4-Amino[1,2,4]triazolo[4,3-a]quinoxalines. A Novel Class of Potent Adenosine Receptor Antagonists and Potential Rapid-Onset Antidepressants

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A series of 4-amino[1,2,4]triazolo[4,3-a]quinoxalines has been prepared. Many compounds from this class reduce immobility in Porsolt's behavioral despair model in rats upon acute administration and may therefore have therapeutic potential as novel and rapid acting antidepressant agents. Optimal activity in this test is associated with hydrogen, CF_3, or small alkyl groups in the 1-position, with NH_2, NH-acetyl, or amines substituted with small alkyl groups in the 4-position, and with hydrogen or 8-halogen substituents in the aromatic ring. Furthermore, many of these 4-amino[1,2,4]triazolo[4,3-a]quinoxalines bind avidly, and in some cases very selectively, to adenosine A_1 and A_2 receptors. A_1 affinity of these compounds was measured by their inhibition of tritiated CHA (N^6-cyclohexyladenosine) binding in rat cerebral cortex membranes and A_2 affinity by their inhibition of tritiated NECA (5'-(*N*-ethylcarbamoyl)adenosine) binding to rat striatal homogenate in the presence of cold N^6-cyclopentyladenosine. Structure-activity relationship (SAR) studies show that best A_1 affinity is associated with ethyl, CF_3, or C_2F_5 in the 1-position, NH-^{i}Pr or NH-cycloalkyl in the 4-position, and with an 8-chloro substituent. Affinity at the A_2 receptor is mostly dependent on the presence of an NH_2 group in the 4-position and is enhanced by phenyl, CF_3, or ethyl in the 1-position. The most selective A_1 ligand by a factor of >3000 is **121** (CP-68,247; 8-chloro-4-(cyclohexylamino)-1-(trifluoromethyl)[1,2,4]triazolo[4,3-a]quinoxaline) with an IC_{50} of 28 nM at the A_1 receptor. The most potent A_2 ligand is **128** (CP-66,713; 4-amino-8-chloro-1-phenyl[1,2,4]triazolo[4,3-a]quinoxaline) with an IC_{50} of 21 nM at the A_2 receptor and a 13-fold selectivity for this receptor. Representatives from this series appear to act as antagonists at both A_1 and A_2 receptors since they antagonize the inhibiting action of CHA on norepinephrine-stimulated cAMP formation in fat cells and they decrease cAMP accumulation induced by adenosine in limbic forebrain slices. Thus certain members of this 4-amino[1,2,4]triazolo[4,3-a]quinoxaline series are among the most potent and A_1 or A_2 selective non-xanthine adenosine antagonists known.

In light of the high suicide potential associated with depression, the discovery of rapidly acting antidepressants is important. Classical antidepressants, such as norepinephrine uptake blockers, require multiple dosing—up to several weeks—in order to manifest therapeutic effects in man. By contrast, they exhibit pharmacological effects in animals, e.g. biochemical effects such as amine uptake blockade, or drug interactions such as reserpine hypothermia reversal, after acute dosing. This discrepancy between the clinical and laboratory time courses has led in recent years to efforts to develop pharmacological models (e.g., β-adrenoceptor desensitization)[1] as well as behavioral models which more closely mimic the time course of antidepressant effects seen in the clinic. Test systems which require multiple dosing might more closely reflect the therapeutic mechanism of action of antidepressant drugs, and these screens might serve to identify novel and possibly more rapidly acting antidepressants.

One model in which classical antidepressants are active only after multiple administrations is the behavioral despair screen in rats developed by Porsolt.[2] In this test rats are forced to swim in a water-filled container from which there is no escape. Over a 15-min training session the rats assume an immobile posture and remain floating in the tank until removed. Repeated, but not acute, treatment with classical antidepressants reduces this immobility period in subsequent test swims. It is assumed that this reduction in immobility is a reflection of decreased behavioral despair as a result of antidepressant treatment.

We discovered during an empirical screening effort that 4-(diethylamino)[1,2,4]triazolo[4,3-a]quinoxaline[3] (**33**; CP-41,475) was effective in reducing immobility in the rat swim test after a single dose. This result suggested that compounds from this class might therefore be rapid-onset antidepressants, and it prompted us to explore systematically the effect of substituents in this ring system on activity in this test. Since biochemical studies indicated early on that certain 4-amino[1,2,4]triazolo[4,3-a]quinoxalines also displayed affinity for adenosine receptors and inhibitory effects on phosphodiesterase, we explored the structure-activity relationship (SAR) of these endpoints as well. Furthermore, since the psychomotor stimulant caffeine has biochemical properties similar to compounds in this series and is also active in the swim test, we carried out behavioral tests to evaluate the contribution of stimulant properties to activity in the swim test.

Chemistry

The [1,2,4]triazolo[4,3-a]quinoxaline derivatives were prepared as shown in Scheme I. The 2,3-dichloroquinoxalines I, which were in some cases obtained from the corresponding substituted *o*-phenylenediamines and diethyl oxalate followed by treatment with $POCl_3$, were treated with hydrazine. With monosubstituted quinoxalines (I, Z = F, Cl, or OMe) this resulted predominantly in the formation of 6-substituted 2-chloro-3-hydrazinoquinoxalines II. Ring closure to III was achieved for X = H, alkyl, methoxy, or aryl by treatment of II with the corresponding ortho esters. Treatment of II with CF_3CO_2H or $C_2F_5CO_2H$ gave VI (X = CF_3 or C_2F_5), which were then converted with $POCl_3$ to III (X = CF_3 or C_2F_5). Reaction of III with NH_3 or various primary or secondary amines gave the desired 8-substituted 4-amino derivatives IV (Y = NR_1R_2). The derivatives of IV with X = OH were obtained by treatment of IV (X = OMe) with HBr in AcOH. For the preparation of the 7-substituted derivatives X the 2,3-dichloroquinoxaline derivatives I were treated with methoxide to block the 3-position by forming V. Subsequent reaction of V with hydrazine produced VII, which on treatment with ortho esters gave VIII. Exposure of VIII to hot HCl in methanol-water selectively cleaved the 4-methoxy group to give the 4-hydroxy derivative, which was in turn converted with $POCl_3$ to IX and then with appropriate amines to the desired 7-substituted 4-amino derivatives X (Y = NR_1R_2). The location of the aromatic substituents was ascertained by NMR analyses of IV or X (X = H, Y = NR_1R_2) involving NOE experiments.[4] The

(1) Sulser, F.; Vetulani, J.; Mobley, P. L. *Biochem. Pharmacol.* **1978**, *27*, 257.
(2) Porsolt, R. D.; Anton, G.; Blavet, N.; Jalfre, M. *Eur. J. Pharmacol.* **1978**, *47*, 379.
(3) This compound was initially prepared by Dr. S. B. Kadin of Pfizer Central Research for antiallergy testing.

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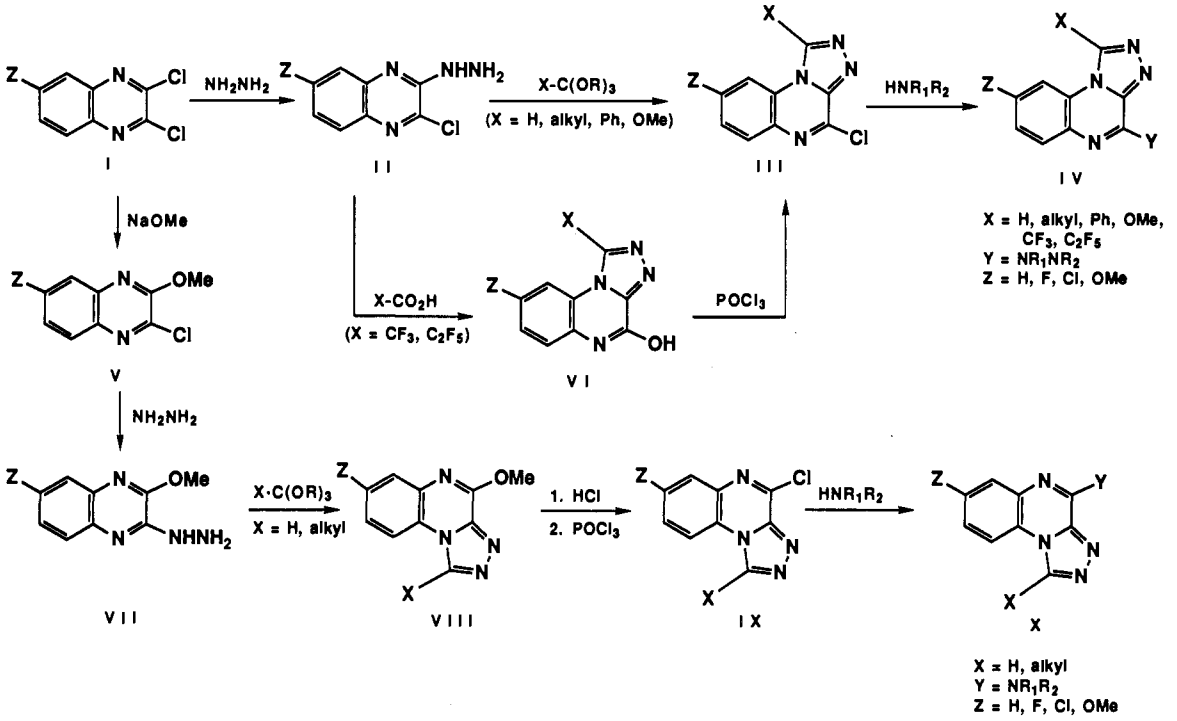
Scheme I



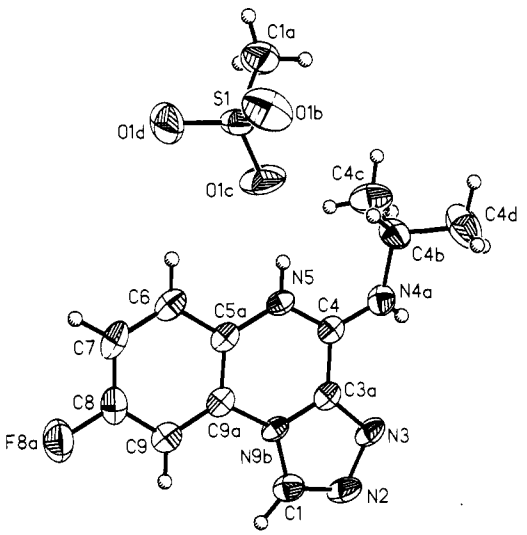


Figure 1. The X-ray structure of compound **21**.

structural assignment of compound **21** was also confirmed by an X-ray analysis,[5] shown in Figure 1.

Biological Results and Discussion

The compounds prepared in this SAR investigation are listed in Tables I–IV and are arranged according to modifications of the substituent X in position 1 which ranges from hydrogen to lower alkyl, trifluoromethyl, pentafluoroethyl, phenyl, methoxy, and hydroxy. Modification of substituent Y in the 4-position is explored within such subgroups and ranges from NH_2 to acylated and mono- or dialkylated amines. Substituents Z in the 6-, 7-, and 8-position vary among hydrogen, halogen, and methoxy. Inspection of these tables shows that acute activity in the Porsolt behavioral despair test[2] is associated with compounds containing hydrogen, small alkyl groups, or CF_3 in the 1-position, provided the amine function in the 4-position is NH_2, NH-acetyl, or nitrogen substituted with small alkyl groups. Among substituents in the aromatic ring, hydrogen or halogen, especially in the 8-position, appear to be beneficial for Porsolt activity. Particularly potent and effective agents are compounds **21**, **22**, **54**, **59**, **61**, **70**, **78**, and **111**.

Since stimulants such as amphetamine or caffeine are also active in the Porsolt test after single dose administration (Table V), it became important to determine if the active 4-amino[1,2,4]triazolo[4,3-*a*]quinoxalines are merely stimulant false positives. Certain Porsolt active members of this series, e.g. **70** (CP-57,103), indeed show mild stimulant activity in rats. However, as discussed in more detail below, the magnitude of this stimulant effect is clearly less than that seen with amphetamine, and compounds such as **70** do not have biochemical properties of amphetamine, such as dopamine reuptake inhibition.[6] On the other hand, many 4-amino[1,2,4]triazolo[4,3-*a*]quinoxalines share two key biological properties with methyl xanthines such as caffeine: inhibition of calcium-dependent and calcium-independent phosphodiesterase from rat brain, and binding to adenosine receptors, with affinity for A_1 receptors measured by inhibition of [^{3}H]-N^6-cyclohexyladenosine (CHA) binding to rat cerebral cortex homogenate, and affinity for A_2 receptors measured by inhibition of [^{3}H]-5′-(*N*-ethylcarbamoyl)adenosine (NECA) binding to rat striatal homogenate in the presence of excess cold N^6-cyclopentyladenosine. These findings prompted us to investigate SAR with regard to these biochemical parameters more fully in this series and to determine whether or not Porsolt SAR paralleled these biochemical properties.

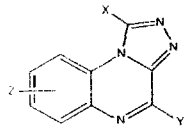
Inspection of the tables shows that A_1 binding activity is widely distributed among these compounds and particularly strong in compounds with CF_3, C_2F_5, or ethyl in the 1-position and with secondary amines such as NH-^{i}Pr or NH-cycloalkyl in the 4-position; with these derivatives

(4) We are grateful to Dr. E. B. Whipple of Pfizer Central Research for these determinations.

(5) The X-ray analysis of **21** was carried out by Dr. J. Bordner and Dr. L. R. Corwin of Pfizer Central Research.

(6) Koe, B. K.; Seymour, P. A.; Browne, R. G.; Sarges, R., manuscript in preparation.

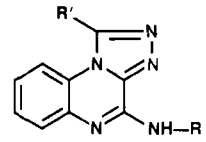
Table I



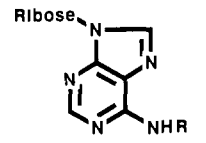
no.	X	Y	Z	mp, °C (recryst solvent)	formula[a]	method	% yield	rat porsolt: MED,[b] mg/kg po	A_1 binding (CHA):[c] IC_{50}, μM	A_2 binding (NECA):[d] IC_{50}, μM	PDEI:[e] IC_{50}, μM $Ca^{2+}D$	PDEI:[e] IC_{50}, μM $Ca^{2+}I$
1	H	NH_2	H	>300 (DMF)	$C_9H_7N_5$	H1	18	3.2–10	4.6 ± 0.7	0.40 ± 0.01		
2	H	NH_2	7-Cl	279–282 dec (EtOH)	$C_9H_6ClN_5 \cdot CH_4O_3S$	H1	48	≤32	1.2	0.21		
3	H	NH_2	8-Cl	>300 (DMF)	$C_9H_6ClN_5$	H1	28	10	3.4	≥1 (43%)		
4	H	NH_2	7,8-Cl_2	>300 (DMF)	$C_9H_5Cl_2N_5$	H1	54	>32				
5	H	NH_2	7-F	246–248 dec (EtOH)	$C_9H_6FN_5 \cdot CH_4O_3S$	H1	25	≤32	6.1	0.56		
6	H	NH_2	8-F	176–178 dec (CH_3OH-Et_2O)	$C_9H_6FN_5 \cdot CH_4O_3S$	H1	19	≤32	2.9 ± 0.3	1.0	>10	4
7	H	NH_2	7-OMe	262–264 dec (EtOH)	$C_{10}H_9N_5O \cdot CH_4O_3S$	H1	38	3.2–32	1.4	0.1	≫10	>10
8	H	NHAc	H	269–272 dec	$C_{11}H_9N_5O \cdot {}^1/_4H_2O$	I1	29	≤32				
9	H	NHAc	7-F	290–292 dec (CH_3OH-$CHCl_3$)	$C_{11}H_8FN_5O \cdot {}^1/_2H_2O$	I1	52	≤32				
10	H	NHAc	8-F	240–240 ($CHCl_3$-Et_2O)	$C_{11}H_8FN_5O \cdot {}^1/_4CHCl_3$	I1	9	≤32				
11	H	NHAc	7-OMe	251–254 ($CHCl_3$)	$C_{12}H_{11}N_5O_2 \cdot {}^1/_4H_2O$	I1	44	≤32				
12	H	NHMe	H	>300	$C_{10}H_9N_5$	H1	69	32	100			
13	H	NHEt	H	254–256 (CH_3OH)	$C_{11}H_{11}N_5$	H1	32	3.2	29	110		
14	H	NHEt	7-F	216–218 dec (EtOH)	$C_{11}H_{10}FN_5 \cdot CH_4O_3S$	H1	63	32	11	26		
15	H	NHEt	8-F	239–242 ($CHCl_3$)	$C_{11}H_{10}FN_5$	H1	37	3.2	3.2 ± 0.1	17		
16	H	NHEt	7,8-F_2	208–211 ($CHCl_3$-Et_2O)	$C_{11}H_9F_2N_5$	H1	26	3.2–32	4.7			
17	H	NHiPr	H	133–135 (IPE)	$C_{12}H_{13}N_5 \cdot {}^1/_3H_2O$	H2	53	3.2–10	4.1	17		
18	H	NHiPr	8-Cl	177–181 ($CHCl_3$-Et_2O)	$C_{12}H_{12}ClN_5$	H2	46	3.2–10	0.37	3.3		
19	H	NHiPr	7,8-Cl_2	218–220 ($CHCl_3$-Et_2O)	$C_{12}H_{11}Cl_2N_5$	H2	39	3.2–10	1.0			
20	H	NHiPr	7-F	214–216 dec (EtOH)	$C_{12}H_{12}FN_5 \cdot CH_4O_3S$	H2	43	>32	4.5	13	>100	6
21	H	NHiPr	8-F	236–237 dec (EtOH)	$C_{12}H_{12}FN_5 \cdot CH_4O_3S$	H2	54	≤3.2	0.68 ± 0.05	6.9	>100	4.5
22	H	NHiPr	7,8-F_2	218–221 ($CHCl_3$-Et_2O)	$C_{12}H_{11}F_2N_5$	H2	21	≤3.2	1.9			
23	H	NHiPr	7-OMe	171–173 ($CHCl_3$-Et_2O)	$C_{13}H_{15}N_5O \cdot {}^1/_4H_2O$	H2	40	>32				
24	H	NHtBu	H	178–180 (cyclohexane)	$C_{13}H_{15}N_5$	H2	36	>32	19			
25	H	NHCH(Me)CH_2OH	8-F	193–194 dec (EtOAc)	$C_{12}H_{12}FN_5O$	H2	47	>32				
26	H	NAc_2	H	211–214 ($CHCl_3$-Et_2O)	$C_{13}H_{11}N_5O_2$	J	14	≤32				
27	H	NAc_2	8-Cl	208–210 ($CHCl_3$-Et_2O)	$C_{13}H_{10}ClN_5O_2$	J	22	≤32				
28	H	NiPrAc	8-Cl	148–151 ($CHCl_3$-Et_2O)	$C_{14}H_{14}ClN_5O$	I1	32	≤32				
29	H	NiPrAc	7,8-Cl_2	207–210 ($CHCl_3$-Et_2O)	$C_{14}H_{13}Cl_2N_5O$	I1	54	>32				
30	H		H	238–241 (EtOAc-Hex)	$C_{13}H_{11}N_5O \cdot {}^1/_2H_2O$	I2	32	>32	>100			
31	H	NMe_2	H	184–186	$C_{11}H_{11}N_5$	H1	44	≤32	59			
32	H	NMe_2	8-F	217–219 (EtOAc)	$C_{11}H_{10}FN_5$	H1	4	≤32				
33	H	NEt_2	H	117–119 (IPO)	$C_{13}H_{15}N_5$	H2	75	10	27 ± 4	42 ± 5	1.2	3
34	H	NEt_2	7,8-Br_2	199–201 (Et_2O)	$C_{13}H_{13}Br_2N_5 \cdot H_2O$	L	13	>32				
35	H	NEt_2	7-Cl	205–207 (EtOH)	$C_{13}H_{14}ClN_5 \cdot CH_4O_3S$	H4	28	32				
36	H	NEt_2	8-F	151–153 ($CHCl_3$-Et_2O)	$C_{13}H_{14}FN_5$	H2	55	3.2–10	>10			
37	H	NEt_2	7,8-F_2	220–223 (EtOH)	$C_{13}H_{13}F_2N_5 \cdot CH_4O_3S$	H2	26	3.2–32	5.0			
38	H	NEt_2	8-OMe	124–126 (cyclohexane)	$C_{14}H_{17}N_5O$	H2	56	>32				
39	H	NPr_2	H	240–242 (cyclohexane)	$C_{15}H_{19}N_5$	H2	41	>32	≥100 (43%)			
40	H		H	208–210 (EtOH)	$C_{13}H_{13}N_5$	H2	69	>32	50			
41	H		H	206–208 (EtOH)	$C_{14}H_{15}N_5$	H2	72	>32	100			
42	H		H	219–221 (EtOH)	$C_{13}H_{13}N_5O$	H2	28	>32				

43	H		H	245–247 (IPO)	$C_{14}H_{16}N_6$	H3	52	>32	>100	
44	H		H	160–162 (DMF)	$C_{13}H_{14}N_6$	H3	65	>32	>10	3 7
45	H		8-Cl	253–256 (DMF)	$C_{13}H_{13}ClN_6 \cdot {}^1/_2H_2O$	H3	46	>32		

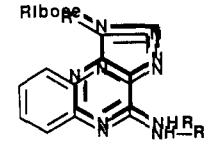
[a] All compounds were analyzed for C, H, N. [b] Minimum effective dose. When a dose range is given, the lower dose showed no statistically significant Porsolt activity, the higher one did. The symbol ≤ means that the compound was active at this dose and not tested at a lower dose to determine the MED. The symbol > means that the compound did not show statistically significant activity at this dose. [c] Inhibition of [^{3}H]-N^6-cyclohexyl adenosine binding in rat cerebral cortex homogenates. [d] Inhibition of [^{3}H]-5′-(*N*-ethylcarbamoyl)adenosine to rat striatal homogenate in the presence of excess cold N^6-cyclopentyl adenosine. [e] Inhibition of Ca^{2+} dependent (Ca^{2+}D) and Ca^{2+} independent (Ca^{2+}I) phosphodiesterase from rat brain homogenate.



4-Amino-[1,2,4]triazolo[4,3-a]quinoxalines



Adenosine Derivatives



Overlap

Figure 2. Proposed overlap between adenosine and the 1-amino[1,2,4]triazolo[4,3-*a*]quinoxalines.

halogen substitution in the ring, particularly 8-chloro, enhances potency. The most potent A_1 ligand is **120** with an IC_{50} of 5.5 nM, being more than 20 000 times more active than caffeine (IC_{50} 117 000 nM). Affinity at the A_2 receptor is clearly dependent on the presence of an NH_2 group in the 4-position, while a phenyl, ethyl, or CF_3 group in the 1-position greatly enhances potency; 7- and/or 8-chloro substituents or the 7-methoxy group amplify the potency. The most potent A_2 ligands are **54**, **55**, **56**, **58**, **128**, and **134** with IC_{50} values between 21 and 36 nM.

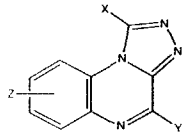
Biochemical studies suggest that compounds **53** and **70** act as antagonists at A_1 as well as at A_2 adenosine receptors, since they antagonize the inhibitory action of CHA on norepinephrine stimulated cAMP formation in fat cells and they decrease cAMP accumulation induced by adenosine in limbic forebrain slices.[6] It is conceivable that most, if not all, compounds from this series act as adenosine antagonists.

It is possible that the 4-amino[1,2,4]triazolo[4,3-*a*]-quinoxalines bind to these adenosine receptors by mimicking adenosine as shown in an overlap of these structures in Figure 2. This overlap prompted us to explore in our series amine substituents such as cycloalkyl, phenyl, and *R*-phenylisopropyl which enhance the affinity of adenosine as agonists for the A_1 receptor.[7] The data obtained with these derivatives are summarized in Table VI and compared with data available for the adenosine agonists. Table VI shows that in our "antagonist" series only the cyclohexyl group gave the expected 3-fold potency enhancement at the A_1 receptor (e.g. **85** vs **55**), whereas the phenyl (**86**) and the *R*- and *S*-phenylisopropyl groups (**87** and **88**) gave less potent derivatives. The *R* isomer of the latter compounds was 3 times more potent than the *S* isomer, at least showing a trend similar to that seen in the adenosine A_1 agonist derivatives.[7] In our series *N*-isopropyl substitution gave compounds with potency similar to the *N*-cyclohexyl derivatives; *N*-cyclopentyl substitution gave the most potent A_1 compounds.

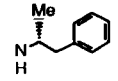
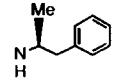
Among adenosine receptor antagonists reported in the literature, certain 1,3-dipropyl-8-phenylxanthine derivatives show extraordinary affinity for A_1 receptors in bovine

(7) Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197.

Table II

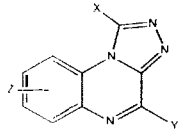


no.	X	Y	Z	mp, °C (recryst solvent)	formula[a]	method	% yield	rat porsolt: MED,[b] mg/kg po	A_1 binding (CHA):[c] IC_{50}, μM	A_2 binding (NECA):[d] IC_{50}, μM	PDEI:[e] IC_{50}, μM $Ca^{2+}D$	PDEI:[e] IC_{50}, μM $Ca^{2+}I$
46	Me	NH_2	8-Cl	213–215 (EtOH)	$C_{10}H_8ClN_5 \cdot CH_4O_3 \cdot 2H_2O$	H1	9	≤32	0.35			
47	Me	NHAc	8-Cl	262–234 ($EtOH \cdot Et_2O$)	$C_{12}H_{10}ClN_5O$	I1	78	3.2–32				
48	Me	NHiPr	H	230–233 (IPO)	$C_{13}H_{15}N_5$	H2	83	10–32	0.85			
49	Me	NHiPr	8-Cl	206–208 ($EtOH \cdot Et_2O$)	$C_{13}H_{14}ClN_5 \cdot CH_4O_3S \cdot {}^1/_2H_2O$	H2	26	3.2	0.1			
50	Me	NEt_2	H	122–124 (cyclohexane)	$C_{14}H_{17}N_5$	H2	55	3.2–10	>10			
51	Me	NEt_2	8-Cl	172–175 ($EtOH \cdot Et_2O$)	$C_{14}H_{16}ClN_5 \cdot CH_4O_3S$	H2	26	≤32	17			
52	Me		H	200–202 (EtOH)	$C_{14}H_{15}N_5$	H2	58	>32	49			
53	Et	NH_2	H	295–298 (EtOH)	$C_{11}H_{11}N_5$	H1	47	3.2–10	0.43 ± 0.05	0.062 ± 0.002	2	12
54	Et	NH_2	7-Cl	240–243 (EtOH)	$C_{11}H_{10}ClN_5 \cdot CH_4O_3S$	H1	25	≤3.2	0.075	0.029		
55	Et	NH_2	8-Cl	248–253 (DMF)	$C_{11}H_{10}ClN_5$	H1	68	≤3.2	0.11 ± 0.02	0.028		
56	Et	NH_2	7,8-Cl_2	>260 (DMF)	$C_{11}H_9Cl_2N_5$	H1	95	3.2–10	0.11	0.023		
57	Et	NH_2	7-F	285–289 dec (DMF)	$C_{11}H_{10}FN_5$	I1	59	3.2–32	0.58	0.17	≫10	40
58	Et	NH_2	7-OMe	255–258 (EtOH)	$C_{12}H_{13}N_5O \cdot CH_4O_3S \cdot {}^1/_4H_2O$	H1	28	>32	0.14	0.036		
59	Et	NHAc	H	193–195 ($CHCl_3 \cdot Et_2O$)	$C_{13}H_{13}N_5O$	I1	82	1–3.2				
60	Et	NHAc	7-Cl	210–212 ($CHCl_3 \cdot Et_2O$)	$C_{13}H_{12}ClN_5O$	I1	58	3.2–32				
61	Et	NHAc	8-Cl	203–205 ($CHCl_3 \cdot Et_2O$)	$C_{13}H_{12}ClN_5O$	I1	34	1–3.2	1.8			
62	Et	NHAc	7,8-Cl_2	230–232 ($CHCl_3 \cdot Et_2O$)	$C_{13}H_{11}Cl_2N_5O$	I1	35	≤3.2	1.3			
63	Et	NHAc	7-F	201–203 ($CHCl_3 \cdot Et_2O$)	$C_{13}H_{12}FN_5O$	I1	81	≤3.2				
64	Et	NHAc	8-F	203–205 ($CHCl_3 \cdot Et_2O$)	$C_{13}H_{12}FN_5O$	I1	72	≤3.2				
65	Et	NHAc	7-OMe	202–205 ($CHCl_3 \cdot Et_2O$)	$C_{14}H_{15}N_5O_2$	I1	35	>32				
66	Et	NHCOEt	8-Cl	212–215 ($CHCl_3 \cdot Et_2O$)	$C_{14}H_{14}ClN_5O$	I1	36	≤3.2				
67	Et	NHCOnPr	8-Cl	185–187 ($CHCl_3 \cdot Et_2O$)	$C_{15}H_{16}ClN_5O$	I1	28	≤32				
68	Et	$NHCOCMe_3$	8-Cl	211–213 ($CHCl_3 \cdot Et_2O$)	$C_{16}H_{18}ClN_5O \cdot {}^1/_2H_2O$	I1	18	≤32				
69	Et	NHMe	H	271–273 (DMF)	$C_{12}H_{13}N_5 \cdot {}^1/_4H_2O$	H1	88	3.2–10	6.0			
70	Et	NHEt	H	237–240 (EtOH)	$C_{13}H_{15}N_5$	H1	64	3.2	1.8 ± 0.2	9.5 ± 1.0	20	2.5
71	Et	NHEt	7-Cl	187–189 (EtOH)	$C_{13}H_{14}ClN_5 \cdot 2CH_4O_3S$	H1	42	3.2–32			>10	0.2
72	Et	NHEt	8-Cl	235–238 dec (EtOH)	$C_{13}H_{14}ClN_5 \cdot CH_4O_3S$	H1	51	3.2–32				
73	Et	NHEt	7-F	215–219 ($EtOH \cdot Et_2O$)	$C_{13}H_{14}FN_5 \cdot CH_4O_3S$	H1	80	≤32				
74	Et	NHEt	8-F	231–233 ($CHCl_3 \cdot Et_2O$)	$C_{13}H_{14}FN_5$	H1	60	3.2–32	0.7			
75	Et	NHEt	8-OMe	234–237 (DMF)	$C_{14}H_{17}N_5O$	H1	77	>32	0.71	4.5		
76	Et	NHiPr	H	222–224	$C_{14}H_{17}N_5$	H2	63	10	0.66	6.4	3	0.3
77	Et	NHiPr	6-Cl	248–251 ($CHCl_3$·hexane)	$C_{14}H_{16}ClN_5$	H2	71	≤32	6.8	>100	≫10	>10
78	Et	NHiPr	8-Cl	189–191 ($CHCl_3 \cdot Et_2O$)	$C_{14}H_{16}ClN_5$	H2	71	≤3.2	0.06	1.7	2	0.5
79	Et	NHiPr	7,8-Cl_2	197–199 ($CHCl_3 \cdot Et_2O$)	$C_{14}H_{15}Cl_2N_5$	H2	80	≤32	0.11	3.2		
80	Et	NHiPr	7-F	178–181 (EtOH)	$C_{14}H_{16}FN_5 \cdot CH_4O_3S$	H2	50	≥32	0.44	48		
81	Et	NHiPr	8-F	209–212 ($CHCl_3 \cdot Et_2O$)	$C_{14}H_{16}FN_5$	H2	73	3.2–10	0.18	3.9		
82	Et	NHiPr	7,8-F_2	151–152 ($EtOH \cdot Et_2O$)	$C_{14}H_{15}F_2N_5 \cdot CH_4O_3S$	H2	50	≤32	0.28	6.4		
83	Et	$NHCH_2CH_2OH$	H	240–242 (EtOH)	$C_{13}H_{15}N_5O \cdot {}^1/_3H_2O$	H2	47	≤32	1.6			
84	Et		8-Cl	183–185 ($CHCl_3$·hexane)	$C_{16}H_{18}ClN_5$	H2	40	>32	0.020	0.89		
85	Et		8-Cl	155–156 (EtOAc)	$C_{17}H_{20}ClN_5$	H2	51	>32	0.044 ± 0.004	4.1		
86	Et		8-Cl	221–223 ($CHCl_3$)	$C_{17}H_{14}ClN_5$	H2	30	>32	0.38	>100		

87	Et		8-Cl	155–157 ($CHCl_3$·Et_2O)[f]	$C_{20}H_{20}ClN_5$	H2	22	≤32	0.22	100		
88	Et		8-Cl	156–157.5 ($CHCl_3$·Et_2O)	$C_{20}H_{20}ClN_5$	H2	47	>32	0.68	>100		
89	Et	NAc_2	H	157–159	$C_{15}H_{15}N_5O_2$	J	38	3.2–10	9.3			
90	Et	NAcEt	H	185–187 ($CHCl_3$·Et_2O)	$C_{15}H_{17}N_5O$	I1	57	≤32				
91	Et	NAciPr	8-Cl	155–158 ($CHCl_3$·Et_2O)	$C_{16}H_{18}ClN_5O$	I1	35	10				
92	Et		H	158–160 (EtOAc)	$C_{15}H_{15}N_5O$	I2	15	>32				
93	Et	NMe_2	H	156–158 ($CHCl_3$·cyclohexane)	$C_{13}H_{15}N_5$	H1	66	≤3.2	12			
94	Et	NMe_2	7-Cl	214–217 dec (EtOH)	$C_{13}H_{14}ClN_5$·CH_4O_3S	H1	87	10–32				
95	Et	NMe_2	7,8-Cl_2	216–219 (EtOH)	$C_{13}H_{13}Cl_2N_5$·CH_4O_3S	H1	76	3.2–32				
96	Et	NEt_2	H	101–103 (cyclohexane)	$C_{15}H_{19}N_5$	H2	28	3.2–10	7.4		0.1	3.7
97	Et	NEt_2	7-Cl	172–175 dec (EtOH)	$C_{15}H_{18}ClN_5$·CH_4O_3S	H2	68	10–32				
98	Et	NEt_2	8-Cl	105–108 dec (Et_2O·PetEth)	$C_{15}H_{18}ClN_5$	H2	19	≤32	6.5			
99	Et	NEt_2	7,8-Cl_2	176–178 dec (EtOH)	$C_{15}H_{17}Cl_2N_5$·CH_4O_3S	H2	40	3.2–10	100			
100	Et	NEt_2	8-F	94–97 ($CHCl_3$·Et_2O)	$C_{15}H_{18}FN_5$	H2	76	10–32				
101	Et	NEt_2	7,8-F_2	109–111 ($CHCl_3$·Et_2O)	$C_{15}H_{17}F_2N_5$	H2	58	3.2–32				
102	Et	NEt_2	8-OMe	135–138 (Et_2O·PetEth)	$C_{16}H_{21}N_5O$·$^1/_8H_2O$	H2	23	≤32				
103	Et		H	196–197.5 (IPO)	$C_{15}H_{17}N_5O$	H2	69	>10	28			
104	Et		H	118–119 (cyclohexane)	$C_{16}H_{19}N_5$	H2	49	10–32	3.5			
105	Et		H	154–156 (IPO)	$C_{15}H_{17}N_5$	H2	59	>3.2	10			
106	Et		7,8-Cl_2	252–255 (EtOH)	$C_{15}H_{16}Cl_2N_6$·CH_4O_3S	H3	27	≤32				
107	Pr	NEt_2	H	92–94 (EtOH·H_2O)	$C_{16}H_{21}N_5$·$^1/_8H_2O$	H2	78	≤32				
108	iPr	NHEt	H	210–211 (IPO)	$C_{14}H_{17}N_5$	H1	42	≤32	2.2			
109	iPr	NEt_2	H	94–96[g]	$C_{16}H_{21}N_5$	H2	75	≤32	19			

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I. [e] See footnote *e* in Table I. [f] $[\alpha]^{20}_D$–5.4° (c 1, MeOH). [g] Distilled with Kugelrohr; bp 140–150 °C at 0.1 mmHg.

Table III



no.	X	Y	Z	mp, °C (recryst solvent)	formula[a]	method	% yield	rat porsolt: MED,[b] mg/kg po	A_1 binding (CHA):[c] IC_{50}, μM	A_2 binding (NECA):[d] IC_{50}, μM	PDEI:[e] IC_{50}, μM	
											$Ca^{2+}D$	$Ca^{2+}I$
110	CF_3	NH_2	8-Cl	259–261 dec (EtOH)	$C_{10}H_5ClF_3N_5 \cdot CH_4O_3S$	H1	36	3.2	0.065 ± 0.005	0.044	>10	2
111	CF_3	NH_2	8-F	260–263 (EtOH)	$C_{10}H_5F_4N_5 \cdot H_2O$	H1	11	1–3.2	0.29	0.10		
112	CF_3	NHAc	8-Cl	215–216 ($CHCl_3 \cdot Et_2O$)	$C_{12}H_7ClF_3N_5O$	I1	10	≤3.2	0.63			
113	CF_3	NHAc	8-F	217–219 ($CHCl_3 \cdot Et_2O$)	$C_{12}H_7F_4N_5O$	I1	20	≤3.2	3.6			
114	CF_3	NHEt	H	223–225 ($CHCl_3 \cdot Et_2O$)	$C_{12}H_{10}F_3N_5$	H1	53	32				
115	CF_3	NHEt	8-Cl	228–230 ($CHCl_3 \cdot Et_2O$)	$C_{12}H_9ClF_3N_5$	H1	20	≤32				
116	CF_3	NHEt	8-F	180–183 ($EtOH \cdot Et_2O$)	$C_{12}H_9F_4N_5 \cdot CH_4O_3S \cdot {}^1/_2H_2O$	H1	62	3.2–32	0.23	14		
117	CF_3	NHiPr	H	185–187 (Et_2O·cyclohexane)	$C_{13}H_{12}F_3N_5$	H2	74	≤10	0.17	4.1		
118	CF_3	NHiPr	8-Cl	183–185 ($EtOH \cdot Et_2O$)	$C_{13}H_{11}ClF_3N_5 \cdot CH_4O_3S$	H2	37	≤3.2	0.024 ± 0.002	2.3	1	1
119	CF_3	NHiPr	8-F	185–188 ($EtOH \cdot Et_2O$)	$C_{13}H_{11}F_4N_5 \cdot CH_4O_3S$	H2	45	≤3.2	0.057 ± 0.006	3.9 ± 0.4	24	0.37
120	CF_3	NH-⬡	8-Cl	171–173 ($CHCl_3$·hexane)	$C_{15}H_{13}ClF_3N_5$	H2	46	>32	0.0055	2.1		
121	CF_3	NH-⬡	8-Cl	200–202 ($CHCl_3$·hexane)	$C_{16}H_{15}ClF_3N_5$	H2	42	>32	0.028 ± 0.005	>100 (44 ± 6%)	≫10	>10
122	CF_3	NH-⬡	8-F	180–183 (toluene)	$C_{16}H_{15}F_4N_5$	H2	47	>32	0.032	60 ± 10	≫10	6
123	CF_3	NEt_2	H	155–157 (Et_2O)	$C_{14}H_{14}F_3N_5$	H2	56	≤32				
124	CF_3	NEt_2	8-Cl	135–136 ($EtOH \cdot Et_2O$)	$C_{14}H_{13}ClF_3N_5 \cdot {}^1/_2H_2O$	H2	38	≤32	>100	>100		
125	CF_3	NEt_2	8-F	146–149 ($CHCl_3$·hexane)	$C_{14}H_{13}F_4N_5$	H2	68	>32				
126	CF_3	NPr_2	8-F	128–130 ($CHCl_3$·hexane)	$C_{16}H_{17}F_4N_5$	H4	31	>32			≫10	≫10
127	C_2F_5	NHiPr	8-Cl	171–174 ($CHCl_3$)	$C_{14}H_{11}ClF_5N_5$	H2	25	32	0.024	3.1		

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I. [e] See footnote *e* in Table I.

Table IV

no.	X	Y	Z	mp, °C (recryst solvent)	formula[a]	method	% yield	rat porsolt: MED,[b] mg/kg po	A_1 binding (CHA):[c] IC_{50}, μM	A_2 binding (NECA):[d] IC_{50}, μM	PDEI:[e] IC_{50}, μM $Ca^{2+}D$	PDEI:[e] IC_{50}, μM $Ca^{2+}I$
128	Ph	NH_2	8-Cl	273–275 (EtOH)	$C_{15}H_{10}ClN_5 \cdot CH_4O_3S$	H1	24	>32	0.27	0.021 ± 0.001		
129	Ph	NHEt	8-Cl	254–256 ($CHCl_3$-hexane)	$C_{17}H_{14}ClN_5$	H1	44	>32			≫10	>10
130	Ph	NHiPr	8-Cl	184–186 (EtOAc-hexane)	$C_{18}H_{16}ClN_5$	H2	41	≤32	3.2	>100		
131	Ph		8-Cl	190–192 ($CHCl_3$-MeOH)	$C_{19}H_{17}ClN_6 \cdot {}^1/_4CH_3OH$	H3	36	>32			≫10	10
132	Ph	NEt_2	H	166–168 (EtOAc-hexane)	$C_{19}H_{19}N_5$	H2	31	1–10				
133	Ph	NEt_2	8-Cl	194–195 ($CHCl_3$-Et_2O)	$C_{19}H_{18}ClN_5$	H2	45	>32				
134	*p*-Cl-Ph	NH_2	8-Cl	321–323 dec (EtOH)	$C_{15}H_9Cl_2N_5 \cdot CH_4O_3S$	H1	27		0.51	0.033		
135	OMe	NHiPr	8-Cl	182–185 dec ($CHCl_3$-Et_2O)	$C_{13}H_{14}ClN_5O \cdot {}^1/_4H_2O$	H2	14	≤32				
136	OMe	NHiPr	8-F	199–201 dec ($CHCl_3$-Et_2O)	$C_{13}H_{14}FN_5O$	H2	55	≤32	0.16	6.5		
137	OH	NH_2	8-F	>250 (EtOH)	$C_9H_6FN_5O \cdot HBr$	K	24	10–32	0.84	0.70		
138	OH	NHiPr	8-F	>300 (MeOH)	$C_{12}H_{12}FN_5O \cdot HBr \cdot H_2O$	K	44	>32	0.73	3.5		

[a] See footnote *a* in Table I. [b] See footnote *b* in Table I. [c] See footnote *c* in Table I. [d] See footnote *d* in Table I. [e] See footnote *e* in Table I.

brain (with IC_{50} values in the pM range)[8] or in rat brain (IC_{50} values in the nM range).[9] In addition, a variety of non-xanthine heterocycles with affinity for the A_1 receptor mostly in the μM range have been described.[10–12] The most potent non-xanthine A_1 and A_2 antagonists yet reported are found in a series of 5,6-dihydro[1,2,4]triazolo-[1,5-*c*]quinazolin-5-amines with CGS 15943 as the best representative.[13] This compound is reported to have IC_{50} values of 21 nM for the A_1 receptor and of 3.3 nM for the A_2 receptor, with an A_2/A_1 selectivity of 6.3-fold.[13] We have confirmed the affinity of CGS 15943 at the A_2 receptor, but we find an IC_{50} value of 6 nM at the A_1 receptor (Table V). Thus CGS 15943 is more potent than our best A_2 ligand (**128** with an IC_{50} value of 21 nM), but we have more selective A_2 antagonists in our series.

Our most potent A_2 ligand is **128** (CP-66,713) with an IC_{50} value of 21 nM at the A_2 receptor and, according to our most recent studies, with an A_2/A_1 potency ratio of 13. Earlier studies from our laboratory indicated an even higher A_2/A_1 potency ratio of >450,[14] but in those experiments less DMSO was used for solubilization of the drug in the binding assay, and the relative insolubility of **128** in aqueous buffer at concentrations above 1 μM may have led to an underestimation of its affinity at the A_1 binding site. We have noticed that when binding assays are run at concentrations at which compounds like **128** precipitate out of solution, ligands like CHA, and particularly NECA, "bind" to the precipitated drug, even in the absence of brain tissue, and give the false impression of diminished inhibition of ligand binding and even of "binding enhancement". However, it is encouraging that an independent laboratory (Dr. Ken Jacobson, NIH) has found a K_i value of 12 nM in rat brain for **128**, using the A_2 selective agonist CGS 21680 [[[2-[4-(2-carboxyethyl)-phenyl]ethyl]amino]-5′-*N*-(ethylcarbamoyl)adenosine] as a ligand, and a K_i value of 300 nM at the A_1 receptor, using *R*-PIA [(*R*)-N^6-(phenylisopropyl)adenosine], and a K_i value of >300 nM, using XAC (xanthine amine congener; 8-[4-[[[(2-aminoethyl)amino]carbonyl]methoxy]phenyl]-1,3-di-*n*-propylxanthine) as the ligand.[15] The most selective A_2 antagonist reported in the literature is HTQZ (3-(3-hydroxyphenyl)-5*H*-thiazolo[2,3-*b*]quinazoline) with a published K_i of 124 nM at the A_2 receptor and an A_2/A_1 potency ratio of 25-fold.[11,12] We have confirmed the A_2 selectivity for HTQZ (Table V), although we find slightly lower affinities for both receptors than those reported in the literature.[12] Thus **128** may not be as selective as HTQZ, but it has a better combination of potency and selectivity than any non-xanthine or xanthine derivative reported in the literature.[11] The chloro congener of **128**,

(8) Bruns, R. F.; Daly, J. W.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 2077.

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

(10) Daly, J. W.; Hong, O.; Padgett, W. L.; Shamim, M. T.; Jacobson, K. A.; Ukena, D. *Biochem. Pharmacol.* **1988**, *37*, 655.

(11) Williams, M.; Jarvis, M. F. *Pharmacol. Biochem. Behav.* **1988**, *29*, 433.

(12) Bruns, R. F.; Coughenour, L. L. *Pharmacologist* **1987**, *29*, 146.

(13) 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.

(14) Sarges, R., Howard, H. R., Browne, R. G.; Koe, B. K. *Abstract E-13*, Purine Nucleosides and Nucleotides in Cell Signalling: Targets for New Drugs; Rockville, MD, September 17–20, 1989; published in *Purines in Cellular Signaling*; Jacobson, K. A., Daly, J. W., Manganillo, V., Eds.; Springer-Verlag: New York, 1990; pp 417–418.

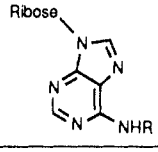
(15) Jacobson, K. A. Private communication.

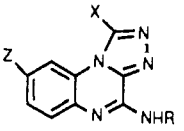
Table V

	rat porsolt: MED,[a] mg/kg po	A_1 binding (CHA):[c] IC_{50}, μM	A_2 binding (NECA):[d] IC_{50}, μM	PDEI:[d] IC_{50}, μM $Ca^{2+}D$	PDEI:[d] IC_{50}, μM $Ca^{2+}I$
caffeine	3.2	117 ± 12	63 ± 4	70	170
IBMX[j]	>32	7.0 ± 0.8	13 ± 1	0.1	3.5
amphetamine sulfate	≤1[e]				
rolipram	>32	>100	>100	>100	0.25
imipramine	>17.8				
CGS 15943[k]	≤32	0.006[f]	0.0028 ± 0.0004[g]		
HTQZ[l]		18 ± 4[h]	0.53 ± 0.11[i]		
CHA[m]		0.0020	0.64		
NECA[n]		0.010 ± 0.001	0.017		

[a] See footnote *b* in Table I. [b] See footnote *c* in Table I. [c] See footnote *d* in Table I. [d] See footnote *e* in Table I. [e] This drug was administered sc. [f] Literature[13] value: IC_{50} = 21 nM. [g] Literature[13] value: IC_{50} = 3.3 nM. [h] Literature[12] value: K_i = 3070 nM. [i] Literature[12] value: K_i = 124 nM. [j] 3-Isobutyl-1-methylxanthine. [k] 9-Chloro-2-(2-furyl)[1,2,4]triazolo[1,5-*c*]quinazolin-5-amine. [l] 3-(3-Hydroxyphenyl)-5*H*-thiazolo[2,3-*b*]quinazoline. [m] N^6-cyclohexyladenosine. [n] 5′-(*N*-ethylcarbamoyl)adenosine.

Table VI. Affinity of Adenosine Agonists and Antagonists for the Adenosine Receptors





	NHR	A_1 binding: affinity constant, nM[a]	A_2 binding: affinity constant, nM[a]	A_1 binding (CHA): IC_{50}, nM[b]	A_1 binding (CHA): K_i, nM[c]	A_2 binding (NECA): K_i, nM[c]	no.	X	Z	NHR	A_1 binding (CHA): IC_{50}, nM[d]	A_2 binding (NECA): IC_{50}, nM[e]
adenosine	NH_2	10	5–10000				**55**	Et	Cl	NH_2	110	28
							111	CF_3	F	NH_2	290	100
							110	CF_3	Cl	NH_2	65	44
N^6-cyclohexyladenosine	NH-cyclohexyl	3	30000	1.7	1.31		**85**	Et	Cl	NH-cyclohexyl	44	4100
							122	CF_3	F	NH-cyclohexyl	32	60000
							121	CF_3	Cl	NH-cyclohexyl	28	>100000
N^6-phenyladenosine	NH-phenyl	3	50000	6.5	4.62		**86**	Et	Cl	NH-phenyl	380	>100000
L-PIA (*R*-PIA)	Me, phenyl	3	30000	2.4	1.17		**87**	Et	Cl	Me, phenyl	220	100000
D-PIA (*S*-PIA)	Me, phenyl	200	100000	105	49.3		**88**	Et	Cl	Me, phenyl	680	>100000
N^6-isopropyladenosine	NH-isopropyl			3.7			**78**	Et	Cl	NH-isopropyl	60	1700
							119	CF_3	F	NH-isopropyl	57	3900
							118	CF_3	Cl	NH-isopropyl	24	2300
N^6-cyclopentyladenosine	NH-cyclopentyl			0.64	0.589	462	**84**	Et	Cl	NH-cyclopentyl	20	890
							120	CF_3	Cl	NH-cyclopentyl	5.5	2100

[a] Affinity constants, estimated from binding constants and efficacy in a variety of tissues; Daly, J. W. *J. Med. Chem.* **1982**, *25*, 197. [b] A_1 binding measured as inhibition of CHA binding in rat brain membranes; Daly, J. W.; Padgett, W.; Thompson, R. D.; Kusachi, S.; Bugni, W. J.; Olsson, R. A. *Biochem. Pharmacol.* **1986**, *35*, 2467. [c] A_1 and A_2 binding measured by inhibition of CHA binding in rat brain homogenate or of NECA binding in rat striatal membranes, respectively; Bruns, R. F.; Lu, G. H.; Pugsley, T. A. *Mol. Pharmacol.* **1986**, *29*, 331. [d] See footnote *c* in Table I. [e] See footnote *d* in Table I.

compound **134**, exhibited binding affinities and selectivity very similar to those of **128**.

The most potent A_1 ligand in our series is **120** with an IC_{50} of 5.5 nM, a value close to that found by us for CGS 15943 (5.7 nM). Compound **120** is selective for the A_1 receptor by a factor of 381. The most selective compound in our series for the A_1 receptor is **121** (CP-68,247) with a selectivity ratio of over 3000. However, as explained in more detail below, it is possible that in vivo this secondary amine could be N-dealkylated to the primary amine and thereby lose its high selectivity since N-dealkylation is a facile process in several species. While we do not know whether or not cycloalkyl derivatives are readily dealkylated, **120** and **121** could, for example, be converted to **110** with a selectivity ratio of 1.5 for the A_2 receptor. Nevertheless, for in vitro binding the selectivity values for the A_1 receptor in our series compare favorably with the best selectivity of 145-fold found in a series of 1,3-dialkyl-xanthines.[9] Thus members from our 4-amino[1,2,4]triazolo[4,3-*a*]quinoxalines are currently the most A_1 selective xanthine or non-xanthine adenosine antagonists known, and compound **120** almost equals CGS 19543 for the potency record among non-xanthine A_1 ligands. A recent study of compounds from our 4-amino[1,2,4]triazolo[4,3-*a*]quinoxaline series, synthesized following our disclosure of their behavioral activity,[16,17] has led to an apparently independent discovery of their adenosine binding properties;[18] the most potent A_1 ligand found by these investigators was the 1-trifluoromethyl-4-cyclopentylamino derivative with a K_i value of 7.3 nM.

Like xanthines, many representatives of the 4-amino[1,2,4]triazolo[4,3-*a*]quinoxalines also show inhibition of Ca^{2+}-dependent and Ca^{2+}-independent phosphodiesterases from rat brain. However, the SAR is somewhat less orderly than and different from the adenosine binding SAR. Compared to standards, one compound (**96**) equals IBMX in potency against the calcium-dependent brain enzyme with an IC_{50} value of 0.1 μM, while others (**71**, **76**, **119**)

(16) Sarges, R. U.S. Patent 4,495,187, Jan. 22, 1985.
(17) Sarges, R. U.S. Patent 4,547,501, Oct. 15, 1985.
(18) Trivedi, B. K.; Bruns, R. F. *J. Med. Chem.* **1988**, *31*, 1011.

Table VII. Plasma and Brain Concentrations in Rats following a 10 mg/kg Oral Dose of **70**, **59**, or **53**

drug administered	time, h	plasma concentration, ng/mL			brain concentration, ng/g		
		70	**59**	**53**	**70**	**59**	**53**
70 (mesylate)	0.25	860		234	476		197
	0.5	1090		349	589		341
	1	506		372	337		445
	2	646		582	428		461
	4	270		445	199		338
59	0.25		565	186		236	131
	0.5		790	499		225	168
	1		921	1337		287	457
	2		1270	1866		325	474
	4		497	484		70	91
53	0.25			236			108
	0.5			137			78
	1			239			124
	2			622			269
	4			608			276

equal rolipram[19] in potency against the calcium-independent enzyme.

These findings raise the question whether the Porsolt activity observed in this series is related to CHA or NECA binding or to PDE inhibitory activity. Resolution of this question is complicated by the fact that the mono- and dialkylated amines of this series are readily N-dealkylated in the rat to the primary amines, suggesting that a significant part of the biological activity may be attributed to the primary amine metabolite.[20] Similarly, N-acetylated compounds such as **59** are rapidly deacylated in the rat to the primary amines. These data are exemplified for compounds **59** and **70** in Table VII.[20] It is apparent from this table that **59** and **70** generate significant plasma and brain levels of the primary amine **53** and may just serve as well absorbed prodrugs for **53** if that were the active species. Similar data were obtained for compounds **21**, **33**, **61**, **72**, and **78**.

Although only a small sample of compounds was examined for effects on PDE, it would appear that the SAR for Porsolt activity is divergent from PDE inhibitory activity, even when considering potential contributions of primary amine metabolites to the in vivo activity. On the other hand, with the exception of the *N*-cycloalkyl derivatives, there is a qualitative correlation between A_1 binding activity and Porsolt activity, suggesting that there may be a link between these activities. Furthermore, with the notable exception of compound **128**, most compounds which have sub-micromolar affinity for the A_2 receptor or which can be metabolized to such compounds have good Porsolt activity. Thus, a role for A_1 or A_2 antagonism in the generation of Porsolt activity can not be excluded without conducting further detailed pharmacokinetic studies on the compounds which were inactive in vivo.

Since no genuine antidepressant effects have yet been attributed to caffeine despite its apparent increase in norepinephrine turnover and its down-regulation of β-adrenoceptors,[21] this raises the question whether 4-amino[1,2,4]triazolo[4,3-a]quinoxalines are caffeine-like false positives in the Porsolt test. Ultimately, this question can only be answered in the clinic. However, we have also tried to obtain evidence in the laboratory that the Porsolt

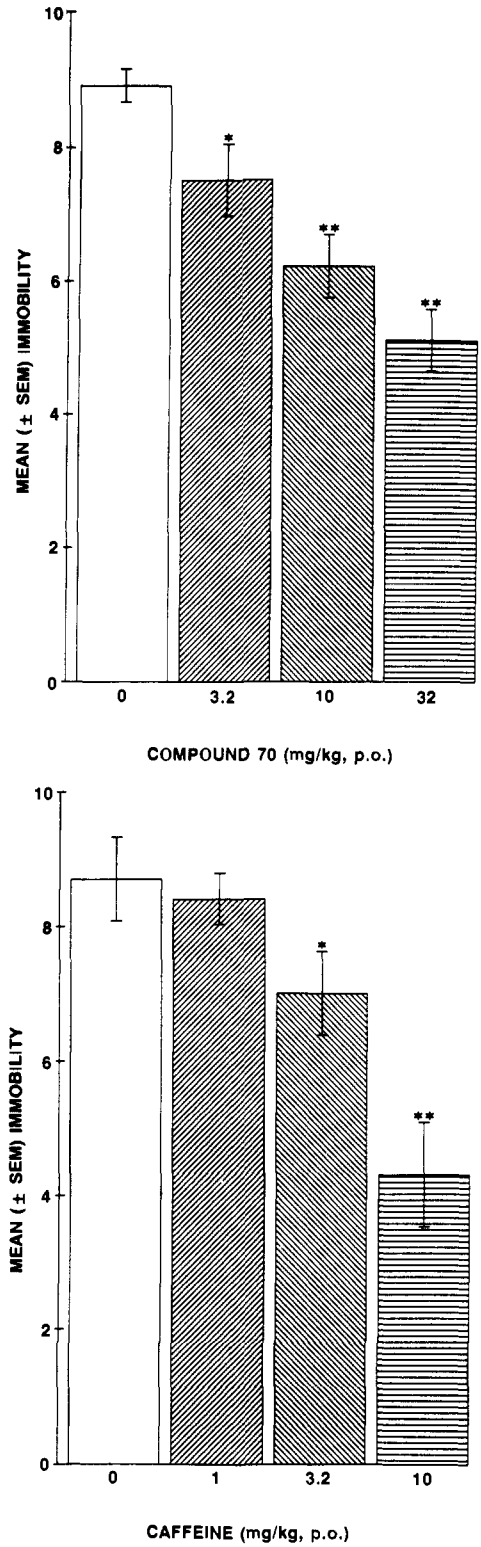


Figure 3. Comparison of the significant reductions in mean (±SEM) immobility in the behavioral despair test in rats, as a function of dose, for compound **70** (CP-57,103) (a) and caffeine (b) after acute administration (*, ** = p <0.05, 0.01, respectively, N = 9–10/group).

activity of 4-amino[1,2,4]triazolo[4,3-a]quinoxalines may be independent of caffeine-like stimulant activity.

When comparing doses which cause locomotor stimulation with doses which cause activity in the standard Porsolt test, there is some differentiation between caffeine and compound **70** (CP-57,103). As shown in Figure 3a,b, both compound **70** and caffeine exhibit activity in the behavioral despair test in a dose-related manner at doses above 3.2 mg/kg orally, significantly prolonging the du-

(19) Schwabe, V.; Mijake, M.; Ohga, Y.; Daly, J. W. *Mol. Pharmacol.* **1976**, *12*, 900.
(20) We are grateful to Dr. R. A. Ronfeld of Pfizer Central Research for these studies.
(21) Goldberg, M. R.; Curatolo, P. W.; Tung, C.-S.; Robertson, D. *Neurosci. Lett.* **1982**, *31*, 47.

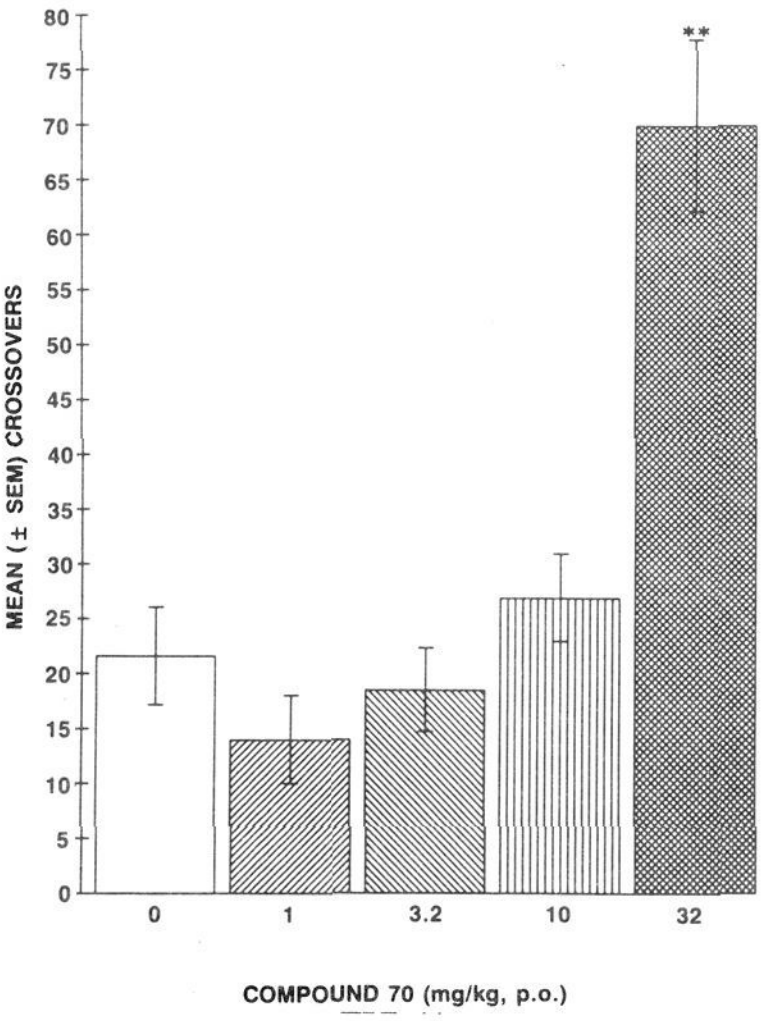


Figure 4. Comparison of the effects of compound **70** (CP-57,103) (a) and caffeine (b) as a function of dose on locomotor activity (mean crossovers ± SEM) in rats over 1 h after drug administration (** = p <0.01).

ration of escape-directed behavior. On the other hand, as shown in Figure 4a,b, despite the fact that Porsolt activity is obtained at 3.2 mg/kg with compound **70**, significant locomotor stimulation is not observed until a dose of 32 mg/kg orally is reached. By contrast caffeine elicits motor stimulation at 3.2 mg/kg, an indication that its activity in the swim test may be related to stimulant properties.

Furthermore, as shown in Figure 5, in the extended swim test[22] compound **70** reduces immobility only during the

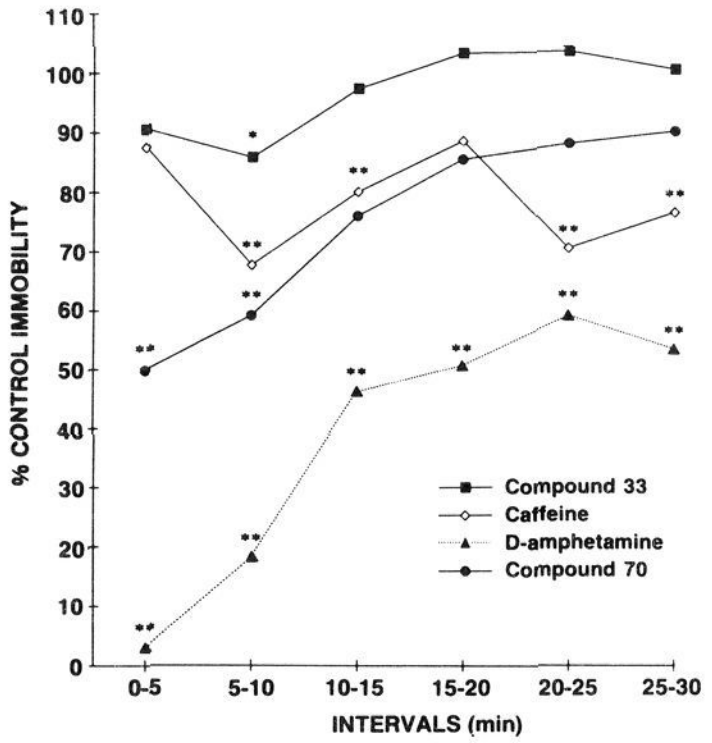


Figure 5. Antidepressant-like activity of compound **70** (32.0 mg/kg, po) and compound **33** (32.0 mg/kg, po), and stimulant-like activity of caffeine (32.0 mg/kg, po) and *d*-amphetamine (3.2 mg/kg, sc) in the extended swim test in rats (*, ** = p <0.05, 0.01, respectively, N = 10/group).

first 10 min of testing, while its analogue **33** (CP-41,475) significantly reduces immobility only during the 5–10-minute interval. Such an effect is characteristic of antidepressants, but not of psychostimulants such as *d*-amphetamine or caffeine.[22] Indeed, in our hands caffeine and *d*-amphetamine reduce immobility for the duration of the 30-minute test. These data support the hypothesis that the 4-amino[1,2,4]triazolo[4,3-*a*]quinoxalines induce activity in the swim test by a prolongation of escape-directed behavior, rather than by a generalized locomotor stimulant effect. Additional support for antidepressant potential comes from sleep studies in cats, shown in Figure 6, which indicate that compounds **33** and **70** selectively suppress REM sleep at a dose of 1 mg/kg, an effect similar to that shown by antidepressants and electroconvulsive shock.[23]

Conclusion

The value of 4-amino[1,2,4]triazolo[4,3-*a*]quinoxalines as antidepressants remains to be determined in clinical studies. In any event, this series has produced very potent, structurally novel, and in some cases highly selective adenosine receptor antagonists which may serve to further define the role of adenosine and adenosine receptors in the brain.

Experimental Section

Chemistry. Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were obtained using a Perkin-Elmer Model 21 spectrophotometer, while mass spectra were obtained with a Hitachi-Perkin-Elmer RMU-6E mass spectrometer for low resolution and an A.E.I. MS-30 for high resolution. ^{1}H NMR were recorded on Varian A-60 (or T-60) or Bruker WM-250 spectrometers, with tetramethylsilane as an internal standard. Microanalyses were performed by the Pfizer Analytical Department and agree within 0.4% of calculated values unless otherwise noted.

(22) Kitada, Y.; Miyauchi, T.; Satoh, A.; Satoh, S. *Eur. J. Pharmacol.* **1981**, *72*, 145.

(23) Vogel, G. W. *Progr. Neuropsychopharmacol. Biol. Psychiatry* **1983**, *7*, 343. The REM sleep studies with compounds **33** and **70** were carried out by Dr. M. B. Sterman of the V. A. Medical Center in Sepulveda, CA.

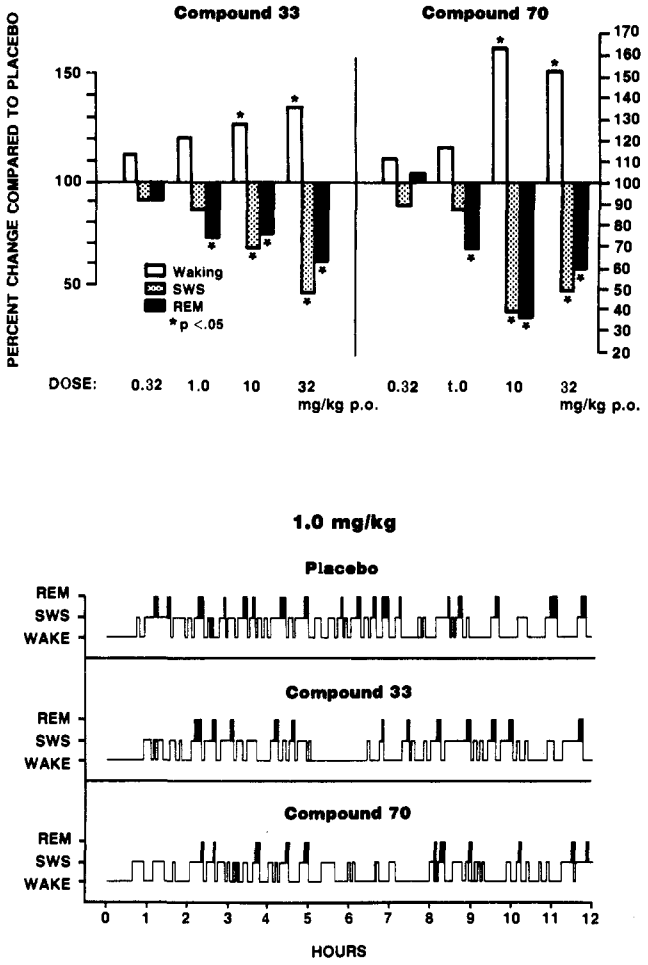


Figure 6. Sleep pattern studies in cats. The top panel shows dose–response comparisons of compounds **33** (CP-41,475) and **70** (CP-57,103) with placebo, indicating selective suppression of REM sleep at 1 mg/kg orally. The bottom panel shows representative records at this dose.

Method A. Preparation of 1,4-Dihydro-2,3-quinoxalinediones. 6-Chloro-1,4-dihydro-2,3-quinoxalinedione. A mixture of 30.0 g of 4-chloro-1,2-phenylenediamine (0.21 mol, Aldrich Chemical Co.) and 175 mL of diethyl oxalate was refluxed for ca. 16 h, cooled to room temperature, and filtered. The product was washed with EtOH and air dried to give the title compound: 40.4 g (98%); mp >320 °C; MS *m/e* 196 (M^+), 198 (M + 2).

In a similar manner, the following 1,4-dihydro-2,3-quinoxalinediones were prepared: 5-chloro (75%, mp >280 °C), 6,7-dichloro (91%, mp >270 °C), 6-fluoro (80%, mp >300 °C), 6,7-difluoro (100%, mp >310 °C), and 6-methoxy (68%, mp >300 °C).

Method B. Preparation of 2,3-Dichloroquinoxalines (I). 2,3,6-Trichloroquinoxaline (I, Z = Cl). A mixture of 140 g (0.70 mol) of 6-chloro-1,4-dihydro-2,3-quinoxalinedione and 326 mL (3.5 mol) of phosphorus oxychloride was refluxed for ca. 16 h, cooled to room temperature, and cautiously poured over ice. The mixture was filtered, and the solids were washed with H_2O and dissolved in $CHCl_3$. The organics were washed with saturated aqueous NaCl, dried ($MgSO_4$), and concentrated in vacuo to a semisolid residue. Recrystallization from $CHCl_3$/EtOH gave the pure product: 120 g (74%); mp 139–142 °C; MS *m/e* 232 (M^+), 234 (M + 2), 236 (M + 4). In a similar manner, the following quinoxaline analogues were prepared: 2,3,5-trichloroquinoxaline (95%, mp 135–137 °C); 2,3,6,7-tetrachloroquinoxaline (66%, mp 165–168 °C); 2,3-dichloro-6-fluoroquinoxaline (100%, mp 148–152 °C); 2,3-dichloro-6,7-difluoroquinoxaline (100%, mp 162–164 °C dec), and 2,3-dichloro-6-methoxyquinoxaline (56%, mp 158–161 °C).

Method C. Preparation of 2-Chloro-3-methoxyquinoxalines (V). 2,6-Dichloro-3-methoxyquinoxaline (V, Z = Cl). A slurry of 11.7 g (0.05 mol) of 2,3,6-trichloroquinoxaline in 140 mL of MeOH was heated to 50 °C and treated dropwise over 6 h with 1.4 g (0.06 mol) of sodium dissolved in 140 mL of MeOH. The mixture was stirred for ca. 16 h at 50 °C, treated with 0.14 g (0.006 mol) of sodium in 20 mL MeOH, heated another 2 h at 50 °C, and cooled to room temperature. The mixture was concentrated in vacuo, and the residue was dissolved in $CHCl_3$ and then washed with H_2O and saturated NaCl. After the mixture was dried ($MgSO_4$), the solvent was removed, and the residue was chromatographed on 250 mL of silica gel (70–230 mesh), with toluene as eluant. The product, a white solid, weighed 9.8 g (86%), mp 92–95 °C. By a similar procedure, 2,3-dichloro-6-fluoroquinoxaline was converted to 2-chloro-6-fluoro-3-methoxyquinoxaline (95%, mp 93–95 °C; MS *m/e* 212 (M^+), 214 (M + 2)), and 2,3-dichloro-6-methoxyquinoxaline gave 2-chloro-3,6-dimethoxyquinoxaline (88%), mp 79–81 °C (Anal. ($C_{10}H_9ClN_2O_2$, C, H, N)).

Method D. Preparation of Hydrazinoquinoxalines (II, VII). 1. 2,6-Dichloro-3-hydrazinoquinoxaline (II, Z = Cl). A mixture of 23.0 g (0.10 mol) of 2,3,6-trichloroquinoxaline and 11.0 g (0.22 mol) of hydrazine hydrate in 500 mL of EtOH was stirred for ca. 16 h at 25 °C. The resulting precipitate was filtered, and the solids were washed with EtOH and air dried to give crude product: 22.2 g (97%); mp >250 °C; MS *m/e* 228 (M^+).

Similarly, the following 3-hydrazinoquinoxalines were also prepared: 2,8-dichloro (72%, mp 153 °C dec), 2,6,7-trichloro (100%, mp >260 °C), 2-chloro-6-fluoro (93%, mp 190–192 °C dec), 2-chloro-6,7-difluoro (67%, mp 212–215 °C dec), 2-chloro-6-methoxy (97%, mp 170–174 °C dec), and 2-chloro (91%, mp 181 °C dec).

2. 6-Chloro-2-hydrazino-3-methoxyquinoxaline (VII, Z = Cl). A mixture of 4.9 g (0.02 mol) of 2,6-dichloro-3-methoxyquinoxaline and 2.7 g (2.6 mL, 0.053 mol) of hydrazine hydrate in 75 mL of EtOH was stirred for ca. 16 h at room temperature. The resulting mixture was filtered, and the solids were washed with EtOH and air dried to give 4.4 g (98%) of product: mp 175–179 °C dec; MS *m/e* 224 (M^+), 226 (M + 2).

Similarly prepared were 6-fluoro-2-hydrazino-3-methoxyquinoxaline (94%, mp 170–174 °C dec) and 3,6-dimethoxy-2-hydrazinoquinoxaline (85%, mp 128–130 °C dec).

Method E. Preparation of 4-Methoxy[1,2,4]triazolo[4,3-a]quinoxalines (VIII). 7-Chloro-4-methoxy[1,2,4]triazolo[4,3-a]quinoxaline (VIII, X = H, Z = Cl). A mixture of 1.4 g (6.2 mmol) of 6-chloro-2-hydrazino-3-methoxyquinoxaline and 20 mL of triethyl orthoformate was heated, with mechanical stirring, with use of a preheated oil bath at 100 °C for ca. 16 h. After the mixture cooled to room temperature, the precipitated solids were filtered, washed with EtOH, and dried to give 1.0 g (69%) of product, mp 250–252 °C.

Similarly, 6-fluoro-2-hydrazino-3-methoxyquinoxaline was converted to 7-fluoro-4-methoxy[1,2,4]triazolo[4,3-a]quinoxaline (72%, mp 245–246 °C dec), and 3,6-dimethoxy-2-hydrazinoquinoxaline gave the 4,7-dimethoxy analogue (96%, mp 238–240 °C dec).

Replacing triethyl orthoformate with triethyl orthopropionate in the above examples gave, respectively, 7-chloro-1-ethyl-4-methoxy[1,2,4]triazolo[4,3-a]quinoxaline (75%, mp 221–223 °C), 1-ethyl-7-fluoro-4-methoxy[1,2,4]triazolo[4,3-a]quinoxaline (64%, mp 200–202 °C dec), and 4,7-dimethoxy-1-ethyl[1,2,4]triazolo[4,3-a]quinoxaline (72%, mp 184–188 °C).

Method F. Preparation of 4-Hydroxy[1,2,4]triazolo[4,3-a]quinoxalines. 1. From 4-Methoxy[1,2,4]triazolo[4,3-a]quinoxaline. 7-Chloro-4-hydroxy[1,2,4]triazolo[4,3-a]quinoxaline. A mixture of 3.4 g (0.014 mol) of 7-chloro-4-methoxy[1,2,4]triazolo[4,3-a]quinoxaline, 35 mL of 1 N HCl and 105 mL of glacial acetic acid was refluxed for 2.5 h, cooled to room temperature, and poured over ice/H_2O. After the mixture stirred for 20 min, the solids were filtered, washed well with H_2O, and air dried to give product: 2.6 g (87%); mp >300 °C.

By a similar process, the following 4-hydroxy[1,2,4]triazolo[4,3-a]quinoxalines were prepared: 7-fluoro (84%, mp >300 °C), 7-methoxy (80%, mp >250 °C), 7-chloro-1-ethyl (94%, mp >300 °C), 1-ethyl-7-fluoro (62%, mp >300 °C), 1-ethyl-7-methoxy (67%, mp >250 °C), and 8-fluoro (85%, mp >285 °C).

2. From 2-Chloro-3-hydrazino[1,2,4]triazolo[4,3-a]quinoxaline. 4-Hydroxy-1-(trifluoromethyl)[1,2,4]triazolo-

[4,3-*a*]quinoxaline (VI, X = CF_3, Z = H). Under N_2 in a flame-dried flask, 3.89 g (0.02 mol) of 2-chloro-3-hydrazinoquinoxaline was added to 22.8 g (0.20 mol) of trifluoroacetic acid with ice bath cooling and mechanical stirring. The mixture was then heated to 100 °C for 3 h and poured over ice/H_2O, and the precipitate was filtered. The solids were washed well with H_2O and air dried to give 3.0 g (60%) of product: mp >300 °C; MS *m/e* 254 (M^+).

Similarly, 2-chloro-6-fluoro-3-hydrazinoquinoxaline gave, after 24 h at 120 °C, 8-fluoro-4-hydroxy-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinoxaline (77%, mp 298–302 °C) and 2,6-dichloro-3-hydrazinoquinoxaline gave, after 24 h at 100 °C, 8-chloro-4-hydroxy-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]-quinoxaline (57%, mp 253–255 °C dec).

Method G. Preparation of 4-Chloro[1,2,4]triazolo[4,3-*a*]quinoxalines. 1. From 2-Chloro-3-hydrazinoquinoxalines. 4-Chloro[1,2,4]triazolo[4,3-*a*]quinoxaline (III, X = H, Z = H). A mixture of 9.0 g (0.046 mol) of 2-chloro-3-hydrazinoquinoxaline and 90 mL of triethyl orthoformate was stirred at 100 °C for 1 h, cooled to room temperature, and filtered. The solids were washed with cyclohexane and dried to give the product: 8.8 g (94%); mp 287–290 °C dec (lit[24] mp 281–283 °C); MS *m/e* 204 (M^+), 206 (M + 2).

In a similar manner, the following [1,2,4]triazolo[4,3-*a*]-quinoxalines were prepared: 4,8-dichloro (76%, mp >250 °C), 4,7,8-trichloro (79%, mp >270 °C), 4-chloro-8-fluoro (91%, mp 310–312 °C), 4-chloro-7,8-difluoro (82%, mp >210 °C dec), and 4-chloro-8-methoxy (76%, mp 280–282 °C dec).

With use of triethyl orthoacetate and heating at 100 °C for 3 h, the following 1-methyl[1,2,4]triazolo[4,3-*a*]quinoxalines were prepared: 4-chloro (45%, mp 215–217 °C from EtOH), 4,8-dichloro (46%, mp >280 °C), and 4,7,8-trichloro (68%, mp 208–210 °C).

Similar, using triethyl orthopropionate gave the following 1-ethyl[1,2,4]triazolo[4,3-*a*]quinoxalines: 4-chloro (85%, mp 158–160 °C), 4,6-dichloro (37%, mp 193–195 °C dec), 4,8-dichloro (62%, mp >250 °C); 4,7,8-trichloro (80%, mp 198–200 °C, from $CHCl_3$/cyclohexane), 4-chloro-8-fluoro (65%, mp 160–163 °C dec), 4-chloro-7,8-difluoro (52%, mp 185–186 °C dec), and 4-chloro-8-methoxy (80%, mp 200–203 °C dec, from EtOH).

Using triethyl orthobenzoate gave the following 1-phenyl-[1,2,4]triazolo[4,3-*a*]quinoxalines: 4-chloro (51%, mp 203–205 °C) and 4,8-dichloro (72%, mp 305–307 °C). Using trimethyl *p*-chloroorthobenzoate and 2,6-dichloro-3-hydrazinoquinoxaline gave 1-(4-chlorophenyl)-4-chloro[1,2,4]triazolo[4,3-*a*]quinoxaline (81%, mp 356–358 dec).

Finally, reacting 2-chloro-3-hydrazinoquinoxaline with triethyl orthobutyrate gave 4-chloro-1-*n*-propyl[1,2,4]triazolo[4,3-*a*]-quinoxaline (53%, mp 173–175 °C, from $CHCl_3$) and with triethyl orthoisobutyrate the product was 4-chloro-1-isopropyl[1,2,4]triazolo[4,3-*a*]quinoxaline (40%, mp 208–210 °C, from EtOH). With tetramethyl orthocarbonate at 100 °C for 18 h were obtained 4,8-dichloro-1-methoxy[1,2,4]triazolo[4,3-*a*]quinoxaline (68%, mp 182–190 °C dec) and 4-chloro-8-fluoro-1-methoxy[1,2,4]triazolo-[4,3-*a*]quinoxaline (72%, mp 203–205 °C dec).

2. From 4-Hydroxy[1,2,4]triazolo[4,3-*a*]quinoxalines. 4,7-Dichloro[1,2,4]triazolo[4,3-*a*]quinoxaline (IX, X = H, Z = Cl). Under N_2 in a flame-dried flask, a mixture of 2.6 g (0.012 mol) of 7-chloro-4-hydroxy[1,2,4]triazolo[4,3-*a*]quinoxaline (from method F1) and 40 mL of phosphorus oxychloride was treated with 2.6 mL tri-*n*-propylamine and refluxed for 16 h. The reactants were cooled, poured cautiously over ice/H_2O, and extracted with EtOAc. The organics were washed (H_2O, saturated $NaHCO_3$, saturated NaCl), dried over $MgSO_4$, and concentrated in vacuo. The resulting crude solid was chromatographed on 200 mL of silica gel (70–230 mesh, 10% MeOH/90% $CHCl_3$) to give a light yellow solid: 1.89 g (66%); mp 253–256 °C dec; MS *m/e* 238 (M^+), 240 (M + 2), 242 (M + 4).

In a similar manner, the following [1,2,4]triazolo[4,3-*a*]-quinoxalines were obtained: 4-chloro-7-fluoro (71%, mp 305–308 °C), 4-chloro-7-methoxy (31%, mp 266–268 °C dec), 4,7-dichloro-1-ethyl (79%, mp 217–220 °C dec), 4-chloro-1-ethyl-7-fluoro (57%, mp 203–205 °C), 4-chloro-1-ethyl-7-methoxy (80%, mp 173–175 °C), 4-chloro-1-trifluoromethyl (61%, mp 190–200 °C dec), 4-chloro-8-fluoro-1-trifluoromethyl (79%, mp 135–138 °C), and 4,8-dichloro-1-trifluoromethyl (75%, mp 133–135 °C).

Method H. Preparation of 4-Amino[1,2,4]triazolo[4,3-*a*]quinoxalines (IV, X). 1. From 4-Chloro[1,2,4]triazolo-[4,3-*a*]quinoxalines and Gaseous Amines. 4-Amino-1-ethyl[1,2,4]triazolo[4,3-*a*]quinoxaline (IV, X = Et, Y = NH_2, 53). A mixture of 4.8 g (0.02 mol) of 4-chloro-1-ethyl[1,2,4]triazolo[4,3-*a*]quinoxaline in 75 mL of DMF was saturated with anhydrous ammonia at 0 °C. After the solution was stirred at 25 °C for 18 h, the solids were filtered, washed well with H_2O, and dried. Recrystallization from EtOH gave **53**: 2.0 g (47%); mp 295–298 °C dec. Anal. ($C_{11}H_{11}N_5$) C, H, N.

The free base of **53** (213 mg, 1 mmol) in 4 mL of hot EtOH was treated with 0.1 mL (1.5 mmol) of methanesulfonic acid to give a clear solution which, on slow cooling, gave the mesylate salt as white crystals: 260 mg (84%); mp 243–245 °C dec. Anal. ($C_{11}H_{11}N_5 \cdot CH_4O_3S$) C, H, N.

2. From 4-Chloro[1,2,4]triazolo[4,3-*a*]quinoxalines and Liquid Amines. 7-Chloro-4-(diethylamino)-1-ethyl[1,2,4]-triazolo[4,3-*a*]quinoxaline Methanesulfonate (X, X = Et, Y = NEt_2, Z = Cl; 97). A mixture of 1.0 g (3.7 mmol) of 4,7-dichloro-1-ethyl[1,2,4]triazolo[4,3-*a*]quinoxaline, 1.2 mL (11.1 mmol) of diethylamine, and 10 mL of DMF was stirred at 25 °C for ca. 18 h to give a homogeneous solution which was poured over ice and stirred for 15 min. The precipitated solids were filtered, washed well with H_2O, dissolved in $CHCl_3$, and washed with saturated NaCl. After drying ($MgSO_4$), the solvent was removed in vacuo to give a semicrystalline residue, 1.10 g (98%). This free base was dissolved in 20 mL of EtOH and treated with 0.55 mL of methanesulfonic acid. After 48 h of standing at 25 °C, the crystalline product was filtered, washed with EtOH, and dried to give pure mesylate of **97**: 1.0 g (68%); mp 172–175 °C dec. Anal. ($C_{15}H_{18}ClN_5 \cdot CH_4O_3S$) C, H, N.

3. From 4-Chloro[1,2,4]triazolo[4,3-*a*]quinoxalines and Solid Amines. 4-Piperazino[1,2,4]triazolo[4,3-*a*]quinoxaline (IV, X = H, Y = piperazin-1-yl, Z = H; 44). A mixture of 6.0 g (0.029 mol) of 4-chloro[1,2,4]triazolo[4,3-*a*]quinoxaline, 25.3 g (0.29 mol) of piperazine, 3.1 g (0.059 mol) of ammonium chloride, and 120 mL of *p*-dioxane was refluxed for ca. 18 h to give a nearly homogeneous yellow solution. After cooling, the mixture was poured over ice/H_2O and extracted several times with EtOAc, and the combined organics were washed with H_2O and saturated NaCl. After drying ($MgSO_4$), the solvent was removed in vacuo to give a crude yellow solid. Chromatography on silica gel (230–400 mesh, 75 × 180 mm column) eluting with 4 L of EtOAc, then increasing polarity with 1% DEA every 1000 mL gave **44** as a yellow solid: 5.28 g (65%); mp 160–162 °C. Anal. ($C_{13}H_{14}N_6$) C, H, N.

Similarly, with 4,8-dichloro[1,2,4]triazolo[4,3-*a*]quinoxaline and piperazine in *p*-dioxane as starting materials, after 48 h at 25 °C, was obtained 8-chloro-4-piperazino[1,2,4]triazolo[4,3-*a*]quinoxaline hemihydrate **45**, (46%, mp 253–256 °C) as a pale yellow solid. Anal. ($C_{13}H_{13}ClN_6 \cdot {}^1/_2H_2O$) C, H, N.

When DMF was used in place of *p*-dioxane as the solvent and the reaction was heated to 100–110 °C for 3 h, 25 °C for approximately 24 h, and then 100–110 °C for an additional 18 h, and then partitioned between EtOAc and H_2O, a yellow insoluble material could be removed (filtration). After washing with H_2O, EtOAc, and MeOH, the solids were identified as 4-(4-formylpiperazino)[1,2,4]triazolo[4,3-*a*]quinoxaline: 38%; mp 310–313 °C dec; MS *m/e* 282 (M^+). Anal. ($C_{14}H_{14}N_6O$) C, H, N.

4. From 4-Hydroxy[1,2,4]triazolo[4,3-*a*]quinoxalines. 7-Chloro-4-(diethylamino)[1,2,4]triazolo[4,3-*a*]quinoxaline Methanesulfonate (X, X = H, Y = NEt_2, Z = Cl; 35). Under an N_2 atmosphere in a flame-dried flask, a mixture of 0.56 g (2.5 mmol) of 7-chloro-4-hydroxy[1,2,4]triazolo[4,3-*a*]quinoxaline, 0.8 mL (6.2 mmol) of triethylamine, and 6.0 mL of phosphorus oxychloride was refluxed for ca. 16 h, cooled to 25 °C, and poured cautiously over ice. The solution was extracted with $CHCl_3$ which was then washed successively with H_2O, saturated $NaHCO_3$, and saturated NaCl, and then dried ($MgSO_4$). Removal of the solvent in vacuo and chromatography of the residue on 125 mL of silica gel (70–230 mesh), eluting with $CHCl_3$, gave an off-white solid, 0.25 g. This solid in 5 mL of EtOH was treated with 0.2 mL methanesulfonic acid to give 0.240 g (28%) **35**, mp 205–207 °C. Anal. ($C_{13}H_{14}ClN_5 \cdot CH_4O_3S$) C, H, N.

Method I. Preparation of 4-(Acylamino)[1,2,4]triazolo-[4,3-*a*]quinoxalines. 1. From Acylation of Triazolo[4,3-

a]quinoxalin-4-amines. 4-(Acetylamino)-1-ethyl[1,2,4]triazolo[4,3-a]quinoxaline (59). A mixture of 0.533 g (2.5 mmol) of 4-amino-1-ethyl[1,2,4]triazolo[4,3-a]quinoxaline and 1.0 g (1.0 mL, 0.01 mol) of acetic anhydride in 20 mL of CH_2Cl_2 was refluxed for ca. 16 h, cooled, and concentrated in vacuo to a white solid. Recrystallization from $CHCl_3/Et_2O$ gave 0.520 g (82%) pure **59**, mp 193–195 °C. Anal. ($C_{13}H_{13}N_5O$) C, H, N. NMR experiments confirmed that acetylation, in contrast to protonation, took place at the exocyclic nitrogen and not at the ring nitrogen.[3b]

2. From 4-Chloro[1,2,4]triazolo[4,3-a]quinoxalines and Amide Anions. 1-Ethyl-4-(2-oxopyrrolidinyl)[1,2,4]triazolo[4,3-a]quinoxaline (92). Under N_2 in a flame-dried flask, 0.48 g (0.01 mol) of 50% NaH was washed with pentane and treated with 10 mL of toluene and 0.76 mL (0.01 mol) of 2-pyrrolidinone. After the mixture was stirred at 25 °C for 1 h, 2.32 g (0.01 mol) of 4-chloro-1-ethyl[1,2,4]triazolo[4,3-a]quinoxaline and 10 mL of toluene were added, resulting in a red suspension. After the suspension was heated to ca. 120–130 °C for 18 h, the mixture was cooled to 25 °C, poured over ice water, and extracted twice with EtOAc. The organics were washed (H_2O, saturated NaCl), dried ($MgSO_4$), and concentrated in vacuo to an orange gum. Chromatography on silica gel (230–400 mesh, 45 × 150 mm) eluting with 1 L of EtOAc, 1 L of 2% MeOH/98% EtOAc and finally 2 L of 3% MeOH/97% EtOAc gave the pure product as a yellow solid: 0.407 g (15%); mp 158–160 °C; MS *m/e* 281 (M^+). Anal. ($C_{15}H_{15}N_5O$) C, H, N.

Method J. Preparation of 4-(Diacylamino)[1,2,4]triazolo[4,3-a]quinoxalines. 4-(Diacetylamino)-1-ethyl[1,2,4]triazolo[4,3-a]quinoxaline (89). A mixture of 5.5 g (25.8 mmol) of 4-amino-1-ethyl[1,2,4]triazolo[4,3-a]quinoxaline and 25 g (25 mL, 0.25 mol) of acetic anhydride in 60 mL of pyridine containing 100 mg of 4-(dimethylamino)pyridine was stirred at 25 °C for ca. 18 h. The mixture was then filtered, and the filtrate was concentrated in vacuo to a dark gum and triturated with H_2O to give pinkish-white crystals. Filtration and washing with H_2O gave, after drying at 50 °C in vacuo, 2.9 g (38%) of **89**, mp 157–159 °C. Recrystallization ($EtOAc/Et_2O$) raised the mp to 158–160 °C. Anal. ($C_{15}H_{15}N_5O_2$) C, H, N.

Method K. Preparation of 1-Hydroxy[1,2,4]triazolo[4,3-a]quinoxalines. 8-Fluoro-1-hydroxy-4-(isopropylamino)-[1,2,4]triazolo[4,3-a]quinoxaline Hydrobromide Hydrate (138). A mixture of 0.54 g (1.96 mmol) of 8-fluoro-4-(isopropylamino)-1-methoxy[1,2,4]triazolo[4,3-a]quinoxaline (**136**), 10 mL of 48% HBr, and 15 mL of acetic acid was refluxed for 4 h to a clear yellow solution and then concentrated in vacuo. The resulting solids (0.63 g) were recrystallized from MeOH to give the white crystalline product: 0.310 g (44%); mp >300 °C. Anal. ($C_{12}H_{12}FN_5O \cdot HBr \cdot H_2O$) C, H, N.

Method L. Bromination of 4-Amino-[1,2,4]triazolo[4,3-a]quinoxalines. 7,8-Dibromo-4-(diethylamino)[1,2,4]triazolo[4,3-a]quinoxaline Hydrate (34). A solution of 2.4 g (0.01 mol) of 4-(diethylamino)[1,2,4]triazolo[4,3-a]quinoxaline (**33**) in 100 mL of MeOH was treated dropwise with 6.2 mL (19.2 g, 0.12 mol) of bromine, the internal temperature rising to 39 °C. After 20 min a precipitate had formed, and after an additional 18 h of stirring the mixture was filtered to give a yellow solid, 3.65 g. The solid was dissolved in $CHCl_3$, washed with saturated $NaHCO_3$, and dried ($MgSO_4$), and the solvent was removed. The resulting white solid was chromatographed on silica gel (70–230 mesh, 150 mL) with $CHCl_3$ as eluant to give crude **34**. Recrystallization from Et_2O gave pure product: 0.530 g (13%); mp 199–201 °C. Anal. ($C_{13}H_{13}Br_2N_5 \cdot H_2O$) C, N; H: calcd, 3.62; found, 3.18.

Pharmacology. Materials. Male CD rats (Charles River, Kingston) weighing 180–200 g on arrival and 225–300 g upon testing were used in the Porsolt swim tests. Animals were housed five per cage on a 12-h light/12-h dark (7 a.m.–7 p.m.) lighting cycle under standard laboratory conditions, for at least 1 week prior to experimentation. All compounds were dissolved or suspended in a saline vehicle containing ethanol (5%) and Emulphor (5%) (GAF Corp.) and were administered orally in a volume of 2 mL/kg.

Porsolt Rat "Behavioral Despair" Test. A modification of the "behavioral despair" test described by Porsolt et al.[2] was used to evaluate the antiimmobility effects of various compounds. On day 1 rats were placed individually in Plexiglas cylinders (height, 18 cm; diameter, 8.5 cm) containing 9.5 cm (depth) of water (25 °C) for 15 min. On day 2 animals were treated orally with vehicle or drug. Compounds were initially tested at 32 mg/kg, po for screening purposes, and interesting actives were tested further for determination of minimal effective doses (MED). After a 60-min period the animals were again placed in the cylinders for a 2-min stabilization period, followed by a 5-min test period. During the test period each animal was rated 10 times for immobility, once every 30 s. Rats were judged as mobile (score = 0) when clear escape-directed behavior was observed, and as immobile (score = 1) when upright floating behavior was observed. Total scores for each animal, therefore, ranged between 0 and 10. Mean immobility scores for each treatment group were calculated and compared with Kruskal–Wallis one-way analyses of variance by ranks, followed by Mann–Whitney U tests comparing each treatment group with its respective control group.

Extended Swim Test. In the extended swim experiments, designed to differentiate between antidepressant-like and psychostimulant activities, the procedure of Kitada et al.[22] was followed. In this test, on day 2, animals were rated for 30 min instead of 5 min. The 30-min test was broken down into six 5-min observation periods. Antidepressants reportedly reduce immobility only during the first 5 or 10 min by prolonging escape-directed behavior. In contrast, psychostimulants such as *d*-amphetamine and caffeine, reduce immobility for the duration of the 30-min test, not by prolonging the escape-directed behavior, but by increasing general motor activity.

Locomotor Activity Studies. Locomotor activity data were recorded in 48 individual Plexiglas behavioral chambers (30 cm × 30 cm) enclosed in sound attenuating cabinets. Locomotor activity was monitored by a PDP 11/34 computer and was measured as the number of crossovers from one quadrant of the grid floor to another. In all locomotor activity experiments, rats were placed in the chambers and allowed to habituate to them overnight. In the morning, during the light portion of the light/dark cycle, each animal was removed from its chamber, treated (po) with vehicle or drug, and placed back into the chamber. Data collection for each animal was initiated individually, immediately after injection and was continued for 60 min, in order to evaluate locomotor activity effects during the time period of interest for activity in the behavioral despair test. Means for each group were compared by using one-way analyses of variance followed by Dunnett's multiple range tests.

Sleep Studies in Cats. Adult cats (20) were prepared surgically for chronic sleep recordings. Pairs of small stainless steel screws were threaded into the skull 4 mm apart over sensory and posterior marginal cortices for EEG recordings. Eye movements were detected from screws placed medial and lateral to the orbit in the frontal sinus. The EMG was recorded from flexible wires insulated except at the tips and inserted in the nuchal musculature. Bipolar electrodes were placed stereotaxically in the lateral geniculate nucleus to monitor phasic phenomena related to the status of sleep. These procedures conform to the standardized sleep recording methods for the cat established by Ursin and Sterman.[25] Each of these leads was attached to a 20-connector Winchester plug and fixed to the skull with dental cement. Following recovery from surgical procedures the animals were housed under normal light/dark cycle conditions in the animal holding facility. State recordings were carried out in sound-attenuated isolation chambers. After the cats were dosed with drugs or placebo, data were collected from 8 a.m. to 8 p.m. under constant light conditions. The scoring of sleep states was based on the convergence of EEG, EMG, and phasic events into patterns as defined by the manual of Ursin and Sterman.[25] Differences between drug-treated and control groups were determined with Student's *t* tests.

Biochemistry. [^{3}H]-N^6-Cyclohexyladenosine A_1 Binding.[26] Sprague-Dawley male CD rats, 200–300 g, from Charles River Breeding Laboratories, Wilmington, MA, were killed by deca-

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pitation. Cerebral cortices were removed rapidly and homogenized in ice-cold 50 mM Tris (tris(hydroxymethyl)aminomethane)·HCl pH 7.7 buffer with a Polytron PT-10 homogenizer (30 mL/g wet weight). The homogenate was centrifuged at 18000*g* for 20 min (0–5 °C), and the pellet was washed by resuspension in fresh buffer and recentrifugation. The final pellet was dispersed in 125 mL of fresh buffer (0.8 mg protein/mL), and the suspension was incubated with 0.75–3 units/mL of adenosine deaminase (Sigma Chemical Co. Type VI) for 30 min at 25 °C. The homogenate was cooled for 30 min in ice and dispensed (1.0-mL aliquots) into glass tubes containing 0.25 mL of 100 μM (−)-(phenylisopropyl)adenosine (for nonspecific binding), inhibitor solution (aqueous dimethyl sulfoxide), or vehicle and 0.75 mL of [^{3}H]CHA (NEN^R DuPont NET-679; 1.0 nM final concentration). The assay mixtures in triplicate were incubated at 25 °C for 2 h and filtered in a Brandell cell harvester containing a Whatman GF/B filter strip. The recovered membranes were washed twice with 5 mL of ice-cold buffer, and the separated filters were placed in 10-mL Aquasol II for determination of radioactivity in a liquid scintillation counter. Wherever possible, assays were carried out in duplicate. When the assays were carried out 3 or more times, standard errors (SEM) are given in the tables.

[^{3}H]-5′-(*N*-Ethylcarbamoyl)adenosine A_2 Binding.[27] Corpora striata were dissected from brains of rats killed by decapitation. The tissue was homogenized in ice-cold 50 mM Tris-HCl pH 7.7 buffer (10 mL/g wet weight), and the homogenate was centrifuged at 50000*g* for 10 min (0–5 °C). After washing the pellet with fresh buffer in this manner, the final pellet was dispersed in 150 mL of fresh buffer (0.7 mg protein/mL) and incubated as above with 0.13 units/mL of adenosine deaminase. The homogenate was cooled for 30 min in ice and dispensed (0.75-mL aliquots) into triplicate assay tubes containing 0.050 mL of 100 μM N^6-cyclopentyladenosine (for nonspecific binding), inhibitor solution, or vehicle and 0.20 ml of [^{3}H]NECA (NEN^R DuPont NET-811; 4.0 nM final concentration in 50 mM Tris·HCl pH 7.7 buffer containing 10 mM $MgCl_2$ and 50 nM cyclopentyladenosine). Assay mixtures were incubated at 25 °C for 1 h and filtered in a Brandell cell harvester containing a Whatman GF/B filter strip. The membranes were washed three times with 4 mL of ice-cold buffer, and the separated filters were placed in Aquasol II for determination of radioactivity. Wherever possible, assays were carried out in duplicate. When the assays were carried out 3 or more times, standard errors (SEM) are given in the tables.

Calcium-Independent and Calcium-Dependent Phosphodiesterase Activity.[28] Partially purified PDE enzymes were prepared by Dr. Craig W. Davis of the University of South Carolina, Columbia, SC.[29] Phosphodiesterase activity was determined by using reaction mixtures (total volume, 0.10 mL) containing Tris·HCl pH 7.5 buffer (5 μmol), $MgCl_2$ (0.5 μmol), and [^{3}H]cAMP (final concentration of cAMP, 1.0 μM containing 400 000 dpm of NEN^R DuPont NET-275 [^{3}H]cAMP). Inhibitor solution or vehicle (0.01 mL) and fresh or boiled PDE (0.01 mL) were added to the [^{3}H]cAMP substrate solution (0.08 mL). Hydrolysis was conducted at 37 °C for 8 min. Reaction mixtures were then placed in hot water (98 °C) for 2 min to stop hydrolysis. Carrier 5′-AMP (0.5 mL of 0.5 mM 5′-AMP in 0.1 M Hepes (*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethanesulfonic acid)/0.1 M NaCl pH 8.5 buffer) was added, and the contents of the incubation tubes were poured onto columns of polyacrylamide/boronate affinity gel (BIO-RAD Affi-Gel 601 Boronate Gel). The unreacted [^{3}H]cAMP was eluted from the gel with 7.5 mL of the 0.1 M Hepes-NaCl buffer. The [^{3}H]5′-AMP product was eluted with 7 mL of 50 mM sodium acetate pH 4.8 buffer. Aliquots (1 mL) of the latter eluates were counted in Aquasol **II** in a liquid scintillation counter to measure the [^{3}H]5′-AMP.

Pharmacokinetics. Three compounds (**70**, **59**, and **53**) were administered orally to rats (10 mg/kg). Rats were sacrificed at 0.25, 0.5, 1, 2, and 4 h after the dose (3 rats per time point), and plasma and whole brain samples were taken for compound analysis. Each brain and plasma sample was assayed for the administered compound and for the presence of compound **53**. Brain homogenates were prepared by adding 2 mL of 66 mM KH_2PO_4 buffer (pH 7.4) to each brain sample. Following homogenization, with use of a glass tube and Teflon pestle, volumes were adjusted to 5 mL. An internal standard (a structural analogue of compound **70** or **59**) was added to an aliquot of plasma (0.5 mL) or brain homogenate (1.0 mL), and the samples were extracted twice with 5 mL of Et_2O; the Et_2O extracts were transferred to a clean tube and evaporated to dryness under nitrogen; the dry residue was reconstituted in 0.25 mL of HPLC mobile phase, and 0.1 mL was injected on the HPLC. The HPLC conditions were as follows: column, μBondapak C^{18}; detector, Waters Model 440 UV operated at 313 nm. For the assay of compounds **59** and **53** after compound **59** administration or compound **53** after compound **53** administration, the mobile phase was CH_3CN/0.02 M KH_2PO_4 (25/75). The mobile phase was CH_3OH/CH_3CN/0.02 M KH_2PO_4 (50/15/35) for the assay of compounds **70** and **53** following compound **70** administration. Concentrations were calculated from relative chromatogram peak heights and an internal standard based standard curve. Concentrations are expressed as ng/mL of plasma or ng/g of brain (wet weight).

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Supplementary Material Available: Additional experimental data concerning the X-ray analysis of compound **21** (10 pages). Ordering information is given on any current masthead page.

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