

7-Deaza-2-phenyladenines: Structure-Activity Relationships of Potent A₁ 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

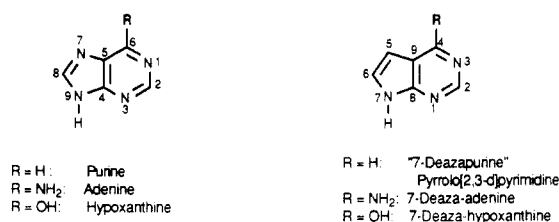
A series of derivatives of 7-deazapurines with varying substituents in the 2-, 6-, and 9-position was synthesized in an attempt to improve the adenosine receptor affinity and A₁ or A₂ selectivity. The adenosine receptor affinities were assessed by measuring the inhibition of [³H]-(R)-N⁶-(phenylisopropyl)adenosine (R-PIA) binding to rat brain A₁ and inhibition of [³H]-5'-(N-ethylcarboxamido)adenosine (NECA) binding to rat striatum A₂ 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 A₁ receptors in rat fat cell membranes and the activation of adenylate cyclase elicited by interaction of NECA with A₂ receptors of pheochromocytoma PC12 cell membranes. The results indicate that 7-deazahypoxanthines have a potential for A₂ selectivity, while all 7-deazaadenines are A₁ selective. Introduction of a phenyl residue in the 2-position of 7-deazaadenines increases A₁ activity tremendously. 2-(p-Chlorophenyl)-7,8-dimethyl-9-phenyl-7-deazaadenine (**29**) is potent and specific for the A₁ receptors of rat brain (K_i = 122 nM), having no affinity for the A₂ receptors of rat striatum. The compound has low activity at the A₂ 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 A₁ affinity. The most potent A₁ antagonist in the present series is the highly A₁ 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 A₁ receptors than its S enantiomer. The solubility of six of the potent 7-deaza-2-phenyladenines was determined by means of an A₁ binding assay. Chloro substitution of the 2-phenyl ring appeared to improve the solubility as well as the solubility over A₁ affinity ratio of 9-phenyl- and 9-(1-phenylethyl)-substituted 7-deazaadenines.

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, A₁ receptors, which can inhibit, and A₂ 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 A₂ receptors, remains a challenge.

In our laboratories 7-deaza-9-phenyladenines and 7-deaza-9-phenylhypoxanthines¹¹ were discovered to have moderate antagonistic activity at A₁ and A₂ receptors. In an effort to improve affinity and selectivity at A₁ and A₂ 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

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



9-position. Activity at A₁ and A₂ 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 A₁ selectivity. A 9-phenylethyl substituent also enhanced A₁ potency and selectivity.

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.; Pfeleiderer, 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.

Table I. 7-Deazapurines: Affinity to A₁ and A₂ Adenosine Receptors

					$K_i \pm SE, \mu M$		
					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
no.	R ¹	R ²	R ³	R ¹ or type			
1	NH ₂	H	H	CH ₃	55 ± 4.6	208 ± 26	3.8
2	NH ₂	H	<i>n</i> -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	<i>p</i> -bromobenzyl	CH ₃	>100	154.9 ± 23.5	
7	NH ₂	H	2-deoxyribose	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 ₂ CH ₃	SO ₂ CH 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	<i>p</i> -chlorophenyl	H	CH ₃	1.24 ± 0.12	35.6 ± 0.58	28.7
25	NH ₂	<i>m</i> -chlorophenyl	H	CH ₃	0.73 ± 0.032	20.8 ± 0.92 ^a	28.5
26	NH ₂	<i>o</i> -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	<i>p</i> -chlorophenyl	phenyl	CH ₃	0.122 ± 0.010	>>250	>>2049
30	NH ₂	<i>o</i> -chlorophenyl	phenyl	CH ₃	0.99 ± 0.087	40.7 ± 0.94	41.1
31	NH ₂	phenyl	1-phenylethyl(<i>R</i>)	CH ₃	0.0047 ± 0.00020	3.71 ± 0.50	789
32	NH ₂	phenyl	1-phenylethyl(<i>S</i>)	CH ₃	0.165 ± 0.0073	80.8 ± 13.2 ^a	490
33	NH ₂	<i>o</i> -chlorophenyl	1-phenylethyl(<i>rac</i>)	CH ₃	0.052 ± 0.0069	28.1 ± 1.5 ^a	540
34	NH ₂	<i>m</i> -chlorophenyl	1-phenylethyl(<i>rac</i>)	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 suggested¹² 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 N⁶-substituents^{12,13}; i.e. the potency at A₁ receptors is enhanced by N⁶-cyclopentyl and N⁶-cyclohexyl substituents, while potency at A₂ 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 A₁ receptors than 9-phenyl-7-deazaadenine (8), while both compounds had comparable potency at rat striatal A₂ receptors (unpublished data). Thus, in this case the 7-deazaadenine was more potent and selective for A₁ 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 A₂ receptors of human fibroblasts.¹⁴ 7-Deazaadenosine apparently has not been investigated at A₁ receptors. Pyrazolo[3,4-*d*]pyrimidine ribonucleosides (7-deaza-8-azaadenosines) are inactive at brain A₁ receptors¹⁵ and human fibroblast A₂ 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 R¹ 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 A₁ and A₂ receptors. Chlorine substitution (compound 13) instead of an oxo at the 6-position decreases the affinity to both A₁ and A₂ receptors 3-fold. Amino substitution at the 6-position (compound 12) yields an analogue that is somewhat more A₁ selective and somewhat less potent than 7-deazahypoxanthine 14.

The effect of N⁶-substitution of 7-deazaadenines has been examined in the type III series. N⁶-Cyclohexyl substitution (16) does not affect A₁ affinity but reduces A₂ affinity to a great extent, the N⁶-substituted compound being 68-fold selective for A₁ receptors. In contrast, N⁶-cyclohexyl substitution of 9-methyladenine¹² and adenosine¹⁶ enhances A₁ receptor affinity, thereby yielding compounds that are highly A₁ selective. N-Cyclohexyl substitution of triazoloquinoxalines also increases A₁ selectivity.^{17,18} In the triazoloquinazoline series aminoalkyl substitution led to a decrease in activity but to an increase in A₁ 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 A₁ selectivity.

In the type II series, 7-deazahypoxanthine 18 is again the most potent compound and shows a slight selectivity for A₂ receptors. The corresponding amino compound 11 was less potent and was about 3-fold selective for A₁ receptors. In the type II series, sulfur-containing substituents were introduced as R¹ and R² 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 A₁ selective, but with relatively low affinities. Remarkably, the bis(methylsulfonyl) compound 21 had a 5 times higher A₁ 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 A₂ 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 A₁ and A₂ 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 A₁ affinity.

If instead of the two R⁴ substituents a 6-membered saturated ring is fused (type II series), a compound (11) with a similar A₁ affinity as the unsubstituted one (compound 9) is obtained, but the A₂ affinity is increased and thus A₁ selectivity is reduced. Compound 12 containing

a fused phenyl ring (type III series) was somewhat more potent at both A₁ and A₂ receptors than the corresponding compound 11 of the type II series.

Substitution of the 2-position in adenosine analogues often provides A₂ receptor selective compounds such as 2-(phenylamino)adenosine,^{19,20} certain 2-alkyladenosines,²¹ and 2-[[*p*-(2-carboxyethyl)phenethyl]amino]-5'-[*N*-ethyl-carboxamido]adenosine (CGS 21680).²² In contrast, 2-phenyladenosine¹⁴ and 2-(*p*-methoxyphenyl)adenosine¹⁶ were reported to be inactive at human fibroblast A₂ receptors. The latter was active at A₁ receptors in rat vas deferens.²³ Subsequently, this 2-(*p*-methoxyphenyl)-adenosine was found to have activity at the high-affinity A₂ receptor in rat striatal membranes and to be somewhat A₂ 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 A₁ and A₂ affinity, while a 2-phenyl group (23) markedly enhances the affinity for the A₁ receptor increasing A₂ 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 A₁ affinity and compound 28 is 400-fold selective for A₁ 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 A₁ 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 A₁ and A₂ receptors while *p*- or *m*-chloro substitution slightly increases the affinities for the A₁, but not for the A₂, receptor. In the 9-phenyl and 9-(1-phenylethyl)-7-deazaadenines the presence of a chloro group on the 2-phenyl decreases both A₁ and A₂ affinity. The *p*-chloro compound 29 is of particular interest. It has fairly high affinity for the A₁ receptor, but no affinity to the A₂ receptor, making it a potent and *specific* A₁ receptor antagonist. In summary, in the 7-deazaadenines the effect of 2-substitution with phenyl appears to enhance A₁ selectivity, a result in marked contrast to adenosines, where 2-substitution including *p*-methoxyphenyl can enhance A₂ selectivity.

The R³ 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 A₁ affinity¹¹ (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 A₁ and A₂ Adenosine Receptors

no.	A ₁ receptor (K_i or $K_B \pm SE, \mu M$)		A ₂ receptor (K_i or $K_B \pm SE, \mu M$)		
	[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	adenylate cyclase NECA stimulation	
				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	<i>b</i>	<i>c</i>
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.

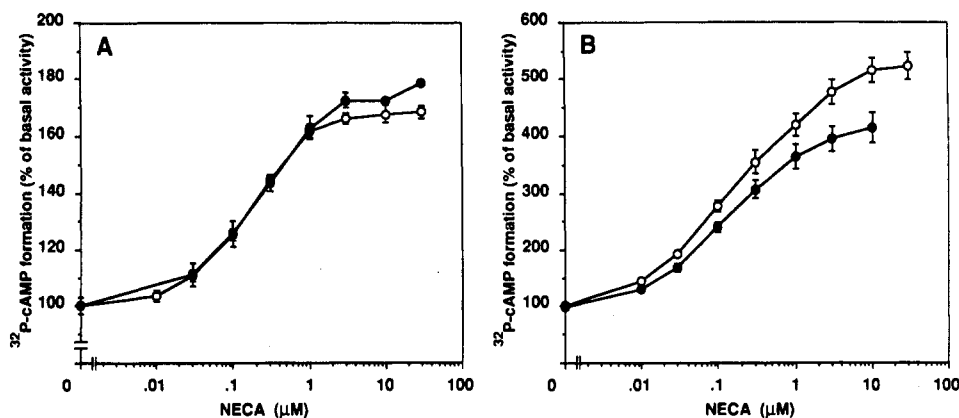


Figure 1. Effects of 2-(*p*-chlorophenyl)-9-phenyl-7-deazaadenine (29) on NECA-stimulated adenylate cyclase activity in membranes from (A) rat striatum and (B) rat PC12 cells. Data represent the means \pm SEM of three separate experiments performed in triplicate. The final two points in A in the presence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

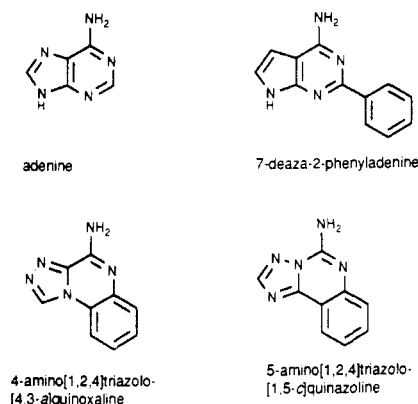
pare 23 and 28), where affinity for A₁ receptors is increased about 50-fold. A 2,3-dimethoxyphenyl (5) or *p*-bromophenyl group (6) at the 9-position decreased affinity, probably due to the bulky residues on the phenyl ring. The presence of a 2-deoxyribosyl group (7) at the 9-position abolished activity. We now find that the (*R*)-1-phenylethyl substituent at the 9-position instead of phenyl results in a further increased receptor affinity and A₁ 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 A₁-binding value of 4.7 nM and an A₁ selectivity of almost 790, which makes it one of the most potent A₁ receptor antagonists known to date.

A subset of representative compounds were selected for studying their effects on adenosine receptor-mediated inhibition (A₁) or activation (A₂) of adenylate cyclase (Table II). The 12 tested compounds all reduced A₁ receptor agonist-elicited inhibition of adenylate cyclase in rat fat cell membranes. The K_B values correlated quite well with the A₁ 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 A₂ 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 A₂

receptors in rat striatum, a result which parallels the lack of binding affinity to rat striatum A₂ receptors. For the three other compounds that were tested in an A₂ receptor mediated activation of adenylate cyclase in rat striatal membranes, the results were consonant with the A₂ 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 A₁ and a 22 times higher affinity to A₂ receptors compared to the *S*-(+) enantiomer. The stereoselectivity of this compound for A₁ 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 A₁ 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-

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

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 N⁶-(*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 A₁ or A₂ 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 Fergus²⁶ 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 Fergus²⁶ have recently introduced an A₁ binding assay approach to measurement of solubilities of such highly water insoluble adenosine agonists and antagonists. This A₁ 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 μ M for 8-(2-amino-4-chlorophenyl)-1,3-dipropylxanthine (PACPX), 1.7 μ M for 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine (CGS 15943), 5.4 μ M for 8-phenyltheophylline to 17 μ M

Table III. A₁ Affinities and Solubilities of Selected 2-Phenyl-7-deazaadenines

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

for 8-cyclopentyl-1,3-dipropylxanthine.²⁶ With the functionalized congener approach²⁷ and introduction of hydrophilic moieties, more water soluble molecules such as XAC (solubility 90 μ M), 8-(*p*-carboxyphenyl)-1,3-dipropylxanthine (180 μ M), and 8-(*p*-sulfophenyl)-1,3-dipropylxanthine (16 mM) have been developed.^{5,27-29}

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 μ M). 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 A₁ 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 A₁ 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 activity²⁴ 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 F₂₄₅ 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)-7H-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; ¹H NMR δ (ppm) 2.1 (s, 3 H, C₆-CH₃), 2.25 (s, 3 H, C₅-CH₃), 5.3 (s, 2 H, CH₂), 6.4 (s, 2 H, NH₂), 7.0 (d, 2 H, arom), 7.5 (d, 2 H, arom), 8.1 (s, 1 H, C₂-H). Anal. (C₁₅H₁₅BrN₄) C, H, N.

4-Amino-5,6,7,8-tetrahydro-9-phenyl-9H-pyrimido[4,5-b]indole (11). 2-Amino-4,5,6,7-tetrahydro-1-phenyl-1H-indole-3-carbonitrile¹¹ (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; ¹H NMR δ (ppm) 1.70 (m, 4 H, C₆-H₂, C₇-H₂), 2.03 (m, 2 H, C₅-H₂), 2.35 (m, 2 H, C₈-H₂), 5.35 (s, 2 H, C₄-NH₂), 7.21–7.54 (m, 5 H, arom), 8.23 (s, 1 H, C₂-H). Anal. (C₁₆H₁₆N₄) C, H, N.

4-Amino-9-phenyl-9H-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; ¹H NMR δ (ppm) 4.35 (s, 2 H, NH₂), 7.35–7.55 (m, 4 H, indole), 7.61 (m, 5 H, aromatic), 8.37 (s, 1 H, C₂-H). Anal. (C₁₆H₁₂N₄) C, H, N.

4-Chloro-9-phenyl-9H-pyrimido[4,5-b]indole (13). A solution of 14¹¹ (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; ¹H NMR δ (ppm) 7.38–7.50 (m, 4 H, indole), 7.55–7.70 (m, 5 H, arom), 8.47 (s, 1 H). Anal. (C₁₆H₁₀ClN₃) C, H, N.

4-Hydrazino-9-phenyl-9H-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; ¹H NMR δ (ppm) 5.85 (s, 2 H, NH₂), 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, C₂-H). Anal. (C₁₆H₁₃N₅) C, H, N.

4-(Cyclohexylamino)-9-phenyl-9H-pyrimido[4,5-b]indole (16). Compound 8¹¹ (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; ¹H NMR δ (ppm) 1.14–2.23 (m, 11 H, cyclohexyl), 6.75 (s, 1 H, NH), 7.28–7.64 (m, 9 H, arom), 8.41 (s, 1 H, C₂-H). Anal. (C₂₂H₂₁N₄) C, H, N.

5,6,7,8-Tetrahydro-9-phenyl-9H-pyrimido[4,5-b]indole-2,4(1H,3H)-dithione (19). 2-Amino-4,5,6,7-tetrahydro-1-phenyl-1H-indole-3-carbonitrile¹¹ (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; ¹H NMR, δ (ppm) 1.69 (m, 4 H, C₆-H₂, C₇-H₂), 2.15 (m, 2 H, C₅-H₂), 2.89 (m, 2 H, C₈-H₂), 7.40–7.60 (m, 5 H, aromatic), 12.57 (s, 2 H, N₁-H, N₃-H). Anal. (C₁₆H₁₅N₃S₂) C, H, N.

5,6,7,8-Tetrahydro-2,4-bis(methylthio)-9-phenyl-9H-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; ¹H NMR, δ (ppm) 1.80 (m, 4 H, C₆-H₂, C₇-H₂), 2.50 (m, 2 H, C₅-H₂), 2.86 (m, 2 H, C₈-H₂), 2.47 (s, 3 H, C₂-SCH₃), 2.63 (s, 3 H, C₄-SCH₃), 7.41–7.55 (m, 5 H, arom). Anal. (C₁₈H₁₉N₃S₂) C, H, N.

5,6,7,8-Tetrahydro-2,4-bis(methylsulfonyl)-9-phenyl-9H-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; ¹H NMR, δ (ppm) 1.86 (m, 4 H, C₆-H₂, C₇-H₂), 2.70 (m, 2 H, C₅-H₂), 3.04 (m, 2 H, C₈-H₂), 3.43 (s, 3 H, C₂-SO₂CH₃), 3.62 (s, 3 H, C₄-SO₂CH₃), 7.63 (m, 5 H, arom). Anal. (C₁₈H₁₉N₃O₄S₂) 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-7H-pyrrolo[2,3-d]pyrimidine (25): reaction time 22 h; yield 86%; mp 216 °C. Anal. (C₁₄H₁₃ClN₄) C, H, N.

4-Amino-2-(*o*-chlorophenyl)-5,6-dimethyl-7H-pyrrolo[2,3-d]pyrimidine (26): reaction time 22 h; yield 82%; mp 278 °C. Anal. (C₁₄H₁₃ClN₄) C, H, N.

(*R*)-4-Amino-5,6-dimethyl-2-phenyl-7H-7-(1-phenylethyl)pyrrolo[2,3-d]pyrimidine (31). (*R*)-1-Phenylethylamine was used as the amine component in the synthesis.³² [α]_D²⁰ = –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-7H-7-(1-phenylethyl)pyrrolo[2,3-d]pyrimidine (32). (*S*)-1-Phenylethylamine was used. [α]_D²⁰ = +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-7H-7-(1-phenylethyl)pyrrolo[2,3-d]pyrimidine (33): reaction time 28 h; yield 45%; mp 196 °C. Anal. (C₂₂H₂₁ClN₄) C, H, N.

4-Amino-2-(*m*-chlorophenyl)-5,6-dimethyl-7H-7-(1-phenylethyl)pyrrolo[2,3-d]pyrimidine (34): reaction time 28 h; yield 43%; mp 175 °C. Anal. (C₂₂H₂₁ClN₄) 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 A₁ 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 A₁ binding assay. The concentration of the saturated solution was then calculated by dividing the known IC₅₀ 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 [³H]-(*R*)-N⁶-(phenylisopropyl)adenosine (*R*-PIA) to A₁ adenosine receptors in rat cerebral cortical membranes and inhibition of binding of [³H]-5'-(*N*-ethylcarboxamido)-adenosine (NECA) to A₂ 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 equation³⁴ and a K_d of 1 nM for [3H]-*R*-PIA and 8.5 nM for [3H]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. Galembo, 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

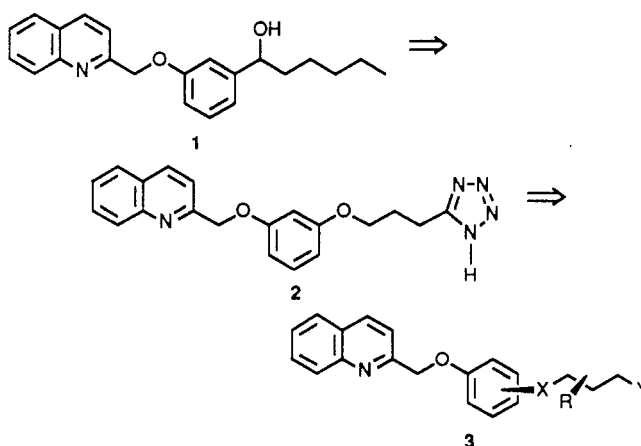
Rorer Central Research, 800 Business Center Drive, Horsham, Pennsylvania 19044. Received October 6, 1989

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 [3H]LTD₄ binding to receptor membrane purified from guinea pig lung ($K_i = 12 \pm 3$ nM) and of the spasmogenic activity of LTC₄, LTD₄, and LTE₄ in guinea pig lung strip. Dosed orally in guinea pigs, this compound blocks LTD₄-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 paper² 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,

Chart I



X = O, Y = CO₂H and 5-tetrazole) or butyl (3: R = H, X = CH₂, Y = CO₂H and 5-tetrazole) spacer group. Sec-

* 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.