Martin Forbes‡

Pharmaceutical and Biotechnology Research, Medical Products Department, E. I. du Pont de Nemours and Co., Medicinal Chemistry, Experimental Station, Wilmington, Delaware 19898, and Chemotherapy Research, Glenolden Laboratory, Glenolden, Pennsylvania 19036. *Received August 31, 1988*

The synthesis and structure/activity studies of the effect of varying the "B" group in a series of oxazolidinone antibacterials (I) are described. Two synthetic routes were used: (1) alkylation of aniline with glycidol followed

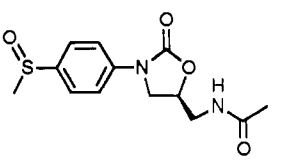


I

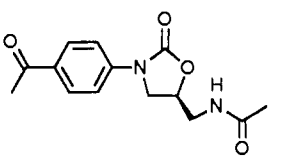
by dialkyl carbonate heterocyclization to afford I (A = H, B = OH), whose arene ring was further elaborated by using electrophilic aromatic substitution methodology; (2) cycloaddition of substituted aryl isocyanates with epoxides to give A and B with a variety of values. I with B = OH or Br were converted to other "B" functionalities by using S_N2 methodology. Antibacterial evaluation of compounds I with A = acetyl, isopropyl, methylthio, methylsulfinyl, methylsulfonyl, and sulfonamido and a variety of different "B" groups against *Staphylococcus aureus* and *Enterococcus faecalis* concluded that the compounds with B = aminoacyl, and particularly acetamido, were the most active of those examined in each A series, possessing MICs in the range of 0.5–4 μg/mL for the most active compounds described.

The oxazolidinones,[1] exemplified by DuP 105 (**1**) and DuP 721 (**2**), are a new class of orally active, synthetic antibacterial agents, derived from a random screening lead, whose in vitro spectrum includes activity against staphylococci, streptococci, enterococci, anaerobic bacteria, and mycobacteria.[2]

As a class, the oxazolidinones have equal activity against methicillin-sensitive and -resistant staphylococcal strains and β-lactamase positive and negative strains.[3,4] Pharmacokinetic studies on DuP 721 indicate that peak serum



1



2

levels exceeding the MIC_{90}'s can readily be achieved following single doses per os.[5] Mechanism of action studies

† Medicinal Chemistry, Experimental Station.
‡ Chemotherapy Research, Glenolden Laboratory.

(1) Fugitt, R. B.; Luckenbaugh, R. W. U.S. Patent 4,340,606, July 20, 1982. Gregory, W. A. U.S. Patent 4,461,773, July 24, 1984. Gregory, W. A. U.S. Patent, 4,705,799, November 10, 1987.

0022-2623/89/1832-1673$01.50/0