on a Rainin Dynamax C-18 (21.4 × 250 mm) column at 10 mL/min with various gradients of acetonitrile/0.1% trifluoroacetic acid. The resulting peptides were analyzed for homogeneity by RP-HPLC and for identity by amino acid analysis with a Beckman 6300 amino acid analyzer and by FAB-MS with a Finnigan TSQ-4C instrument (Table II).

Anticoagulant Assay. Inhibition of plasma clot formation was determined as previously described.[7] Human plasma from a healthy female (fasting for 12 h) was collected in a final EDTA concentration of 0.1%. The plasma was immediately sterilized by filtration through a 0.2-μM filter disk (Gelman). It was then aliquoted as 1 mL/vial and stored at −20 °C. All peptide samples were assessed by using the same plasma preparation. $Hirudin_{45-65}$ was always included[7] as a standard for quality control. Intra- and interassay coefficients of variation were less than 5% and 10%, respectively. Briefly, 50 μL (0.2 pmol) of bovine thrombin (Sigma) was added to the wells of a 96-well microtiter plate (Falcon) containing 50 μL of a solution of the synthetic peptide to be tested (0.25 nmol). After 1 min of agitation and additional incubation for 10 min at 24 °C, 100 μL of diluted human plasma (1:10) in 0.1 M NaCl and 0.012 M sodium phosphate (PBS) was added and vortexed for 20 s. The turbidity of the solution was monitored by an autoreader (EL 309, Bio-Tek Instruments) at 405 nm at 5-min intervals. Typically turbidity at 30 min for various doses of peptide was used to construct dose–response curves for the IC_{50} values. All of the above reagents were diluted in an assay buffer containing 0.12 M sodium chloride, 0.01 M sodium phosphate, 0.01% sodium azide, and 0.1% bovine serum albumin, pH 7.4.

Acknowledgment. We thank Dr. Brad Ackerman and Dr. John Coutant for the FAB-MS analyses and Sue Treadway for preparation of the manuscript.

Registry No. 1, 113274-55-8; 2, 113274-56-9; 3, 113274-57-0; 4, 113274-58-1; 5, 113274-59-2; 6, 113274-60-5; 7, 113274-61-6; 8, 113274-62-7; 9, 113274-63-8; 10, 113274-64-9; 11, 113274-65-0; 12, 113274-66-1; 13, 113274-67-2.

[1,2,4]Triazolo[4,3-*a*]quinoxalin-4-amines: A New Class of A_1 Receptor Selective Adenosine Antagonists

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 October 30, 1987*

Several [1,2,4]triazolo[4,3-*a*]quinoxalines that were reported as antidepressants in the patent literature were found to possess moderate affinity for the adenosine A_1 and A_2 receptors. On the basis of structural parallels with adenine and adenosine, the *N*-cyclopentyl derivative was synthesized and found to have improved affinity and selectivity for the A_1 receptor. In the *N*-cyclopentyl series, affinity was optimal with trifluoromethyl substitution at the 1-position, resulting in a compound (**9**) with 7.3 nM A_1 affinity and 138-fold selectivity for the A_1 receptor.

Adenosine elicits a wide variety of physiological actions via membrane-bound receptors, which have been divided into A_1 and A_2 subtypes.[1] Xanthine derivatives such as caffeine and theophylline have long been known to block adenosine receptors.[2] However, their low potency and nonspecific actions such as phosphodiesterase inhibition and calcium modulation have limited their utility as pharmacological tools.[3] Attempts to improve upon the potency of theophylline have led to the discovery of potent xanthine antagonists such as 8-cyclopentyltheophylline[4] and the xanthine amine congener *N*-(2-aminoethyl)-2-[4-(2,3,6,7-tetrahydro-2,6-dioxo-1,3-dipropyl-1*H*-purine-8-yl)phenoxy]acetamide (XAC).[5] Although the search for more potent and receptor-selective xanthine antagonists continues,[6] there are key features of these molecules (for instance, poor aqueous solubility) that impede their utility as pharmacological tools for in vivo studies and tend to limit their development as potential therapeutic agents. Because of these limitations of the xanthines, there is considerable interest in identifying nonxanthine adenosine antagonists that might not share these drawbacks.

Chronic administration of caffeine and theophylline has been shown to result in down-regulation of β-adrenergic receptors,[7-9] an activity that is considered a useful preclinical predictor of antidepressant activity. In light of these reports, our attention was drawn to two accounts[10,11] describing [1,2,4]triazolo[4,3-*a*]quinoxalin-4-amines with antidepressant-like activity of unknown mechanism. An examination of these molecules (Figure 1) revealed several striking features that suggest similarity to the adenine moeity, including (1) a 6:5 fused ring system, (2) an exocyclic amino function, and (3) the presence of four ring nitrogen atoms arranged in a manner similar to those of

Table I. Physical and Chemical Properties of Novel [1,2,4]Triazolo[4,3-*a*]quinoxalin-4-amines

example	method of preparation[a]	mp, °C[b]	(formula) anal.
4	A	257–260	($C_{14}H_{15}N_5$) C, H, N
5	B	196–198	($C_{15}H_{17}N_5$) C, H, N
6	B	166–168	($C_{16}H_{19}N_5$) C, H, N
7	A	186–188	($C_{17}H_{21}N_5$) C, H, N
8	A	195–197	($C_{18}H_{23}N_5$) C, H, N
9	C	183–184	($C_{15}H_{14}N_5F_3$) C, H, N, F
10	A	190–192	($C_{14}H_{15}N_5$) C, H, N
11	B	215–217	($C_{15}H_{17}N_5$) C, H, N
12	A	217–220	($C_{17}H_{21}N_5$) C, H, N
13	B	170–172	($C_{18}H_{21}N_5$) C, H, N
14	B	172–174	($C_{17}H_{15}N_5$) C, H, N
15	A	236–238	($C_{14}H_{17}N_5O$) C, H, N

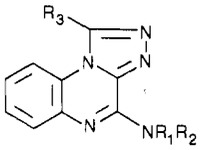
[a] Methods of preparation A–C are described in the Experimental Section. [b] Melting points are uncorrected.

† Department of Chemistry.
‡ Department of Pharmacology.

(1) Van Calker, D.; Müller, M.; Hamprecht, B. *J. Neurochem.* **1979**, *33*, 999.
(2) Sattin, A.; Rall, T. W. *Mol. Pharmacol.* **1970**, *6*, 13.
(3) Rall, T. W. *Pharmacologist* **1982**, *24*, 277.
(4) Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331.
(5) Jacobson, K. A.; Kirk, K. L.; Padgett, W. L.; Daly, J. W. *J. Med. Chem.* **1985**, *28*, 1334.
(6) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Bristol, J. A.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1987**, *335*, 59.
(7) Goldberg, M. R.; Curalto, P. W.; Tung, C. S.; Robertson, D. *Neurosci. Lett.* **1982**, *31*, 47.
(8) Fredholm, B. B.; Jonzon, B.; Lindgren, E. *Acta Physiol. Scand.* **1984**, *122*, 55.
(9) Green, R. M.; Stiles, G. L. *J. Clin. Invest.* **1986**, *77*, 222.

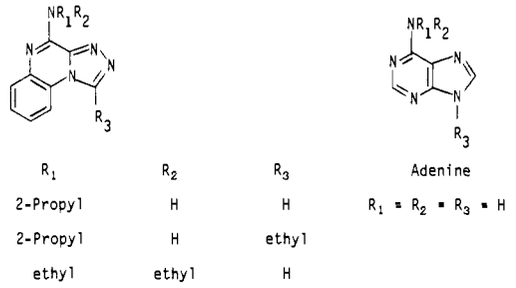
0022-2623/88/1831-1011$01.50/0

Table II. A_1 and A_2 Adenosine Receptor Affinities of [1,2,4]Triazolo[4,3-*a*]quinoxalin-4-amines



example	R_1	R_2	R_3	K_i,[a] nM A_1	K_i,[a] nM A_2	ratio A_2/A_1
1	2-propyl	H	H	2300	7000	3.0
2	2-propyl	H	ethyl	240	4200	17.5
3	ethyl	ethyl	H	8900	18800	2.1
4	cyclopentyl	H	H	181	6700	37
5	cyclopentyl	H	methyl	34	2700	79
6	cyclopentyl	H	ethyl	28	3000	107
7	cyclopentyl	H	propyl	39	1830	47
8	cyclopentyl	H	butyl	55	>10000	>182
9	cyclopentyl	H	CF_3	7.3	1010	138
10	cyclopropyl	H	ethyl	260	4500	17.3
11	cyclobutyl	H	ethyl	58	1710	29
12	cyclohexyl	H	ethyl	80	4000	50
13	exo-2-norbornyl	H	ethyl	44	6100	139
14	phenyl	H	ethyl	630	11800	18.7
15	(*S*)-2-hydroxypropyl	H	ethyl	330	22000	67

[a] A_1 binding of [^{3}H]CHA to rat brain membranes and A_2 binding of [^{3}H]NECA to rat striatal membranes was performed as described.[4] All values are means of three or more independent experiments.



Example	R_1	R_2	R_3	Adenine
1	2-Propyl	H	H	$R_1 = R_2 = R_3 = H$
2	2-Propyl	H	ethyl	
3	ethyl	ethyl	H	

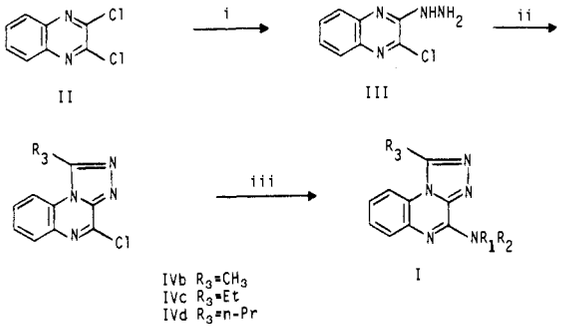
Figure 1.

the adenine moeity. Because of their similarities to adenine, we synthesized several of these compounds and evaluated them in adenosine A_1 and A_2 receptor binding assays (see Table II). These compounds did bind to the adenosine receptors, but IC_{50} values indicated only moderate potency. This paper describes several adenosine antagonists with improved A_1 affinity and selectivity developed from this initial chemical lead.

Chemistry. All compounds (Table I) were prepared according to the literature procedure[10,11] in which commercially available 2,3-dichloroquinoxaline (II) was reacted with hydrazine hydrate in ethanol to give 2-chloro-3-hydrazinoquinoxaline (III). Treatment of III with the appropriate triethyl orthoalkanoate followed by reaction with the appropriate amine in DMF afforded the quinoxaline derivatives in high yields (Scheme I). In the case of the trifluoromethyl derivative (**9**), 2-chloro-3-hydrazinoquinoxaline (III) was reacted with trifluoroacetic acid to afford the quinoxaline derivative (V), which upon chlorination under modified conditions[12] with phosphorous oxychloride, tetraethylammonium chloride, and *N,N*-dimethylaniline in refluxing acetonitrile gave the chloro derivative (VI) in good yield. Compound VI upon treatment with the appropriate amine in DMF afforded the trifluoromethyl derivative **9** (Scheme II).

Receptor Binding and Structure–Activity Relationships. Affinities of the quinoxaline derivatives were

Scheme I[a]

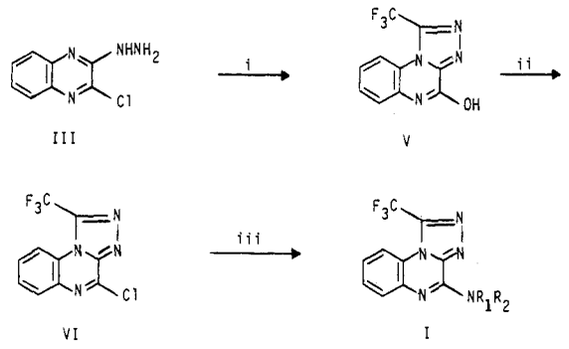


[a] (i) $NH_2NH_2 \cdot H_2O$, EtOH, room temperature; (ii) $R_3C(OEt)_3$, Δ; (iii) HNR_1R_2, DMF, room temperature.

determined in A_1 receptor binding, with [^{3}H]N^6-cyclohexyladenosine in rat whole brain membranes, and in A_2 binding, with [^{3}H]-1-(6-amino-9*H*-purin-9-yl)-1-deoxy-*N*-ethyl-β-D-ribofuranuronamide ([^{3}H]NECA) in rat striatal membranes.[4] Evaluation of the binding affinities of the known agents revealed some interesting features of these molecules. Incorporation of an ethyl group at R_3 (**2**) increased the A_1 affinity and selectivity 10-fold. Dialkyl substitution at the exocyclic amine (**3**) reduced A_1 binding affinity, implying that one of the two amino-group protons was required for high affinity. This particular observation is striking since it is well known that similar structural requirements exist for the N^6-substituted adenosine agonists.[13] This suggested the possibility that structure–activity relationships at this position might bear other similarities to the N^6-substituted adenosines. In light of this conjecture, we synthesized the corresponding cyclopentylquinoxaline derivative (**6**), on the basis of the known potency and selectivity of N^6-cyclopentyladenosine for the A_1 receptor.[14] As anticipated, compound **6** showed greatly improved A_1 affinity and selectivity compared to example 2 (Table II). With **6** as the prototype, several further

(10) Sarges, R. U.S. Pat. 4547501, 1985.
(11) Sarges, R. U.S. Pat. 4495187, 1985.
(12) Robins, M. J.; Uznanski, B. *Can. J. Chem.* **1981**, *59*, 2601.
(13) Olsson, R. A.; Khouri, E. M.; Bedynek, J. L., Jr.; McLean, J. *Circ. Res.* **1979**, *45*, 468.
(14) Moos, W. H.; Szotek, D. S.; Bruns, R. F. *J. Med. Chem.* **1985**, *28*, 1383.

Scheme II[a]



[a] (i) CF_3CO_2H, Δ; (ii) $POCl_3$, *N,N*-dimethylaniline, $Et_4N^+Cl^-$, CH_3CN, Δ; (iii) HNR_1R_2, DMF, room temperature.

modifications were made to explore structure–activity relationships at R_1 and R_3. Alteration of the alkyl chain length at R_3 reduced A_1 binding affinity compared to the R_3-ethyl derivative **6**. However, the corresponding trifluoromethyl compound **9** showed a fivefold increase in A_1 receptor affinity and a twofold increase in selectivity when compared to the methyl analogue **5**. Since the methyl and trifluoromethyl groups have similar steric bulk, the improved affinity of the trifluoromethyl analogue is undoubtedly due to its greater electron-withdrawing activity. A likely explanation is that due to the inductive effect, the proton on the amine function is relatively more acidic in the case of the trifluoromethyl analogue and hence available for hydrogen bonding at the receptor site to a greater extent.

On varying the size of the cycloalkyl moiety (Examples **6** and **10–12**), it is apparent that the cyclopentyl ring is optimal for both receptor affinity and selectivity. Furthermore, the *exo*-2-norbornyl compound **13** also showed significant A_1 affinity and a high degree of selectivity for the A_1 receptor; this is in agreement with the known high affinity of N^6-(*exo*-2-norbornyl)adenosine.[15] However, the parallels between the exocyclic amino group in the present series and the N^6 of adenosine are not unlimited. Although (*S*)-2-hydroxypropyl substitution at N^6 of adenosine results in an adenosine agonist with greater A_1 selectivity than CPA,[16] the corresponding antagonist **15** is less A_1 selective than **6**. It may be that the exact orientations of the amino groups relative to the receptor differ in the two series. Interestingly, similar parallels have also been reported between the N^6 of adenosine and the C-8 of theophylline.[4,17] As in the N^6-modified adenosines and the present series, cyclopentyl substitution at the 8-position of theophylline results in optimal A_1 affinity and selectivity.[4,17,18]

Aqueous solubility is an important prerequisite for in vivo activity. The solubility of **9** in physiological buffer at pH 7.7 is 4.1 μM.[19] The ratio of solubility to A_1 affinity of 560 for this compound, although less than the ratio of 5400 seen with theophylline,[19] should be sufficient to provide useful activity in vivo.

Shortly after the conclusion of the studies described in the present paper, the potent adenosine antagonist CGS 15943 [9-chloro-2-(2-furanyl)[1,2,4]triazolo[1,5-c]quinazolin-5(6*H*)-imine] was described.[20] Although the CGS compound bears some structural resemblance to the compounds in the present study, the former is A_2-selective whereas the present compounds exhibit a preference for the A_1 receptor.

In conclusion, we have demonstrated that [1,2,4]triazolo[4,3-*a*]quinoxalin-4-amines are potentially interesting nonxanthine adenosine antagonists. Additionally, *N*-cyclopentyl-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (**9**) represents the first example of a nonxanthine antagonist with high A_1 receptor potency and selectivity, suggesting potential utility as a pharmacological tool.

Experimental Section

Melting points are uncorrected. Analytical thin-layer chromatography (TLC) was done with precoated glass plates (EM Science silica gel 60 F-254). Mass spectra were recorded on a Finnegan 4500 mass spectrometer with an INCO5 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 Warner-Lambert/Parke-Davis.

General Method for the Preparation of Compounds of Scheme I. Procedure A. *N*-Cyclopentyl-1-propyl[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (7). A reaction mixture of 5 g of 2-chloro-3-hydrazinoquinoxaline[10] in 15 mL of triethyl orthobutyrate was heated at 100 °C for 3 h. Upon cooling, the precipitated solid was filtered, washed with cyclohexane, and dried, yielding 4 g (63.2 %) of 4-chloro-1-propyl[1,2,4]triazolo[4,3-*a*]quinoxaline (IVd): mp 172–175 °C [lit.[10] mp 173–175 °C]; mass spectrum, *m/e* 246.

A reaction mixture of IVd (1.0 g, 0.004 mol) and cyclopentanamine (1.0 g, 0.012 mol) in 15 mL of DMF was stirred at room temperature for 20 h. The precipitated solid was filtered, washed with ethanol, and dried, affording 0.92 g (82%) of *N*-cyclopentyl-1-propyl[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine: mp 186–188 °C; mass spectrum, *m/e* 295 (M^+). Anal. ($C_{17}H_{21}N_5$) C, H, N.

Procedure B. *N*-Cyclopentyl-1-methyl[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (5). A reaction mixture of 5 g of 2-chloro-3-hydrazinoquinoxaline (III) in 50 mL of triethyl orthoacetate was heated at 100 °C for 3 h. Upon cooling, the precipitated solid was filtered, washed with cyclohexane, and dried, affording 4.27 g (76%) of 4-chloro-1-methyl[1,2,4]triazolo[4,3-*a*]quinoxaline (IVb): mp 215–218 °C (lit.[10] mp 215–222 °C); mass spectrum, *m/e* 218 (M^+).

A reaction mixture of IVb (1.0 g, 0.0045 mol) and cyclopentanamine (1.17 g, 0.013 mol) in 15 mL of DMF was stirred at room temperature for 20 h. The solution was poured over ice–water, and the precipitated solid was filtered, washed with water, and dried, affording 0.9 g (74%) of *N*-cyclopentyl-1-methyl[1,2,4]triazolo[4,3-*a*]quinoxaline: mp 196–198 °C; mass spectrum, *m/e* 267 (M^+). Anal. ($C_{15}H_{17}N_5$) C, H, N.

General Method for the Preparation of Compounds of Scheme II. Procedure C. *N*-Cyclopentyl-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (9). a. 1-(Trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ol (V). 2-Chloro-3-hydrazinoquinoxaline (III) (3.8 g, 0.02 mol) was added to 15 mL of ice-cold trifluoroacetic acid. The reaction was then heated at 100 °C for 3 h and poured over ice–water. The precipitated solid was filtered, washed with water, and dried, affording 3.7 g of 1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ol, mp >325 °C.

b. 4-Chloro-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxaline (VI). To a suspension of 1-(trifluoromethyl)-[1,2,4]triazolo[4,3-*a*]quinoxalin-4-ol (V) (2.5 g, 0.01 mol) in 60 mL of acetonitrile were added $Et_4N^+Cl^-$ (2.4 g) followed by *N,N*-dimethylaniline (1.7 mL) and $POCl_3$ (3.75 mL). The reaction

(15) Paton, D. M.; Olsson, R. A.; Thompson, R. T. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **1986**, *333*, 313.
(16) Hamilton, H. W.; Taylor, M. D.; Steffen, R. P.; Haleen, S. J.; Bruns, R. F. *Life Sci.* **1987**, *41*, 2295.
(17) Ukena, D.; Jacobson, K. A.; Padgett, W. L.; Ayala, C.; Shamim, M. T.; Kirk, K. L.; Olsson, R. A.; Daly, J. W. *FEBS Lett.* **1986**, *209*, 122.
(18) Martinson, E. A.; Johnson, R. A.; Wells, J. N. *Mol. Pharmacol.* **1987**, *31*, 247.
(19) Bruns, R. F.; Fergus, J. H., unpublished results.
(20) Williams, M.; Francis, J.; Ghai, G.; Braunwalder, A.; Psychoyos, S.; Stone, G. A.; Cash, W. D. *J. Pharmacol. Exp. Ther.* **1987**, *241*, 415.

mixture was refluxed for 20 h. Volatiles were removed under reduced pressure; the residue was dissolved in $CHCl_3$ (100 mL) and added to ice-water. The layers were separated, and the aqueous layer was extracted with chloroform (2 × 200 mL). The combined extract was washed with 5% $NaHCO_3$ (2 × 120 mL) followed by brine (2 × 50 mL). It was dried over $MgSO_4$ and filtered, and the volatiles were removed. The residue was crystallized from chloroform–hexane, affording 1.78 g of 4-chloro-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxaline (**VI**): mass spectrum, *m/e* 272 (M^+).

c. *N*-Cyclopentyl-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine (9). A solution of 4-chloro-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxaline (**VI**) (0.6 g, 0.002 mol) and cyclopentanamine (1.0 g, 0.011 mol) in 10 mL of DMF was stirred at room temperature for 20 h and then added to ice–water. Precipitated solid was filtered, dissolved in 100 mL of chloroform, washed with brine (1 × 25 mL), dried over $MgSO_4$, and filtered, and the volatiles were removed. The residue was dissolved in ether–2-propanol and diluted with hexane. Solid material obtained was filtered and dried, affording 0.42 g (60%) of *N*-cyclopentyl-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxalin-4-amine: mp 183–184 °C; mass spectrum, *m/e* 321 (M^+). Anal. ($C_{15}H_{14}N_5F_3$) C, H, N, F.

Receptor Binding. A_1 binding was carried out with [^{3}H]CHA in rat whole brain membranes,[4] and A_2 binding was carried out with [^{3}H]NECA in the presence of 50 nM unlabeled N^6-cyclopentyladenosine in rat striatal membranes.[4]

Acknowledgment. We thank James Fergus and Gina Lu for providing biochemical screening results.

Registry No. **I** (R_1 = 2-Pr, R^2 = R_3 = H), 91895-47-5; **I** (R_1 = 2-Pr, R^2 = H, R_3 = Et), 91895-54-4; **I** (R_1 = Et, R_2 = Et, R_3 = H), 91895-45-3; **I** (R_1 = Cyclopentyl, R_2 = R_3 = H), 113181-11-6; **I** (R_1 = cyclopentyl, R_2 = H, R_3 = CH_3), 113181-12-7; **I** (R_1 = cyclopentyl, R_2 = H, R_3 = Et), 113181-13-8; **I** (R_1 = cyclopentyl, R_2 = H, R_3 = Pr), 113181-14-9; **I** (R_1 = cyclopentyl, R_2 = H, R_3 = $H_3C(CH_2)_3$, 113181-15-0; **I** (R_1 = cyclopentyl, R_2 = H, R_3 = CF_3), 113181-16-1; **I** (R_1 = cyclopropyl, R_2 = H, R_3 = Et), 113181-17-2; **I** (R_1 = cyclobutyl, R_2 = H, R_3 = Et), 113181-18-3; **I** (R_1 = cyclohexyl, R_2 = H, R_3 = Et), 113181-19-4; **I** (R_1 = *exo*-2-norbornyl, R_2 = H, R_3 = Et), 113181-20-7; **I** (R_1 = phenyl, R_2 = H, R_3 = Et), 113181-21-8; **I** (R_1 = (*S*)-$H_3CCH(OH)CH_2$, R_2 = H, R_3 = Et), 113181-22-9; **III**, 91895-39-5; **IVc**, 91895-40-8; **IVd**, 91895-41-9; **IV** (R_3 = H), 62603-54-7; **IV** (R_3 = $H_3D(CH_2)_3$), 113181-23-0; **V**, 91895-67-9; **VI**, 91895-68-0; $C_6H_5NH_2$, 62-53-3; (*S*)-$H_3CCH(OH)CH_2NH_2$, 2799-17-9; $CH(OEt)_3$, 122-51-0; $H_3C(OEt)_3$, 78-39-7; $Et(OEt)_3$, 115-80-0; $Pr(OEt)_3$, 24964-76-9; $PrCH_2(OEt)_3$, 919-29-9; cyclopentylamine, 1003-03-8; cyclopropylamine, 765-30-0; cyclobutylamine, 2516-34-9; cyclohexylamine, 108-91-8; *exo*-2-aminonorbornane, 7242-92-4; adenosine, 58-61-7.

Structure–Activity Profile of a Series of Novel Triazoloquinazoline Adenosine Antagonists

John E. Francis,* William D. Cash, Stacy Psychoyos, Geetha Ghai, Paul Wenk, Robert C. Friedmann, Charlotte Atkins, Vivian Warren, Patricia Furness, James L. Hyun, George A. Stone, Mahesh Desai, and Michael Williams

Research Department, Pharmaceuticals Division, CIBA-GEIGY Corporation, Summit, New Jersey 07901.
Received October 13, 1987

During a search for benzodiazepine receptor modulators, a highly potent adenosine antagonist (CGS 15943) was discovered. The compound was defined as a resonance-stabilized hybrid of the canonical structures 9-chloro-2-(2-furyl)[1,2,4]triazolo[1,5-c]quinazolin-5-amine (**2a**) and 9-chloro-2-(2-furyl)-5,6-dihydro[1,2,4]triazolo[1,5-*c*]-quinazolin-5-imine (**2b**). Spectroscopic evidence and chemical reactivity in polar media favor the amine form **2a** as the major contributor of the two canonical structures. The synthesis of **2** and some of its analogues and the structure–activity relationships in four biological test systems are described. Replacement of the 9-chloro group by hydrogen, hydroxyl, or methoxyl gave compounds with comparable binding potency at the A_1 and A_2 receptors but much less activity as antagonists of 2-chloroadenosine in guinea pig tracheal strips. Alkylation of the 5-amino group caused, in general, a loss of binding activity, particularly at the A_2 receptor, as well as complete loss of activity in the tracheal model. Modification of the 2-furyl group caused a pronounced loss of activity in all of the test systems.

The discovery of CGS 8216 (**1**) in these laboratories as a potent benzodiazepine receptor antagonist[1,2] led to the screening of other tricyclic heterocyclic structures for similar activity coupled with a search for an understanding of the mechanism of action of this novel compound. In 1979, Phillis and co-workers reported that theophylline, an adenosine antagonist,[3] antagonized the depressant action of flunitrazepam on cerebral cortical neurons in rats.[4] Other investigators had reported that theophylline and other xanthines block diazepam binding sites in brain tissue. Furthermore, inosine, an adenosine metabolite, interacted with brain benzodiazepine receptors.[5,6] These observations led to the suggestion that benzodiazepines and adenosine depress central neurons by acting at the same receptor. A comparison of **1** and theophylline in the adenosine-stimulated adenylate cyclase system present in guinea pig synaptoneurosomes[7] revealed that **1** indeed blocked adenosine activation more potently than theophylline.[2] Accordingly, this test system was used in screening other chemical structures prepared as potential anxiomodulators. Subsequently, the triazoloquinazoline structure **2** (CGS 15943) was discovered to be more potent than any adenosine antagonist reported at that time (January, 1983). It was approximately 500 times as active as theophylline and 250 times as potent as **1**.

(1) Yokoyama, N.; Ritter, B.; Neubert, A. *J. Med. Chem.* **1982**, *25*, 337.
(2) Czernik, A. J.; Petrack, B.; Kalinsky, H. J.; Psychoyos, S.; Cash, W. D.; Tsai, C.; Rinehart, R. K.; Granat, F. R.; Lovell, R. A.; Brundish, D. E.; Wade, R. *Life Sci.* **1982**, *30*, 363.
(3) Nichols, R. E.; Walaszek, E. J. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1963**, *22*, 308.
(4) Phillis, J. W.; Edstrom, J. P.; Ellis, S. W.; Kirkpatrick, J. R. *Can. J. Physiol. Pharmacol.* **1979**, *57*, 917.
(5) Asano, T.; Spector, S. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 977.
(6) Marango, P. J.; Paul, S. M.; Parma, A. M.; Goodwin, F. K.; Syapin, P.; Skolnick, P. *Life Sci.* **1979**, *24*, 851.
(7) Psychoyos, S.; Dove, J.; Strawbridge, B.; Nusynowitz, I. *J. Neurochem.* **1982**, *38*(5), 1437.

0022-2623/88/1831-1014$01.50/0