7-Deaza-2-phenyladenines: Structure–Activity Relationships of Potent A_1 Selective Adenosine Receptor Antagonists

Christa E. Müller,[†,§] Izumi Hide,[†] John W. Daly,*[,†] Klaus Rothenhäusler,[‡] and Kurt Eger[‡]

Laboratory of Bioorganic Chemistry, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, and Pharmazeutisches Institut, Pharmazeutische Chemie, Universität Tübingen, D-7400 Tübingen, Federal Republic of Germany. *Received March 12, 1990*

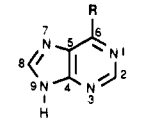
A series of derivatives of 7-deazapurines with varying substituents in the 2-, 6-, and 9-position was synthesized in an attempt to improve the adenosine receptor affinity and A_1 or A_2 selectivity. The adenosine receptor affinities were assessed by measuring the inhibition of [^{3}H]-(*R*)-N^6-(phenylisopropyl)adenosine (*R*-PIA) binding to rat brain A_1 and inhibition of [^{3}H]-5′-(*N*-ethylcarboxamido)adenosine (NECA) binding to rat striatum A_2 adenosine receptors. A selected set of compounds representing the main structural variations was further examined in adenosine receptor coupled adenylate cyclase assays. All tested compounds antagonized the inhibition of adenylate cyclase elicited by interaction of *R*-PIA with A_1 receptors in rat fat cell membranes and the activation of adenylate cyclase elicited by interaction of NECA with A_2 receptors of pheochromocytoma PC12 cell membranes. The results indicate that 7-deazahypoxanthines have a potential for A_2 selectivity, while all 7-deazaadenines are A_1 selective. Introduction of a phenyl residue in the 2-position of 7-deazaadenines increases A_1 activity tremendously. 2-(*p*-Chlorophenyl)-7,8-dimethyl-9-phenyl-7-deazaadenine (**29**) is potent and specific for the A_1 receptors of rat brain (K_i = 122 nM), having no affinity for the A_2 receptors of rat striatum. The compound has low activity at the A_2 receptors of rat PC12 cell membranes where it appears to act as a noncompetitive inhibitor. A 1-phenylethyl substituent at the 9-position was found to be superior to a phenyl residue in terms of A_1 affinity. The most potent A_1 antagonist in the present series is the highly A_1 selective (790-fold) (*R*)-7,8-dimethyl-2-phenyl-9-(1-phenylethyl)-7-deazaadenine (**31**, K_i = 4.7 nM), which is 30–35 times more potent at A_1 receptors than its *S* enantiomer. The solubility of six of the potent 7-deaza-2-phenyladenines was determined by means of an A_1 binding assay. Chloro substitution of the 2-phenyl ring appeared to improve the solubility as well as the solubility over A_1 affinity ratio of 9-phenyl- and 9-(1-phenylethyl)-substituted 7-deazadenines.

Introduction

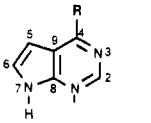
Adenosine receptors occur in virtually all tissues and cell types. The endogenous ligand adenosine modulates a variety of physiological functions and adenosine receptor ligands have a potential as therapeutic agents.[1,2] Adenosine receptors are divided into two main classes, A_1 receptors, which can inhibit, and A_2 receptors, which can stimulate adenylate cyclase activity.[3,4] Adenosine receptors also can be coupled to other second messenger systems, namely ion channels and phospholipase C. The best known class of adenosine receptor antagonists are the xanthines,[5-7] although certain other heterocyclic compounds also are active.[8-10] Many antagonists have poor water solubility, ancillary activity as phosphodiesterase inhibitors, and low potency or lack of selectivity for adenosine receptor subclasses; for these reasons the potential as pharmacological tools and for therapeutic use is limited. Development of antagonists with improved properties, particularly selectivity toward A_2 receptors, remains a challenge.

In our laboratories 7-deaza-9-phenyladenines and 7-deaza-9-phenylhypoxanthines[11] were discovered to have moderate antagonistic activity at A_1 and A_2 receptors. In an effort to improve affinity and selectivity at A_1 and A_2 receptors, a series of 34 pyrrolo[2,3-*d*]pyrimidines, tetrahydropyrimido[4,5-*b*]indoles and pyrimido[4,5-*b*]indoles, all of which can be envisaged as purine analogues lacking a nitrogen in the 7-position (see Chart I), have been synthesized. Substituents in positions 7 and 8 were, when present, identical, usually methyl groups or a fused, partly saturated 6-membered ring (type II, tetrahydropyrimido-[4,5-*b*]indoles) or a phenyl ring (type III, pyrimido[4,5-*b*]indoles). Substituents were varied in the 2-, 6-, and 9-position. Activity at A_1 and A_2 adenosine receptors was assessed by using binding and adenylate cyclase assays in membranes from rat brain, rat striatum, rat pheochromocytoma PC12 cells, and rat fat cell membranes. Phenyl substitution in the 2-position was found to greatly enhance antagonistic activity and A_1 selectivity. A 9-phenylethyl substituent also enhanced A_1 potency and selectivity.

Chart I. Structures and Numbering Systems of Purines and Pyrrolo[2,3-*d*]pyrimidines (7-Deazapurines)



R = H: Purine
R = NH_2: Adenine
R = OH: Hypoxanthine



R = H: "7-Deazapurine" Pyrrolo[2,3-d]pyrimidine
R = NH_2: 7-Deaza-adenine
R = OH: 7-Deaza-hypoxanthine

Results and Discussion

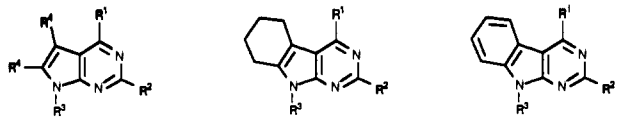
A variety of heterocyclic compounds have been reported as antagonists at adenosine receptors.[8-10] Two classes of these heterocycles can be considered very closely related

* Correspondence: LBC:NIDDK, NIH, Bldg. 8, Rm. 1A-15, Bethesda, MD 20892.
† Laboratory of Bioorganic Chemistry, NIH.
‡ Universität Tübingen.
§ On leave from the Pharmazeutisches Institut, Pharmazeutische Chemie, Universität Tübingen, F.R.G., with support of the Deutsche Forschungsgemeinschaft.

(1) Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197.
(2) Daly, J. W. *Adv. Cyclic Nucleotide Protein Phosphorylation Res.* **1985**, *19*, 29.
(3) Stiles, G. L. *Trends Pharmacol. Sci.* **1986**, 486.
(4) Van Calker, D.; Müller, M.; Hamprecht, B. *Neurochemistry* **1979**, *33*, 999.
(5) Shamim, M. T.; Ukena, D.; Padgett, W. L.; Daly, J. W. *J. Med. Chem.* **1988**, *31*, 613.
(6) Shamim, M. T.; Ukena, D.; Padgett, W. L.; Daly, J. W. *J. Med. Chem.* **1989**, *32*, 123.
(7) Jacobson, K. A.; Kiriasis, L.; Barone, S.; Bradbury, B. J.; Kammula, U.; Campagne, J. M.; Secunda, S.; Daly, J. W.; Neumeyer, J. L.; Pfleiderer, W. *J. Med. Chem.* **1989**, *32*, 1873.
(8) Daly, J. W.; Hong, O.; Padgett, W. L.; Shamim, M. T.; Jacobson, K. A.; Ukena, D. *Biochem. Pharmacol.* **1988**, *37*, 655.
(9) Peet, N. P.; Dickerson, G. A.; Abdallah, A. H.; Daly, J. W.; Ukena, D. *J. Med. Chem.* **1988**, *31*, 2034.
(10) Bruns, R. F. *Biochem. Pharmacol.* **1981**, *30*, 325.
(11) Daly, J. W.; Padgett, W. L.; Eger, K. *Biochem. Pharmacol.* **1988**, *37*, 3749.

0022-2623/90/1833-2822$02.50/0

Table I. 7-Deazapurines: Affinity to A_1 and A_2 Adenosine Reactors



type I: Pyrrolo[2,3-*d*]pyrimidines

type II: Pyrimido[4,5-*b*]tetrahydroindoles

type III: Pyrimido[4,5-*b*]indoles

					$K_i \pm$ SE, μM		
no.	R^1	R^2	R^3	R^1 or type	A_1 receptor versus [^{3}H]-*R*-PIA binding rat brain membrane	A_2 receptor versus [^{3}H]NECA binding rat striatal membrane	ratio A_2/A_1
1	NH_2	H	H	CH_3	55 ± 4.6	208 ± 26	3.8
2	NH_2	H	*n*-hexyl	CH_3	60 ± 2.6	263 ± 24.9	4.4
3	NH_2	H	allyl	CH_3	49 ± 8.0	83 ± 9.9	1.7
4	NH_2	H	phenyl	CH_3	18 ± 4.1	123 ± 11.3	6.8
5	NH_2	H	2,3-dimethoxyphenyl	CH_3	54.3 ± 4.2	224 ± 13.2[a]	4.1
6	NH_2	H	*p*-bromobenzyl	CH_3	>100	154.9 ± 23.5	
7	NH_2	H	2-deoxyribosyl	CH_3	≫250	>250	
8	NH_2	H	phenyl	H	3.1 ± 0.1	25.0 ± 1.0	8.1
9	NH_2	H	phenyl	CHO	50.1 ± 7.1	76.3 ± 5.2	1.5
10	NH_2	H	phenyl	COOH	67 ± 5.9	150[b]	2.2
11	NH_2	H	phenyl	II	3.24 ± 0.46	10.9 ± 1.1	3.4
12	NH_2	H	phenyl	III	1.95 ± 0.27	5.98 ± 0.65	3.1
13	Cl	H	phenyl	III	2.42 ± 0.18	4.06 ± 0.32	1.7
14	OH	H	phenyl	III	0.88 ± 0.04	1.44 ± 0.05	1.6
15	$NHNH_2$	H	phenyl	III	8.42 ± 0.98	7.71 ± 0.41	0.9
16	cyclohexylamino	H	phenyl	III	1.96 ± 0.23	133 ± 3.3	68
17	OH	H	H	CH_3	58 ± 5.4	176 ± 14.6	3.0
18	OH	H	phenyl	II	2.07 ± 0.11	1.35 ± 0.27	0.65
19	SH	SH	phenyl	II	84.8 ± 10.5	≫250	≫3
20	SCH_3	SCH_3	phenyl	II	97.8 ± 19.4	≫250	≫2.6
21	SO_2CH_3	SO_2CH_3	phenyl	II	16.9 ± 0.18	157 ± 20.4	9.3
22	NH_2	CH_3	H	CH_3	88.0 ± 10.4	≫250	≫2.8
23	NH_2	phenyl	H	CH_3	1.49 ± 0.053	21.3 ± 0.97	14.3
24	NH_2	*p*-chlorophenyl	H	CH_3	1.24 ± 0.12	35.6 ± 0.58	28.7
25	NH_2	*m*-chlorophenyl	H	CH_3	0.73 ± 0.032	20.8 ± 0.92[a]	28.5
26	NH_2	*o*-chlorophenyl	H	CH_3	5.17 ± 0.18	85.6 ± 2.11[a]	16.6
27	NH_2	CH_3	phenyl	CH_3	30.4 ± 3.84	203 ± 6.9	6.7
28	NH_2	phenyl	phenyl	CH_3	0.036 ± 0.0042	14.3 ± 1.44[a]	397
29	NH_2	*p*-chlorophenyl	phenyl	CH_3	0.122 ± 0.010	≫250	≫2049
30	NH_2	*o*-chlorophenyl	phenyl	CH_3	0.99 ± 0.087	40.7 ± 0.94	41.1
31	NH_2	phenyl	1-phenylethyl(*R*)	CH_3	0.0047 ± 0.00020	3.71 ± 0.50	789
32	NH_2	phenyl	1-phenylethyl(*S*)	CH_3	0.165 ± 0.0073	80.8 ± 13.2[a]	490
33	NH_2	*o*-chlorophenyl	1-phenylethyl(*rac*)	CH_3	0.052 ± 0.0069	28.1 ± 1.5[a]	540
34	NH_2	*m*-chlorophenyl	1-phenylethyl(*rac*)	CH_3	0.028 ± 0.0054	59.9 ± 10.1[a]	2139

[a] Value estimated by extrapolation of the inhibition curve since solubility precluded determination of full inhibition curve. [b] K_B value from adenylate cyclase assay with rat PC12 cell membranes, inhibition of NECA stimulation.[11]

to the endogenous agonist adenosine in structure. Those are the 9-substituted adenines, in which the 9-ribose ring has been replaced by a 9-methyl substituent,[12] and the 9-substituted 7-deazaadenines, in which in most cases the 9-substituent is a phenyl or substituted-phenyl ring.[11] Both classes show activity as adenosine receptor antagonists.

It has been suggested[12] that the 9-methyladenines bind to adenosine receptors in a similar orientation to adenosines. This is based on the observation that the binding affinities of 9-methyladenines and adenosines are influenced in a similar manner by the N^6-substituents[12,13]; i.e. the potency at A_1 receptors is enhanced by N^6-cyclopentyl and N^6-cyclohexyl substituents, while potency at A_2 receptors is either reduced (adenosine) or little affected (9-methyladenines). As yet, there are insufficient analogous compounds in the 9-substituted adenine series and the 9-substituted 7-deazaadenine series to justify any generalized conclusions as to possible structure–activity correlations between the two series. However, 9-phenyladenine, provided by Dr. R. A. Olsson (University of South Florida), was 3-fold less potent at rat brain A_1 receptors than 9-phenyl-7-deazaadenine (8), while both compounds had comparable potency at rat striatal A_2 receptors (unpublished data). Thus, in this case the 7-deazaadenine was more potent and selective for A_1 receptors than the adenine.

A comparison of ribosides in the purine and deazapurine series is possible, and 7-deazaadenosine (tubercidin), the 7-deaza analogue of adenosine, is a very weak agonist at A_2 receptors of human fibroblasts.[14] 7-Deazaadenosine apparently has not been investigated at A_1 receptors. Pyrazolo[3,4-*d*]pyrimidine ribonucleosides (7-deaza-8-azaadenosines) are inactive at brain A_1 receptors[15] and human fibroblast A_2 receptors.[14]

In the 9-phenyl-7-deazapurine series certain "hypoxanthines" were active,[11] suggesting that the interaction with adenosine receptors is different than that of agonists, where an amino function at position 6 is essential for activity. In the 9-methylpurines, the compounds with 6-phenoxy substituents were inactive.[12]

(12) Ukena, D.; Padgett, W. L.; Hong, O.; Daly, J. W.; Daly, D. T.; Olsson, R. A. *FEBS Lett.* **1987**, *215*, 203.
(13) Bruns, R. F. *Biochem. Pharmacol.* **1988**, *37*, 655.
(14) Bruns, R. F. *Can. J. Physiol. Pharmacol.* **1980**, *58*, 673.
(15) Hamilton, H. W.; Bristol, J. A. *J. Med. Chem.* **1983**, *26*, 1601.

An analysis—position by position—of structure–activity relationships for the present set of 34 7-deazapurines provides some insights into the nature of the binding site for this class of adenosine antagonists. Substituents have been varied at what corresponds to the 6-, 7,8-, the 2-, and the 9-position of purines.

The R^1 substituent in 7-deazapurines corresponds to the 6-substituent in purines (see Table I). The most potent compound in the 9-phenyl-7-deazapurine type III series is hypoxanthine **14**, which is nearly nonselective toward A_1 and A_2 receptors. Chlorine substitution (compound **13**) instead of an oxo at the 6-position decreases the affinity to both A_1 and A_2 receptors 3-fold. Amino substitution at the 6-position (compound **12**) yields an analogue that is somewhat more A_1 selective and somewhat less potent than 7-deazahypoxanthine **14**.

The effect of N^6-substitution of 7-deazaadenines has been examined in the type III series. N^6-Cyclohexyl substitution (**16**) does not affect A_1 affinity but reduces A_2 affinity to a great extent, the N^6-substituted compound being 68-fold selective for A_1 receptors. In contrast, N^6-cyclohexyl substitution of 9-methyladenine[12] and adenosine[16] enhances A_1 receptor affinity, thereby yielding compounds that are highly A_1 selective. *N*-Cyclohexyl substitution of triazoloquinoxalines also increases A_1 selectivity.[17,18] In the triazoloquinazoline series aminoalkyl substitution led to a decrease in activity but to an increase in A_1 selectivity. In general, the result of alkyl substitution of the exocyclic nitrogen in 7-deazaadenines and other classes of heterocyclic adenosine receptor antagonists is to enhance A_1 selectivity.

In the type II series, 7-deazahypoxanthine **18** is again the most potent compound and shows a slight selectivity for A_2 receptors. The corresponding amino compound **11** was less potent and was about 3-fold selective for A_1 receptors. In the type II series, sulfur-containing substituents were introduced as R^1 and R^2 residues, corresponding to the 6- and 2-position, respectively, of purines. Thio and methylthio at these two positions afforded compounds **19** and **20**, which were A_1 selective, but with relatively low affinities. Remarkably, the bis(methylsulfono) compound **21** had a 5 times higher A_1 affinity than dithio compound **19**.

The 5- and 6-positions of pyrrolo[2,3-*d*]pyrimidines correspond to the 7- and 8-positions of purines. Because of the synthetic route, both positions will bear the same substituent, which usually has been methyl. In case of 7-deazaadenosine, a very weak agonist at human fibroblast A_2 receptors, substitution with a carboxamido or cyano group at the 7-position yielded 7-deazaadenosines that were inactive.[14] In the 9-phenyl-7-deazaadenine series the presence of methyl groups at the 7- and 8-position lowered A_1 and A_2 affinity markedly compared to that of the unsubstituted analogue **8**. Replacement of the methyl groups by aldehyde or carboxylic functions (compounds **9**, **10**) further decreased A_1 affinity.

If instead of the two R^4 substituents a 6-membered saturated ring is fused (type II series), a compound (**11**) with a similar A_1 affinity as the unsubstituted one (compound **9**) is obtained, but the A_2 affinity is increased and thus A_1 selectivity is reduced. Compound **12** containing a fused phenyl ring (type III series) was somewhat more potent at both A_1 and A_2 receptors than the corresponding compound **11** of the type II series.

Substitution of the 2-position in adenosine analogues often provides A_2 receptor selective compounds such as 2-(phenylamino)adenosine,[19,20] certain 2-alkyladenosines,[21] and 2-[[*p*-(2-carboxyethyl)phenethyl]amino]-5′-[*N*-ethylcarboxamido)adenosine (CGS 21680).[22] In contrast, 2-phenyladenosine[14] and 2-(*p*-methoxyphenyl)adenosine[16] were reported to be inactive at human fibroblast A_2 receptors. The latter was active at A_1 receptors in rat vas deferens.[23] Subsequently, this 2-(*p*-methoxyphenyl)-adenosine was found to have activity at the high-affinity A_2 receptor in rat striatal membranes and to be somewhat A_2 selective.[20]

We investigated the effect of various substituents in the 2-position on activity of 7-deazaadenines. In the 9-unsubstituted series a 2-methyl substituent (**22**) decreases A_1 and A_2 affinity, while a 2-phenyl group (**23**) markedly enhances the affinity for the A_1 receptor increasing A_2 affinity to a lesser extent, compared with compound **1**. Similar effects occur in the 9-phenyl-7-deazaadenines. Thus, 2-methyl substitution (**27**) decreases the affinities for both receptors compared with those of **4**. 2-Phenyl substitution (**28**) dramatically increases the A_1 affinity and compound **28** is 400-fold selective for A_1 receptors. Compound **28** has no inhibitory effect on phosphodiesterase in contrast to the xanthines and to some 7-deazaadenines with an unsubstituted 2-position.[24] In the 9-(1-phenylethyl)-7-deazaadenines, the effect of the 2-phenyl substitution (compounds **31**, **32**) is not known, since the corresponding parent compound, lacking the 2-phenyl group, has not been made. Both compounds **31** and **32** have high affinity and selectivity for A_1 receptors (see below). The effect of chloro groups on the 2-phenyl ring was investigated in three different 7-deazaadenines. The 9-unsubstituted compound **23** was compared to its chlorinated analogues **24**–**26**. *o*-Chloro substitution decreases the affinities for both A_1 and A_2 receptors while *p*- or *m*-chloro substitution slightly increases the affinities for the A_1, but not for the A_2, receptor. In the 9-phenyl and 9-(1-phenylethyl)-7-deazaadenines the presence of a chloro group on the 2-phenyl decreases both A_1 and A_2 affinity. The *p*-chloro compound **29** is of particular interest. It has fairly high affinity for the A_1 receptor, but no affinity to the A_2 receptor, making it a potent and *specific* A_1 receptor antagonist. In summary, in the 7-deazaadenines the effect of 2-substitution with phenyl appears to enhance A_1 selectivity, a result in marked contrast to adenosines, where 2-substitution including *p*-methoxyphenyl can enhance A_2 selectivity.

The R^3 substituent of pyrrolo[2,3-*d*]pyrimidines (see Table I) corresponds to the 9-position in purines. 9-Phenyl substitution of such 7-deazaadenines was found to be superior to alkyl substitution or hydrogen, particularly with respect to A_1 affinity[11] (see compounds 1–4, Table I). The effect of 9-phenyl substitution is even more pronounced in the novel, very potent 2-phenyl-7-deazaadenines (com-

(16) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 5547.
(17) Trivedi, B. K.; Bruns, R. F. *J. Med. Chem.* **1988**, *31*, 1011.
(18) Francis, J. E.; Cash, W. D.; Psychoyos, S.; Ghai, G.; Wenk, P.; Friedmann, R. C.; Atkins, C.; Warren, V.; Furness, P.; Hyun, J. L.; Stone, G. A.; Desai, M.; Williams, M. *J. Med. Chem.* **1988**, *31*, 1014.
(19) Ukena, D.; Olsson, R. A.; Daly, J. W. *Can. J. Physiol. Pharmacol.* **1987**, *65*, 365.
(20) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.
(21) Matsuda, A.; Ueda, T. *Nucleosides Nucleotides* **1987**, *6*, 85.
(22) Hutchison, A. J.; Webb, R. L.; Oei, H. H.; Ghai, G. R.; Zimmerman, M. B.; Williams, M. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 47.
(23) Paton, D. M. *J. Pharm. Pharmacol.* **1980**, *32*, 133.
(24) Klumpp, S.; Frey, M.; Kleefeld, G.; Sauer, A.; Eger, K. *Biochem. Pharmacol.* **1989**, *38*, 949.

Table II. Comparison of Certain 7-Deazaadenines and 7-Deazahypoxanthines as Antagonists at A_1 and A_2 Adenosine Receptors

	A_1 receptor (K_i or K_B ± SE, μM)		A_2 receptor (K_i or K_B ± SE, μM)		
				adenylate cyclase NECA stimulation	
no.	[^{3}H]-*R*-PIA binding for rat brain membranes	adenylate cyclase *R*-PIA inhibition: rat fat cell membranes	[^{3}H]NECA binding for rat striatal membranes	rat striatal membranes	rat PC12 cell membranes
1	55 ± 4.6	110 ± 27	208 ± 26.5		190 ± 20
2	60 ± 2.6		263 ± 24.9		42 ± 1.9
3	49 ± 8.0	170 ± 20	83 ± 9.9		40 ± 3.9
4	18 ± 4.1	22 ± 1.3	123 ± 11.3		62 ± 4.1
8	3.1 ± 0.1	4.6 ± 0.2	25.0 ± 1.0		17 ± 2.5
11	3.2 ± 0.5		10.9 ± 1.1	31.6 ± 6.4	7.5 ± 1.4
12	1.95 ± 0.27	3.30 ± 0.64	5.98 ± 0.65	14.0 ± 4.3	1.73 ± 0.47
14	0.88 ± 0.01	1.4 ± 0.3	1.44 ± 0.05		0.22 ± 0.06
17	58 ± 5.4	300 ± 70	177 ± 15		33 ± 3
18	2.1 ± 0.11		1.35 ± 0.27	1.76 ± 0.83	0.87 ± 0.23
23	1.49 ± 0.05	0.80 ± 0.13	21.3 ± 1.0		5.5 ± 1.2
28	0.036 ± 0.0042	0.024 ± 0.0033	14.3 ± 1.4[a]		22.1 ± 4.6
29	0.122 ± 0.010	0.057 ± 0.0082	≫250	*b*	*c*
31	0.0047 ± 0.0002	0.0038 ± 0.0009	3.71 ± 0.50		3.06 ± 0.35
32	0.165 ± 0.0073	0.112 ± 0.020	81 ± 13.2[a]		

[a] Value estimated, see Table I. [b] No effect, see Figure 1. [c] Noncompetitive inhibition, see Figure 1.

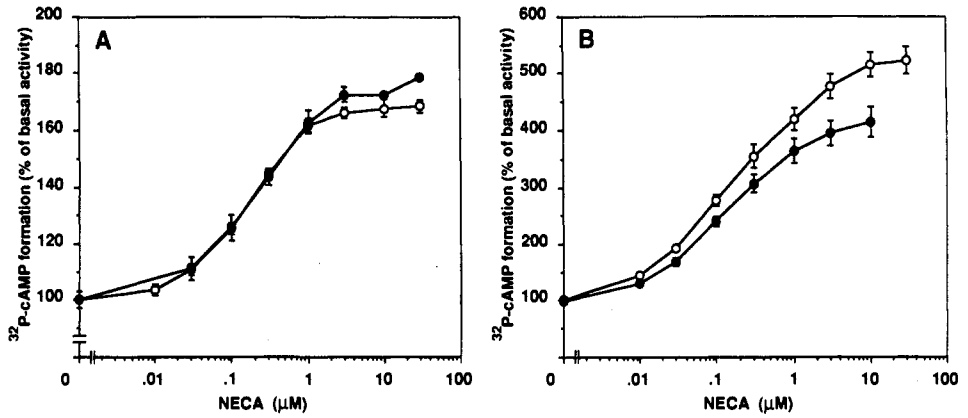


Figure 1. Effects of 2-(*p*-chlorophenyl)-9-phenyl-7-deazaadenine (**29**) on NECA-stimulated adenylate cyclase activity in membranes from (A) rat striatum and (B) rat PC12 cells. Data represent the means ± SEM of three separate experiments performed in triplicate. The final two points in A in the presence of **29** are from a single experiment in triplicate (○, control; ● 30 μM **29**).

pare **23** and **28**), where affinity for A_1 receptors is increased about 50-fold. A 2,3-dimethoxyphenyl (**5**) or *p*-bromophenyl group (**6**) at the 9-position decreased affinity, probably due to the bulky residues on the phenyl ring. The presence of a 2-deoxyribosyl group (**7**) at the 9-position abolished activity. We now find that the (*R*)-1-phenylethyl substituent at the 9-position instead of phenyl results in a further increased receptor affinity and A_1 selectivity (compare **28** and **31**). The increased flexibility of the side chain compared to that of a phenyl appears to be advantageous for a better fitting into a receptor pocket. The most potent compound in the present series of 7-deazaadenines is **31** with an A_1-binding value of 4.7 nM and an A_1 selectivity of almost 790, which makes it one of the most potent A_1 receptor antagonists known to date.

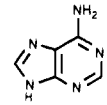
A subset of representative compounds were selected for studying their effects on adenosine receptor-mediated inhibition (A_1) or activation (A_2) of adenylate cyclase (Table II). The 12 tested compounds all reduced A_1 receptor agonist-elicited inhibition of adenylate cyclase in rat fat cell membranes. The K_B values correlated quite well with the A_1 binding data from rat brain membranes. All but one of the 15 tested compounds competitively blocked the stimulation of adenylate cyclase in rat PC12 cell membranes mediated by A_2 receptors. In that one case, compound **29**, the inhibition curve indicated a noncompetitive mechanism (see Figure 1). The same compound has no inhibitory effect on activation of adenylate cyclase via A_2 receptors in rat striatum, a result which parallels the lack of binding affinity to rat striatum A_2 receptors. For the three other compounds that were tested in an A_2 receptor mediated activation of adenylate cyclase in rat striatal membranes, the results were consonant with the A_2 binding values obtained from rat striatal membranes (Table II).

The most active compound synthesized in the present series of 7-deazaadenines was, in preliminary screening, the racemic mixture of (*R*)- and (*S*)-9-(1-phenylethyl)-2-phenyl-7,8-dimethyl-7-deazaadenine. The two pure enantiomers **31** and **32**, therefore, were synthesized and their activities compared. We found that the binding of the compounds to the receptors shows a high degree of stereoselectivity with the *R*-(−) enantiomer having a 35 times higher affinity to A_1 and a 22 times higher affinity to A_2 receptors compared to the *S*-(+) enantiomer. The stereoselectivity of this compound for A_1 adenosine receptors is in about the same order of magnitude as the stereoselectivity displayed by N^6-(phenylisopropyl)adenosines. The *R*-(−) enantiomer of the latter has an about 39 times higher affinity to A_1 receptors than the *S*-(+) enantiomer.

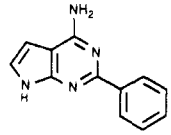
It has been suggested that the N^6-substituents of adenosine derivatives might bind to the same receptor region as 8-substituents of xanthine derivatives, since the SAR for both substituents are quite similar.[25] Our find-

(25) Bruns, R. F.; Fergus, J. H. *J. Pharm. Pharmacol.* **1989**, *41*, 590.

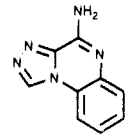
Chart II. Comparison of the Basic Structures of Potent Non-Xanthine Adenosine Receptor Antagonists



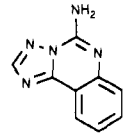
adenine



7-deaza-2-phenyladenine



4-amino[1,2,4]triazolo-[4,3-*a*]quinoxaline



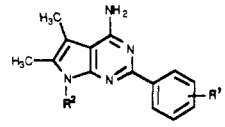
5-amino[1,2,4]triazolo-[1,5-*c*]quinazoline

ings raise the question of whether the 9-substituent in the 2-phenyl-7-deazaadenines is binding to the same receptor region. Certainly, the similar stereochemical preference for the N^6-(*R*)-phenylisopropyl residue of PIA and the 9-(*R*)-phenylethyl residue of deazaadenine **31** would appear to support this proposal.

While all known adenosine receptor agonists are derived from adenosine, various classes of compounds have been found to possess antagonistic activity besides the xanthines. A closer look at the structures of 2-phenyl-7-deazaadenines and other classes of potent adenosine receptor antagonists however reveals striking similarities (see Chart II): A 6:5 fused, rigid, unsaturated ring system, an exocyclic amino (or oxo or thio) function in a specified position, and from three to five ring nitrogen atoms differently arranged. A phenyl ring fused (triazoloquinazolines, triazoloquinoxalines) or attached (2-phenyl-7-deazaadenines) can increase activity and this aryl ring may be chlorine substituted. A lipophilic substituent in the "9-position" also seems to be favorable for receptor affinity.

Most of the known adenosine receptor antagonists show very poor water solubility, a property which limits their application as pharmacological tools and their development as drugs, since in vitro and in vivo studies are naturally carried out in aqueous media mostly at physiological pH values. A significant number in this context is ratio of the solubility to A_1 or A_2 affinity since this determines the magnitude of the "right shift" of dose–response curves for agonists that can be obtained at the solubility limit for the antagonist. Bruns and Fergus[26] proposed that compounds with a solubility over affinity ratio of <100 will not have potential for in vivo use. The solubility of the 2-phenyl-7-deazaadenines is difficult to determine by UV spectrophotometry due to the very low water solubility of the compounds. Bruns and Fergus[26] have recently introduced an A_1 binding assay approach to measurement of solubilities of such highly water insoluble adenosine agonists and antagonists. This A_1 binding assay was used to measure the solubility of the compounds **23**, **24**, **28**, **29**, **31**, and **34** in TRIS buffer solution, pH 7.4, as a representative series of 2-phenyl-7-deazaadenine derivatives. The results are shown in Table III. For comparison, the solubilities of some potent adenosine receptor antagonists, which have been reported, range from 0.14 μM for 8-(2-amino-4-chlorophenyl)-1,3-dipropylxanthine (PACPX), 1.7 μM for 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine (CGS 15943), 5.4 μM for 8-phenyltheophylline to 17 μM for 8-cyclopentyl-1,3-dipropylxanthine.[26] With the functionalized congener approach[27] and introduction of hydrophilic moieties, more water soluble molecules such as XAC (solubility 90 μM), 8-(*p*-carboxyphenyl)-1,3-dipropylxanthine (180 μM), and 8-(*p*-sulfophenyl)-1,3-dipropylxanthine (16 mM) have been developed.[5,27-29]

Table III. A_1 Affinities and Solubilities of Selected 2-Phenyl-7-deazaadenines



no.	R^1	R^2	A_1 affinity, μM	solubility, μM	ratio solubility/A_1 affinity
23		H	1.49	7.4	5.0
24	*p*-Cl	H	1.24	5.8	4.7
28		phenyl	0.036	0.10	2.8
29	*p*-Cl	phenyl	0.122	3.50	29
31		(*R*)-1-phenylethyl	0.0047	0.26	55
34	*m*-Cl	(*rac*)-1-phenylethyl	0.028	2.8	100

2-Phenyl-7-deazaadenines (pyrrolopyrimidines) **23**, **24**, **29**, and **34** exhibit a solubility on the same order of magnitude as most of the reported potent adenosine receptor antagonists (3–7 μM). Two of the 9-substituted 7-deazaadenines, **28** and **31**, were roughly 1 order of magnitude less soluble. We also investigated the effect of the lipophilic electron-withdrawing chloro substituent at the 2-phenyl ring on the solubility of the compounds. As expected the 9-unsubstituted 7-deazaadenines are more soluble than 9-phenyl or 9-(1-phenylethyl) derivatives and chloro substitution slightly diminishes the solubility and enhances the receptor affinity of the 9-unsubstituted compounds. Surprisingly in the 9-phenyl- and 9-(1-phenylethyl)-substituted compounds, chlorine substitution of the 2-phenyl ring has the opposite effect. The solubilities of compounds **28** and **31**, are increased 35 times (**28**) or 11 times (**31**) by chlorine substitution in the para position (**29**) or in the meta position (**34**). Although the receptor affinity is reduced by chloro substitution, the solubility over A_1 affinity ratio is improved 10-fold for **29** and at least 2-fold for **34**. The reason for this unexpected effect of chloro substitution may be due to different steric arrangements of the substituted and the unsubstituted phenyl ring with respect to the heterocyclic ring.

In conclusion, structure–activity relationships for 7-deazapurines at adenosine receptors have been analyzed and, where possible, activities of analogous compounds in the 7-deazapurine and purine series have been contrasted. 7-Deaza-2-phenyladenines were among the most potent and most A_1 selective antagonists reported to date. Such compounds are of interest as pharmacological tools and potential therapeutics as an alternative to the xanthines, because of high potency and selectivity and because they appear to lack phosphodiesterase inhibitory activity[24] characteristic of many xanthines.

Experimental Section

Synthetic Procedures. NMR spectra were performed on a Bruker AC-80 spectrometer (80 MHz) in DMSO-d_6 as solvent and tetramethylsilane as internal standard. For thin-layer chroma-

(26) Bruns, R. F. *Purines in Cell Signalling: Targets for New Drugs*, Jacobson, K. A., Daly, J. W., Manganiello, V., Eds.; Springer Verlag: New York, 1990.

(27) Jacobson, K. A.; Kirk, K. L.; Padgett, W. L.; Daly, J. W. *J. Med. Chem.* **1985**, *28*, 1334.

(28) Daly, J. W.; Padgett, W. L.; Shamim, M. T. *J. Med. Chem.* **1986**, *29*, 1520.

(29) Daly, J. W.; Padgett, W.; Shamim, M. T.; Butts-Lamb, P.; Waters, J. *J. Med. Chem.* **1985**, *28*, 487.

tographic analysis alumina silica gel plates 60 F_{245} from Merck were used and developed with chloroform/methanol/formic acid (85%) = 85:10:5. Melting points were taken on a Büchi melting point apparatus 510 and are uncorrected.

Compounds 1–5, 7, 8–10, 14, 17, and 18 were prepared as described.[11,24,30,31]

4-Amino-5,6-dimethyl-7-(4-bromobenzyl)-7*H*-pyrrolo[2,3-*d*]pyrimidine (6). 3-Hydroxy-2-butanone (17.6 g, 0.2 mol), 4-bromobenzylamine (18.6 g, 0.2 mol), and malononitrile (3.2 g, 0.2 mol) were condensed as described for the synthesis of other pyrroles.[11] The resulting crystalline pyrrole was refluxed with 150 mL of formamide, 50 mL of dimethylformamide, and 20 mL of formic acid (85%) for 8–10 h. The product was recrystallized from ethanolic sodium hydroxide solution: yield 26.8 g (81%); mp 223 °C; ^{1}H NMR, δ (ppm) 2.1 (s, 3 H, C_6-CH_3), 2.25 (s, 3 H, C_5-CH_3), 5.3 (s, 2 H, CH_2), 6.4 (s, 2 H, NH_2), 7.0 (d, 2 H, aromat), 7.5 (d, 2 H, aromat), 8.1 (s, 1 H, C_2-H). Anal. ($C_{15}H_{15}BrN_4$) C, H, N.

4-Amino-5,6,7,8-tetrahydro-9-phenyl-9*H*-pyrimido[4,5-*b*]indole (11). 2-Amino-4,5,6,7-tetrahydro-1-phenyl-1*H*-indole-3-carbonitrile[11] (5.0 g, 21 mmol) was dissolved in a mixture of 30 mL of formamide, 10 mL of *N*,*N*-dimethylformamide, and 4 mL of formic acid (85%) and refluxed for 5 h. The resulting crystalline product was collected by filtration and recrystallized from an ethanolic solution of potassium hydroxide: yield 5.1 g (65%); mp 219 °C; ^{1}H NMR δ (ppm) 1.70 (m, 4 H, C_6-H_2, C_7-H_2), 2.03 (m, 2 H, C_5-H_2), 2.35 (m, 2 H, C_8-H_2), 5.35 (s, 2 H, C_4-NH_2), 7.21–7.54 (m, 5 H, aromat), 8.23 (s, 1 H, C_2-H). Anal. ($C_{16}H_{16}N_4$) C, H, N.

4-Amino-9-phenyl-9*H*-pyrimido[4,5-*b*]indole (12). Compound 13 (2.0 g, 7.2 mmol) was added to ethanol, saturated with ammonia gas, and stirred in a steel vessel. The temperature was kept at 100 °C. The reaction was finished after 30 h. The excess ethanol was removed in vacuo and the resulting residue was dissolved in dichloromethane/isopropyl ether. The precipitate was collected by filtration and recrystallized from ethanol: yield 52%; mp 225 °C; ^{1}H NMR δ (ppm) 4.35 (s, 2 H, NH_2), 7.35–7.55 (m, 4 H, indole), 7.61 (m, 5 H, aromatic), 8.37 (s, 1 H, C_2-H). Anal. ($C_{16}H_{12}N_4$) C, H, N.

4-Chloro-9-phenyl-9*H*-pyrimido[4,5-*b*]indole (13). A solution of 14[11] (4.0 g, 14.3 mmol) in phosphoryl chloride (120 mL) was refluxed for 5 h. After cooling, the excess phosphoryl chloride was evaporated in vacuo. The residue was hydrolyzed with ice water. The precipitate was collected by filtration and recrystallized from toluene: yield 2.3 g (58%); mp 145 °C; ^{1}H NMR δ (ppm) 7.38–7.50 (m, 4 H, indole), 7.55–7.70 (m, 5 H, aromat), 8.47 (s, 1 H). Anal. ($C_{16}H_{10}ClN_3$) C, H, N.

4-Hydrazino-9-phenyl-9*H*-pyrimido[4,5-*b*]indole (15). Compound 13 (2.0 g, 7.2 mmol) was dissolved in ethanol (80 mL). After addition of 20.0 g of hydrazine hydrate (100%), the reaction mixture was refluxed for 5 h. After cooling, the precipitate was collected by filtration and recrystallized from ethanol: yield 60%; mp 170 °C; ^{1}H NMR δ (ppm) 5.85 (s, 2 H, NH_2), 6.57 (s, 1 H, NH), 7.20–7.38 (m, 4 H, indole), 7.57 (m, 5 H, aromatic), 8.44 (s, 1 H, C_2-H). Anal. ($C_{16}H_{13}N_5$) C, H, N.

4-(Cyclohexylamino)-9-phenyl-9*H*-pyrimido[4,5-*b*]indole (16). Compound 8[11] (3.0 g, 10.7 mmol) was dissolved in 60 mL of ethanol, and 12 g of cyclohexylamine, and 2 drops of concentrated HCl were added. The mixture was refluxed for 8 h. After evaporation of the solvent, the residue was dissolved in dichloromethane. The product was precipitated by addition of diisopropyl ether: yield 2.3 g (65%); mp 132 °C; ^{1}H NMR δ (ppm) 1.14–2.23 (m, 11 H, cyclohexyl), 6.75 (s, 1 H, NH), 7.28–7.64 (m, 9 H, aromat), 8.41 (s, 1 H, C_2-H). Anal. ($C_{22}H_{21}N_4$) C, H, N.

5,6,7,8-Tetrahydro-9-phenyl-9*H*-pyrimido[4,5-*b*]indole-2,4(1*H*,3*H*)-dithione (19). 2-Amino-4,5,6,7-tetrahydro-1-phenyl-1*H*-indole-3-carbonitrile[11] (7.0 g, 28.3 mmol) was refluxed in a mixture of potassium xanthogenate (7.0 g, 43.8 mmol) in 1-butanol (100 g) for 5 h. After cooling, the solid product was collected by filtration, dissolved in DMF/water (40 mL/60 mL), and acidified with 2 N hydrochloric acid. The precipitate was collected by filtration and recrystallized from methanol: yield 85% (7.5 g); mp 305 °C; ^{1}H NMR, δ (ppm) 1.69 (m, 4 H, C_6-H_2, C_7-H_2), 2.15 (m, 2 H, C_5-H_2), 2.89 (m, 2 H, C_8-H_2), 7.40–7.60 (m, 5 H, aromatic), 12.57 (s, 2 H, N_1-H, N_3-H). Anal. ($C_{16}H_{15}N_3S_2$) C, H, N.

5,6,7,8-Tetrahydro-2,4-bis(methylthio)-9-phenyl-9*H*-pyrimido[4,5-*b*]indole (20). Compound 19 (3.8 g, 12.14 mmol) was added to an ammonium hydroxide solution (100 mL, 0.75%) and methyl iodide (20.0 g, 141 mmol). After refluxing for 1 h, the reaction mixture was evaporated. The residue was acidified with acetic acid (20%) and extracted with chloroform (3 × 40 mL). The organic solvent was removed in vacuo: yield 70% (2.9 g); mp 140 °C; ^{1}H NMR, δ (ppm) 1.80 (m, 4 H, C_6-H_2, C_7-H_2), 2.50 (m, 2 H, C_5-H_2), 2.86 (m, 2 H, C_8-H_2), 2.47 (s, 3 H, C_2-SCH_3), 2.63 (s, 3 H, C_4-SCH_3), 7.41–7.55 (m, 5 H, aromat). Anal. ($C_{18}H_{19}N_3S_2$) C, H, N.

5,6,7,8-Tetrahydro-2,4-bis(methylsulfonyl)-9-phenyl-9*H*-pyrimido[4,5-*b*]indole (21). Compound 20 (2.0 g, 5.85 mmol) was dissolved in ethanol (90 mL) and *m*-chloroperbenzoic acid (4.2 g, 46.7 mmol) was added. The mixture was stirred at room temperature for 36 h. The resulting crystals were collected by filtration and recrystallized from ethanol: Yield 72% (1.7 g); mp 262 °C; ^{1}H NMR, δ (ppm) 1.86 (m, 4 H, C_6-H_2, C_7-H_2), 2.70 (m, 2 H, C_5-H_2), 3.04 (m, 2 H, C_8-H_2), 3.43 (s, 3 H, C_2-SO_2CH_3), 3.62 (s, 3 H, C_4-SO_2CH_3), 7.63 (m, 5 H, aromat). Anal. ($C_{18}H_{19}N_3O_4S_2$) C, H, N. Compounds 22–24 and 27–30 were prepared as described.[32] 25, 26, and 31–34 were prepared analogously.

4-Amino-2-(*p*-chlorophenyl)-5,6-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (25): reaction time 22 h; yield 86%; mp 216 °C. Anal. ($C_{14}H_{13}ClN_4$) C, H, N.

4-Amino-2-(*o*-chlorophenyl)-5,6-dimethyl-7*H*-pyrrolo[2,3-*d*]pyrimidine (26): reaction time 22 h; yield 82%; mp 278 °C. Anal. ($C_{14}H_{13}ClN_4$) C, H, N.

(*R*)-4-Amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3-*d*]pyrimidine (31). (*R*)-1-Phenylethylamine was used as the amine component in the synthesis.[32] $[\alpha]^{20}_D$ = −6.80° (*c* 0.5%, DMSO); mp 174 °C; yields and further analytical data were identical with those of the racemic mixture.[32]

(*S*)-4-Amino-5,6-dimethyl-2-phenyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3-*d*]pyrimidine (32). (*S*)-1-Phenylethylamine was used. $[\alpha]^{20}_D$ = +7.60° (*c* 0.5%, DMSO); mp 175 °C; yields and further analytical data were identical with those of the racemic mixture.[32]

4-Amino-2-(*o*-chlorophenyl)-5,6-dimethyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3-*d*]pyrimidine (33): reaction time 28 h; yield 45%; mp 196 °C. Anal. ($C_{22}H_{21}ClN_4$) C, H, N.

4-Amino-2-(*m*-chlorophenyl)-5,6-dimethyl-7*H*-7-(1-phenylethyl)pyrrolo[2,3-*d*]pyrimidine (34): reaction time 28 h; yield 43%; mp 175 °C. Anal. ($C_{22}H_{21}ClN_4$) C, H, N.

Biological Assays. Solubility Determination. The compounds were dissolved in DMSO, diluted 1:100 in TRIS-HCl buffer, 50 mM, pH 7.4, and incubated at room temperature overnight with slow shaking. After centrifugation, the supernatant was filtered through cotton. Several dilutions of this saturated stock solution were made in the buffer, on the basis of the estimated solubility and the known affinity of each compound. An A_1 binding assay was performed as described below with the various dilutions of the saturated stock solution. Since the drug dilutions were done in aqueous buffer solution, an appropriate amount of DMSO was added to the incubation tubes to maintain the same conditions as for the standard A_1 binding assay. The concentration of the saturated solution was then calculated by dividing the known IC_{50} value of a compound by the fold dilution of the saturated solution required to give 50% inhibition.

Receptor Binding and Adenylate Cyclase Assays. Inhibition of binding of [^{3}H]-(*R*)-N^6-(phenylisopropyl)adenosine (*R*-PIA) to A_1 adenosine receptors in rat cerebral cortical membranes and inhibition of binding of [^{3}H]-5′-(*N*-ethylcarboxamido)-adenosine (NECA) to A_2 receptors in rat striatal membranes were assayed as described.[16,20,33] Theophylline (5 mM) was used to

(30) Roth, H. J.; Eger, K. *Arch. Pharm.* **1975**, *308*, 252.
(31) Eger, K.; Junginger, G.; Spätling, S. *J. Heterocycl. Chem.*, in press.
(32) Eger, K.; Pfahl, J. G.; Folkers, G.; Roth, H. J. *J. Heterocycl. Chem.* **1987**, *24*, 425.
(33) Jacobson, K. A.; Ukena, D.; Kirk, K. L.; Daly, J. W. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 4089.

define nonspecific binding and 50 nM N^6-cyclopentyladenosine was present to block A_1 adenosine receptors in the A_2 binding assay. Inhibition of binding by a range of concentrations of 7-deazapurines was determined in triplicate in three separate experiments. K_i values were calculated from IC_{50} values with the Cheng-Prusoff equation[34] and a K_d of 1 nM for [^{3}H]-*R*-PIA and 8.5 nM for [^{3}H]NECA. Inhibition of the stimulation of adenylate cyclase via A_2 receptors by NECA in pheochromocytoma PC12 cells and rat striatal membranes and reversal of the inhibition of adenylate cyclase via A_1 receptors by *R*-PIA in rat fat cells were essentially assayed as described.[35,36] K_B values were calculated with the Schild equation and the ratio of EC_{50} values for NECA activation or the ratio of IC_{50} values for *R*-PIA inhibition in the presence or absence of antagonist.

Acknowledgment. We thank Dr. H. M. Garraffo for his generous assistance in the preparation of this manuscript. C. E. Müller and K. Eger were supported by grants from the Deutsche Forschungsgemeinschaft. I. Hide was supported by a grant from the International Life Sciences Institute (Washington, DC).

(34) Cheng, Y. C.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.
(35) Ukena, D.; Daly, J. W.; Kirk, K. L.; Jacobson, K. A. *Life Sci.* **1986**, *38*, 797.
(36) Daly, J. W.; Butts-Lamb, P.; Padgett, W. *Cell. Mol. Neurobiol.* **1983**, *3*, 69.

The Development of a Novel Series of (Quinolin-2-ylmethoxy)phenyl-Containing Compounds as High-Affinity Leukotriene Receptor Antagonists. 3. Structural Variation of the Acidic Side Chain To Give Antagonists of Enhanced Potency

Robert A. Galemmo, Jr.,* William H. Johnson, Jr., Keith S. Learn, Thomas D. Y. Lee,† Fu-Chih Huang, Henry F. Campbell, Raymond Youssefyeh, Susan V. O'Rourke, Glenn Schuessler, Dennis M. Sweeney, Jeffrey J. Travis, Charles A. Sutherland, George W. Nuss, Gilbert W. Carnathan, and Richard G. Van Inwegen

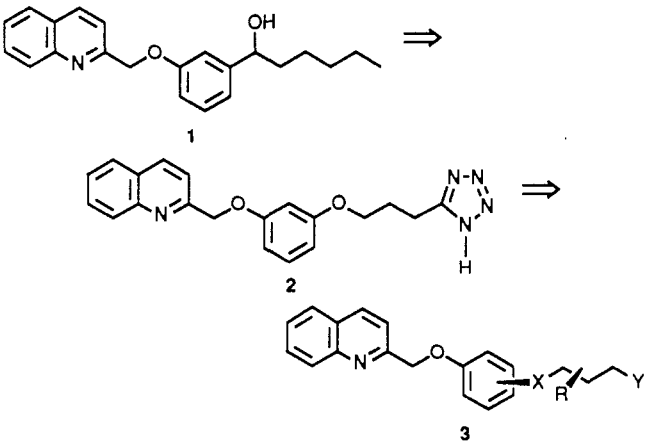
Rorer Central Research, 800 Business Center Drive, Horsham, Pennsylvania 19044. *Received October 6, 1989*

This paper is the third in a series outlining the development of orally active sulfido peptide leukotriene antagonists containing a (quinolin-2-ylmethoxy)phenyl moiety. In this work the systematic variation of the acid side chain substituents led to dramatic and reproducible changes in the oral activity of these compounds, presumably due to alterations in their pharmacokinetic properties. The most potent compound identified, 5-[4-[4-(quinolin-2-ylmethoxy)phenyl]-3-methylbutyl]tetrazole (**32**), represents a convergence of good in vitro antagonist activity and a 3–10-fold improvement in oral potency over the current clinical candidate **2**. The new findings from these optimization studies are as follows: oxygen substitution in the acid side chain was not necessary for antagonist activity, in vitro and in vivo activity was enhanced by alkyl or phenyl substitution on the γ-carbon of the acid side chain of para-substituted (quinolin-2-ylmethoxy)phenyl derivatives, and free rotation about the side chain carbon atom adjacent to the (quinolin-2-ylmethoxy)phenyl ring was required for activity. The lead compound of this report (**32**) is a competitive inhibitor of [^{3}H]LTD_4 binding to receptor membrane purified from guinea pig lung (K_i = 12 ± 3 nM) and of the spasmogenic activity of LTC_4, LTD_4, and LTE_4 in guinea pig lung strip. Dosed orally in guinea pigs, this compound blocks LTD_4-induced bronchoconstriction (ED_{50} 0.8 mg/kg) and antigen-induced systemic anaphylaxis (ED_{50} = 1.2 mg/kg).

The problem of elucidating the role of endogenous sulfido peptide leukotrienes in the pathophysiology of human asthma demands the development of receptor-specific, bioavailable, and long-acting leukotriene antagonists.[1] Meeting these criteria requires the continued refinement of existing antagonists. A preceding paper[2] outlined the development of a specific and orally active leukotriene antagonist, **2** (RG 7152, Chart I), derived from **1** (RG 5901), a competitive inhibitor of 5-lipoxygenase and a weak but competitive antagonist of leukotrienes.[3] This initial study evaluated a number of carbo- and heterocyclic ethers and found the (quinolin-2-ylmethoxy)phenyl ether the best suited for leukotriene antagonist activity. The addition of an acidic functional group, connected at either the meta or the para position of the (quinolin-2-ylmethoxy)phenyl ring by an oxypropyl spacer, gave a potent series of antagonists from which **2** emerged.[4]

In this study we improved upon the activity of **2** by the systematic modification of the acid side chain. First, we explored the role of the right-hand side-chain oxygen upon leukotriene receptor affinity and antagonist activity with compounds employing an oxypropyl (Chart I, **3**: R = H, X = O, Y = CO_2H and 5-tetrazole) or butyl (**3**: R = H, X = CH_2, Y = CO_2H and 5-tetrazole) spacer group. Sec-

Chart I



† Present address: General Food Corp., Tarrytown, NY, 10591.

(1) Fleisch, J. H.; Rinkema, L. E.; Whitesitt, C. A.; Marshall, W. S. *The Leukotrienes: Their Biological Significance*; Piper, P. J., Ed.; Raven Press: New York, 1986; p 109.

0022-2623/90/1833-2828$02.50/0