present may not be those seen in the solid state and complicated distributions of polyoxometalate species may be established on time scales that are short with respect to the time for the drug to be administered and to take effect.

Experimental Section

Materials. The polyoxometalates were prepared by standard literature procedures.[4a,5a,14a,18] All polyoxometalates were purified by recrystallization, and their purity was assessed spectroscopically with ^{183}W and ^{31}P NMR, UV-visible, and infrared absorption spectroscopies.

Spectroscopic Methods. The ^{31}P NMR spectra were taken on an IBM WP-200SY instrument. Chemical shifts are referenced to a 1% TMP (trimethyl phosphate) solution in D_2O, by running the reference in a 5-mm coaxial tube, inside the 10-mm sample tube. The D_2O in the coaxial tube proved sufficient to provide a lock, so D_2O was not used in the buffer solutions. Infrared spectra were run on a Perkin-Elmer 1430 ratio recording spectrophotometer. Samples were run as KBr pellets (2-4 wt % in KBr). Electronic absorption spectra were run on a Hewlett-Packard Model 8451A diode-array spectrophotometer.

Cell Culture Assays. The compounds were evaluated in human mitogen-stimulated PBM cells infected with HIV-1 (strain LAV-1), as described previously.[19] Virus was harvested after 6 days. Stock solutions (2 mM) of the compounds were freshly prepared in water prior to testing. Compounds **7**, **15**, **16**, and **20** were dissolved in DMSO and then diluted with medium so that the final concentration of DMSO was less than 0.05%. At that concentration, DMSO has no effect on the viral yield.

Cytotoxicity Assays in PBM Cells. The drugs were evaluated for their potential toxic effects on uninfected mitogen-stimulated human PBM cells. Flasks were seeded so that the final cell concentration was 2×10^5 cells/mL. The cells were cultured with and without drug for 6 days at which time aliquots were counted for cell viability, as assessed by the trypan blue dye-exclusion method using a hemacytometer.[19] The EC_{50} and IC_{50} were calculated by using the median effect method.[20]

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2,4-Dihydro-3*H*-1,2,4-triazol-3-ones as Anticonvulsant Agents

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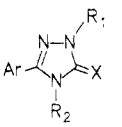
A series of 5-aryl-2,4-dihydro-3*H*-1,2,4-triazol-3-ones was evaluated for anticonvulsant activity. In general the members of this series were prepared by the alkaline cyclization of 1-aroyl-4-alkylsemicarbazides. The resulting 2-unsubstituted 3*H*-1,2,4-triazol-3-ones were then alkylated, yielding 2,4-dialkyl-3*H*-1,2,4-triazol-3-ones. Approximately one-third of the compounds examined exhibited activity against both maximal electroshock- and pentylenetetrazole-induced seizures in mice. Receptor-binding studies suggest that this activity was not a consequence of activity at either benzodiazepine or NMDA-type glutamate receptors. From this series, compound **45** was selected for further evaluation where it was also found to be active against 3-mercaptopropionic acid, bicuculline, and quinolinic acid induced seizures in mice. In addition, **45** also protected gerbils from hippocampal neuronal degeneration produced by either hypoxia or intrastriatal quinolinic acid injection.

We have recently been investigating a series of 2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **1** as potential antidepressant agents.[1] Selected members of this series exhibited potent behavioral effects in test systems traditionally used to detect these agents. The mechanism of action of these compounds, however, remains uncertain. While attempting to establish some correlation between the structure and the activity of these triazoles, we prepared several 2,4-dihydro-3*H*-1,2,4-triazol-3-ones **2**. Surprisingly, these compounds were completely devoid of antidepressant-like activity and instead exhibited anticonvulsant activity against a variety of convulsant stimuli. In order to more fully investigate these findings, we have prepared additional derivatives of **2** and we now report the anticonvulsant activities associated with members of this series.



1, X=S
2, X=O

Chemistry

The triazol-3-ones (Table I) which were evaluated in this study were prepared via the three routes depicted in Scheme I. The synthesis of 2,4-dihydro-5-phenyl-3*H*-1,2,4-triazol-3-one (**3**) was accomplished by the method of Lipkin.[2] Thus, condensation of benzoic acid hydrazide (**4**) and urea (**5**) gave **3** in a single, low-yielding step. The synthesis of 2,4-dihydro-2-methyl-5-phenyl-3*H*-1,2,4-triazol-3-one (**6**) was accomplished via a new route which involved S-methylation of triazole-3-thione **7**.[3] The resultant thioether **8**[4] was then oxidized with 2.5 equiv of *m*-chloroperoxybenzoic acid (MCPBA), affording sulfone **9**. Alkaline hydrolysis of **9** cleanly afforded **6**, which was

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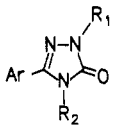
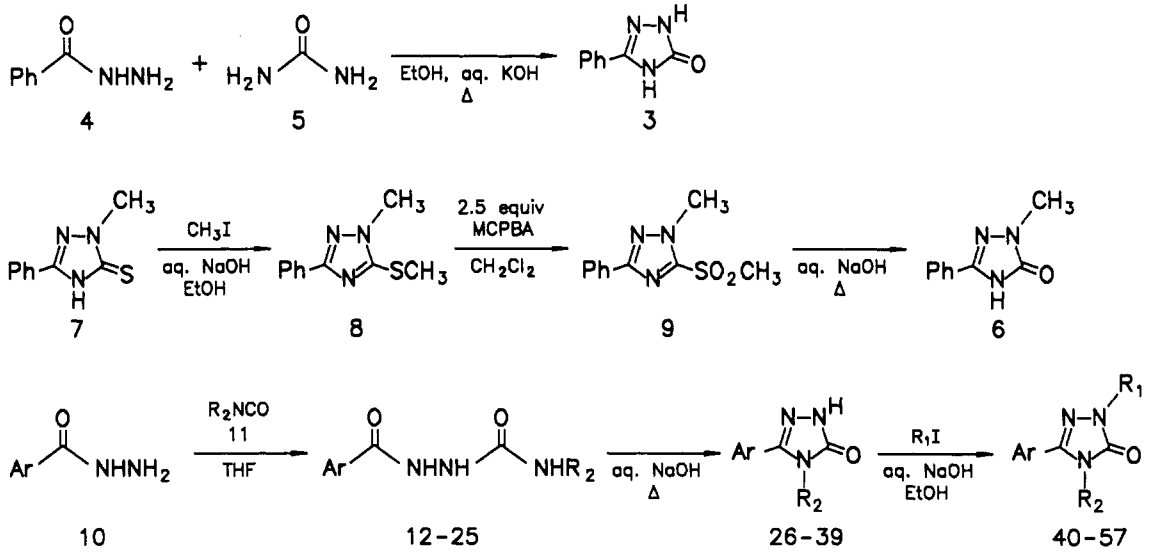
Table I. 2,4-Dihydro-3*H*-1,2,4-triazol-3-ones



compd	Ar	R_1	R_2	mp, °C	crystn solvent[a]	% yield	formula[b]
3	C_6H_5	H	H	>310[c]	A	10	$C_8H_7N_3O$
6	C_6H_5	CH_3	H	218–219[d]	B	90	$C_9H_9N_3O$
26	C_6H_5	H	CH_3	177–178[e]	A	74	$C_9H_9N_3O$
27	C_6H_5	H	C_2H_5	163–165[f]	A	69	$C_{10}H_{11}N_3O$
28	2-ClC_6H_4	H	CH_3	168–170	C	71	$C_9H_8ClN_3O$
29	4-ClC_6H_4	H	CH_3	213–215[g]	D	69	$C_9H_8ClN_3O$
30	4-ClC_6H_4	H	CH_3	199–201[h]	A	83	$C_{10}H_{10}ClN_3O$
31	3,4-$Cl_2C_6H_3$	H	CH_3	170–172	C	72	$C_9H_7Cl_2N_3O$
32	2-FC_6H_4	H	CH_3	189–191	E	54	$C_9H_8FN_3O$
33	4-FC_6H_4	H	CH_3	216–218[i]	D	53	$C_9H_8FN_3O$
34	3-$CF_3C_6H_4$	H	C_2H_5	130–131	F	52	$C_{11}H_{10}F_3N_3O$
35	4-$CH_3C_6H_4$	H	CH_3	206–208	D	78	$C_{10}H_{11}N_3O$
36	4-$CH_3OC_6H_4$	H	CH_3	174–176	A	86	$C_{10}H_{11}N_3O_2$
37	4-CH_3O-3-(n-C_4H_9O)C_6H_3	H	CH_3	96–98	C	70	$C_{14}H_{19}N_3O_3$
38	2-C_4H_3S	H	CH_3	183–185[j]	A	82	$C_7H_7N_3OS$
39	4-C_5H_4N	H	CH_3	249–251[k]	D	71	$C_8H_8N_4O$
40	C_6H_5	CH_3	CH_3	140–141[l]	D	64	$C_{10}H_{11}N_3O$
41	C_6H_5	CH_3	C_2H_5	oil[m]		64	$C_{11}H_{13}N_3O$
42	C_6H_5	C_2H_5	CH_3	87–89[n]	G	61	$C_{11}H_{13}N_3O$
43	2-ClC_6H_4	CH_3	CH_3	61–63	G	51	$C_{10}H_{10}ClN_3O$
44	4-ClC_6H_4	CH_3	CH_3	126–128[o]	C	48	$C_{10}H_{10}ClN_3O$
45	4-ClC_6H_4	CH_3	C_2H_5	73–75[p]	G	58	$C_{11}H_{12}ClN_3O$
46	4-ClC_6H_4	C_2H_5	CH_3	79–81[q]	G	53	$C_{11}H_{12}ClN_3O$
47	4-ClC_6H_4	C_2H_5	C_2H_5	62–64[r]	H	50	$C_{12}H_{14}ClN_3O$
48	4-ClC_6H_4	n-C_3H_7	C_2H_5	oil		55	$C_{13}H_{16}ClN_3O$
49	3,4-$Cl_2C_6H_3$	CH_3	CH_3	107–109	B	65	$C_{10}H_9Cl_2N_3O$
50	2-FC_6H_4	CH_3	CH_3	69–71	G	57	$C_{10}H_{10}FN_3O$
51	4-FC_6H_4	CH_3	CH_3	104–106[s]	G	63	$C_{10}H_{10}FN_3O$
52	3-$CF_3C_6H_4$	CH_3	C_2H_5	49–51	I	41	$C_{12}H_{12}F_3N_3O$
53	4-$CH_3C_6H_4$	CH_3	CH_3	92–94	G	56	$C_{11}H_{13}N_3O$
54	4-$CH_3OC_6H_4$	CH_3	CH_3	109–111	G	36	$C_{11}H_{13}N_3O_2$
55	4-CH_3O-3-(n-C_4H_9O)C_6H_3	CH_3	CH_3	112–114	C	33	$C_{15}H_{21}N_3O_3$
56	2-C_4H_3S	CH_3	CH_3	108–110[t]	C	59	$C_8H_9N_3OS$
57	2-C_4H_3S	C_2H_5	CH_3	47–49	J	53	$C_9H_{11}N_3OS$

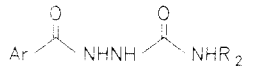
[a] A = 2-propanol, B = aqueous ethanol, C = ethyl acetate/hexane, D = ethanol, E = 2-butanone, F = aqueous 2-propanol, G = cyclohexane, H = hexane, I = Kugelrohr distilled 130–135 °C (0.5 mm), J = Kugelrohr distilled 210–225 °C (0.3 mm). [b] Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds. [c] Literature[2] mp 326–327 °C. [d] Literature[21] mp 218–219 °C. [e] Literature[4] mp 179–180 °C. [f] Literature[26] mp 168 °C. [g] Literature[27] mp 213–215 °C. [h] Literature[28] mp 199–200 °C. [i] Literature[27] mp 216–218 °C. [j] Literature[29] mp 183–185 °C. [k] Literature[29] mp 249–251 °C. [l] Literature[4] mp 144–145 °C. [m] Literature[30] bp 130 °C (0.2 mm). [n] Literature[27] mp 87–89 °C. [o] Literature[27] mp 126–128 °C. [p] Literature[27] mp 73–75 °C. [q] Literature[27] mp 79–81 °C. [r] Literature[27] mp 62–64 °C. [s] Literature[27] mp 104–106 °C. [t] Literature[29] mp 108–110 °C.

Scheme I



isolated in 90% yield. It should be noted that, under the same conditions, attempted hydrolysis of thioether 8 returned only starting material. With the above two exceptions, all the remaining triazol-3-ones were prepared

Table II. 1-Aroyl-4-alkylsemicarbazides



compd	Ar	R_2	mp, °C	crystn solvent[a]	% yield	formula[b]
12	C_6H_5	CH_3	181–183[c]	A	77	$C_9H_{11}N_3O_2$
13	C_6H_5	C_2H_5	175–177[d]	B	85	$C_{10}H_{13}N_3O_2$
14	2-ClC_6H_4	CH_3	184–186	C	84	$C_9H_{10}ClN_3O_2$
15	4-ClC_6H_4	CH_3	234–236[e]	C	91	$C_9H_{10}ClN_3O_2$
16	4-ClC_6H_4	C_2H_5	237–239[f]	C	89	$C_{10}H_{12}ClN_3O_2$
17	3,4-$Cl_2C_6H_3$	CH_3	245–247	C	56	$C_9H_9Cl_2N_3O_2$
18	2-FC_6H_4	CH_3	122–124	B	42	$C_9H_{10}FN_3O_2$
19	4-FC_6H_4	CH_3	222–224[g]	C	75	$C_9H_{10}FN_3O_2$
20	3-$CF_3C_6H_4$	C_2H_5	188–189	C	29	$C_{11}H_{12}F_3N_3O_2$
21	4-$CH_3C_6H_4$	CH_3	194–196	C	86	$C_{10}H_{13}N_3O_2$
22	4-$CH_3OC_6H_4$	CH_3	180–181	C	52	$C_{10}H_{13}N_3O_3$
23	4-CH_3O-3-(n-C_4H_9O)C_6H_3	CH_3	195–197	C	83	$C_{14}H_{21}N_3O_4$
24	2-C_4H_3S	CH_3	165–167	C	84	$C_7H_9N_3O_2S$
25	4-C_5H_4N	CH_3	224–225	C	80	$C_8H_{10}N_4O_2$

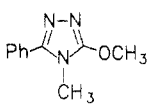
[a] A = water, B = 2-propanol, C = ethanol. [b] Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds. [c] Literature[31] mp 181 °C. [d] Literature[31] mp 175 °C. [e] Literature[27] mp 234–236 °C. [f] Literature[27] mp 237–239 °C. [g] Literature[27] mp 222–224 °C.

by the method of Kubota and Uda.[4] Thus, reaction of hydrazides **10** and isocyanates **11** gave aroylsemicarbazides **12–25** (Table II). Cyclization of these semicarbazides in refluxing aqueous sodium hydroxide afforded 2-unsubstituted triazol-3-ones **26–39**, which were subsequently N-alkylated with alkyl iodides, yielding 2,4-dialkyltriazol-3-ones **40–57**.

Results and Discussion

The anticonvulsant activities of the triazoles listed in Table I were initially evaluated against maximal electroshock- and pentylenetetrazole-induced seizures in mice. The results are listed in Table III, where it can be seen that approximately one-third of the compounds exhibited activity against both convulsant stimuli. Also listed in Table III are LD_{50} estimates which show that the acute toxicity associated with the use of these compounds is reasonably low, three-fourths of the compounds having an estimated LD_{50} of greater than 400 mg/kg. In terms of structure it can be observed that all of the least toxic compounds (LD_{50} > 800 mg/kg) are unsubstituted in the 2-position of the triazole ring.

The correlation between structure and activity in this series was not straightforward; however, some trends could be observed. As alluded to earlier, the most obvious structural feature necessary for anticonvulsant activity was the carbonyl moiety. Changing this group to either a thiocarbonyl group, e.g. **1** (Ar = C_6H_5, R_1 = R_2 = CH_3, X = S),[5] or a methyl ether, e.g. **58**,[4] resulted in a loss of



58

anticonvulsant activity. In general, the aryl groups of the active compounds were monohalogenated (compounds **28, 29, 33, 43, 44, 45,** and **47**) although phenyl derivative **26** and 3-(trifluoromethyl)phenyl derivative **52** also had activity. In contrast, aromatic rings containing either alkyl groups (compound **53**) or alkoxy groups (compounds **36, 37,** and **54**) were generally inactive as were compounds containing heteroaryl groups (compounds **38, 39, 56,** and **57**). Substitution at the 2-position of the triazole nucleus did not appear to dramatically affect the anticonvulsant activity. For example, compounds which were either unsubstituted (compounds **26, 28, 29,** and **33**), methylated (compounds **43, 44, 45,** and **52**), or ethylated (compound

Table III. Biological Activities for 2,4-Dihydro-3*H*-1,2,4-triazol-3-ones

compd	estimated LD_{50}, mg/kg ip	activity[a,b] MES[c]	activity[a,b] PTZ[d]	IC_{50}, μM [^{3}H]fluni-trazepam	IC_{50}, μM [^{3}H]CPP[e]
1[f]	100–200	–	–		
3	>800	–	–		
6	400–800	–	++		
26	>800	++	+++	>100	>100
27	>800	–	++		
28	400–800	++	+++	>100	>100
29	>800	+++	+++	>100	>100
30	200–400	–	+++	>100	>100
31	200–400	+	+	>100	>100
32	>800	–	+++		
33	>800	+++	+++	>100	>100
34	200–400	–	++		
35	>800	–	++		
36	>800	–	–		
37	100–200	–	–		
38	200–400	–	–	>100	>100
39	>800	–	–	>100	>100
40	400–800	–	++		
41	400–800	++	+		
42	400–800	–	++		
43	400–800	+	+++		
44	400–800	++	+++	>100	>100
45	200–400	++	+++	>100	>100
46	400–800	–	+++		
47	200–400	++	++		
48	400–800	–	–		
49	100–200	+	+		
50	400–800	–	++		
51	400–800	+	+		
52	400–800	++	+++	>100	>100
53	400–800	–	–		
54	400–800	–	–		
55	400–800	–	++	33.2 ± 1.2	>100
56	400–800	–	–		
57	400–800	–	–		
58	>800	–	–		
N-methyl-D-aspartate					6
diazepam				0.012	

[a] Activity = protection of >50% of the treated mice. [b] + = activity at $^1/_4$ LD_{50} range, ++ = activity at $^1/_8$ LD_{50} range, +++ = activity at $^1/_{16}$ LD_{50} range. [c] MES = antagonism of maximal electroshock-induced seizures in mice. [d] Antagonism of pentylenetetrazole-induced seizures in mice. [e] 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid. [f] Ar = C_6H_5, R_1 = R_2 = CH_3, X = S (ref 5).

(5) Sandström, J.; Wennerbeck, I. *Acta Chem. Scand.* **1966**, *20*, 57.

Table IV. Anticonvulsant Activities for **45** and Reference Compounds

compound	ED_{50}, mg/kg ip					
	MES[a]	PTZ[b]	MPA[c]	BIC[d]	STR[e]	QA[f]
45	48	17	31	15	~101	26
carbamazepine	13–25	>200	86		61	25
phenobarbital	20	24	30	9	52	52
phenytoin	26	>32	89	>200	>200	*g*
valproate	>200	100–200	183	28	>200	>256
clonazepam	25–50	0.06	0.20	0.07	~2[h]	<8[i]

[a] Antagonism of maximal electroshock-induced seizures in mice. [b] Antagonism of pentylenetetrazole-induced seizures in mice. [c] Antagonism of 3-mercaptopropionic acid induced seizures in mice. [d] Antagonism of bicuculline-induced seizures in mice. [e] Antagonism of strychnine-induced seizures in mice. [f] Antagonism of quinolinic acid induced seizures in mice. [g] Maximum effect, 50% at 20 mg/kg. [h] Irregular dose–response function. [i] Irregular dose–response function; 90% effect at 8 mg/kg.

47) at this position had activity. On the other hand, alkyl substitution at the 4-position of the triazole ring appeared to be more necessary, accepting either methyl (compounds **26**, **28**, **29**, **33**, **43**, and **44**) or ethyl (compounds **45**, **47**, and **52**) substitution. Of the two examples which were unsubstituted at this position, one (compound **3**) was inactive against both seizure stimuli while the other (compound **6**) was active against only pentylenetetrazole-induced seizures.

From the examples listed in Table I, we selected compound **45** for further evaluation. Thus, **45** was examined in several additional seizure models, and these results are presented in Table IV. With the exception of strychnine-induced seizures, **45** was active against all of the seizure models within a relatively narrow dose range. The anticonvulsant profile observed for **45** appeared to be somewhat better than those observed for either carbamazepine, phenytoin, or sodium valproate and was similar to that observed for phenobarbital. On the other hand, **45** was considerably less potent than clonazepam in all of the seizure models with the exception of maximal electroshock-induced seizures, where their activities were comparable.

Among the possible mechanisms which are known to inhibit seizure activity are enhancement of GABA transmission[6,7] and antagonism of glutamate transmission.[8,9] Accordingly, a number of the triazoles listed in Table I were examined for affinity at the benzodiazepine binding site on the $GABA_A$ receptor complex and at the glutamate site on the NMDA subtype of the glutamate receptor. The results are presented in Table III, showing that none of the compounds tested had any appreciable affinity for either of these sites. In addition, compound **45** did not exhibit any appreciable affinity for either the kainate or quisqualate receptor subtypes (IC_{50} values > 100 μM).

Recent studies have suggested that excessive glutamate receptor activation may be involved in various neurodegenerative disorders.[10–13] Since quinolinic acid is an agonist at glutamate receptors, the activity we observe against quinolinic acid induced seizures might represent a functional antagonism at the glutamate receptor.[14–17] Accordingly, three of the triazoles which had exhibited activity against quinolinic acid induced seizures were examined for activity against the hippocampal neurodegeneration produced by hypoxia in gerbils.[18,19] As can be seen in Table V, compound **45** produced a significant protection, decreasing hypoxic damage to only 33% of that seen in controls, while **28** and **30** were essentially inactive. This protective activity was equal to that produced by phencyclidine, a known NMDA antagonist with neuroprotective activity.[14]

Table V. Activity against Quinolinic Acid Induced Seizures and Effect on Hypoxia-Induced Neuronal Damage of PCP and Compounds **28**, **30**, and **45**

compound	QA[b]	damage scores[a] (mean ± SEM)	
		sham	hypoxic
water		0.33 ± 0.09	2.98 ± 0.24
PCP[c]		1.06 ± 0.48	1.14 ± 0.38[d]
28	~65	0.22 ± 0.16	2.95 ± 0.19
30	~51	0.33 ± 0.16	2.64 ± 0.48
45	26	0.33 ± 0.24	0.97 ± 0.52[d]

[a] Hippocampal (CA1 region) neuronal damage scores assessed in gerbils pretreated with drug and subjected to bilaterial carotid occlusion for 5 min (or sham operation). Compounds **28**, **30**, and **45** were administered at 40 mg/kg ip 20 min prior to occlusion. [b] ED_{50}, mg/kg ip in mice against quinolinic acid induced seizures. [c] Phencyclidine hydrochloride (15 mg/kg ip). [d] $p < 0.05$ vs water.

In summary, a series of 3*H*-1,2,4-triazol-3-ones has been examined for anticonvulsant activity. Approximately one-third of the compounds in this series exhibited activity against both maximal electroshock- and pentylenetetrazole-induced seizures in mice. Of these examples, triazole **45** was examined in several additional seizure models where it was found to be active. This generalized antiseizure activity suggests that compounds such as **45** might find utility against a variety of seizure disorders. Mechanistically, these compounds do not directly interact with either the benzodiazepine binding site on the $GABA_A$ receptor complex or the glutamate binding site on the NMDA subtype of the glutamate receptor. In addition, triazole **45** also protected against hypoxic neuronal degeneration in gerbils, suggesting that it also might find utility as a neuroprotective agent.

Experimental Section

Melting points were determined in open capillaries on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed on site and were within ±0.4% of the theoretical values. All compounds were routinely examined by proton NMR (Varian FT80A, EM390, XL300, and Gemini 300), IR (Perkin-Elmer 180), and TLC (silica gel).

1-Methyl-5-(methylsulfonyl)-3-phenyl-1*H*-1,2,4-triazole (9). MCPBA (14.7 g, 68.1 mmol, commercial 80% mixture) was added portionwise to a stirred, 0 °C, solution of 1-methyl-5-(methylthio)-3-phenyl-1*H*-1,2,4-triazole[4] (5.6 g, 27 mmol) and

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CH_2Cl_2 (180 mL). After being stirred overnight at room temperature, the reaction was diluted with CH_2Cl_2 (300 mL). The reaction was transferred to a separatory funnel where it was washed two times with saturated aqueous $NaHCO_3$ and once with saturated aqueous NaCl. The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4 before it was evaporated at reduced pressure. The resulting solid was purified by flash chromatography[20] (2% EtOAc/CH_2Cl_2). Subsequent crystallization from EtOAc/hexane afforded colorless needles: 4.7 g (73%); mp 112–114 °C; ^1H NMR ($CDCl_3$) δ 3.49 (s, 3 H), 4.24 (s, 3 H), 7.42–7.51 (m, 3 H), 8.04–8.12 (m, 2 H); ^{13}C NMR ($CDCl_3$) 37.91, 42.91, 126.39, 128.63, 129.36, 129.94, 151.72, 160.79 ppm. Anal. ($C_{10}H_{11}N_3O_2S$), C, H, N.

2,4-Dihydro-2-methyl-5-phenyl-3*H*-1,2,4-triazol-3-one (6). A stirred mixture of **9** (2.84 g, 12.0 mmol) and 1 M aqueous NaOH (30 mL) was heated to reflux. After refluxing 30 min, the reaction was allowed to cool to room temperature at which time it was acidified by the addition of 1 M aqueous HCl (32 mL). The resulting precipitate was collected by filtration. Crystallization from aqueous EtOH afforded colorless needles: 1.88 g (90%); mp 218–219 °C (lit.[21] mp 218–219 °C).

General Procedure for the Preparation of 1-Aroyl-4-alkylsemicarbazides 12–25. A stirred mixture of hydrazide **10** (30 mmol) and dry THF was warmed until a homogeneous solution was obtained. The alkyl isocyanate **11** (33 mmol) was then added dropwise. After 17 h the mixture was diluted with Et_2O (80 mL) and the precipitate was collected by filtration. The collected solid was washed with Et_2O and subsequently purified by crystallization from one of the solvents listed in Table II.

General Procedure for the Preparation of 4-Alkyl-5-aryl-2,4-dihydro-3*H*-1,2,4-triazol-3-ones 26–39. A stirred mixture of the 1-aroyl-4-alkylsemicarbazide (98.1 mmol) and 1 M aqueous NaOH (118 mL) was heated to reflux. After 23 h the mixture was allowed to cool to room temperature when it was acidified by the addition of 1 M aqueous HCl (130 mL). The mixture was cooled in an ice bath and the precipitate was collected by filtration. With the exception of **31**, which was flash chromatographed (EtOAc) prior to crystallization, all of the products were purified by crystallization from one of the solvents listed in Table I.

General Procedure for the Preparation of 5-Aryl-2,4-dialkyl-2,4-dihydro-3*H*-1,2,4-triazol-3-ones 40–57. To a stirred solution of the 4-alkyl-5-aryl-2,4-dihydro-3*H*-1,2,4-triazol-3-one (26.8 mmol) and 1 M aqueous NaOH (30 mL) was added a solution of the alkyl iodide (40 mmol) in EtOH (10 mL). The reaction was routinely monitored by removal of a small aliquot, acidification, and TLC of an EtOAc extract. In general, methylations were complete in 24 h whereas ethylation and propylation took considerably longer (27 days in the case of **48**). The reaction was transferred to a separatory funnel where it was extracted several times with EtOAc. The extracts were combined, washed with saturated aqueous NaCl, and dried over anhydrous Na_2SO_4. The drying agent was removed by filtration and the filtrate was evaporated at reduced pressure, leaving the crude product which was purified by a combination of flash chromatography (generally 50% EtOAc/CH_2Cl_2) and crystallization from one of the solvents listed in Table I.

Anticonvulsant Activity. Groups of five or ten CD-1 male mice were housed individually before being administered the test compound ip as a solution in distilled H_2O or a suspension in distilled H_2O/Tween 80. Reference compounds were prepared similarly. The H_2O/Tween 80 vehicle had no effect in any of the test systems. Thirty minutes after administration of the test compound, mice were administered the convulsant stimulus: (a) maximal electroshock (MES, 50 mA, 0.2 s, corneal electrodes); (b) pentylenetetrazole (PTZ, 60 mg/kg iv); (c) 3-mercaptopropionic acid (MPA, 100 mg/kg iv); (d) bicuculline (BIC, 0.6 mg/kg iv); (e) strychnine sulfate (STR, 2.7 mg/kg ip); (f) quinolinic acid (QA, 7.7 μg icv). In each of these tests, mice were considered protected according to the following criteria: (a) MES, absence of tonic hind-limb extension; (b) PTZ, absence of clonic seizures for 2 min after PTZ administration; (c) MPA, absence of seizures for 5 min after MPA administration; (d) BIC, absence of the typical clonic-tonic seizure syndrome for 5 min after BIC administration; (e) STR, absence of tonic extension for more than 15 min after STR administration; (f) QA, absence of clonic-tonic seizures for 15 min after QA administration. The ED_{50} was defined as that dose causing significant protection from seizures in 50% of the mice and it was calculated, when appropriate, with a computer program for analysis of quantal data. For all calculated ED_{50} values, 95% confidence limits are within the range 0.5–2.0 ED_{50}. All ED_{50} values were calculated from the results of at least four doses, each administered to at least one group of 10 mice.

Intraperitoneal LD_{50} Estimation. Groups of three CD-1 male mice (18–26 g) were administered graded doses of the test compounds prepared as either solutions in distilled H_2O or suspensions in distilled H_2O/Tween 80. Standard compounds were prepared similarly. Deaths occurring during the next 7 days were recorded, and the dose range encompassing the 50% lethal effect was recorded as the estimated LD_{50}.

Receptor Binding. Male, Sprague–Dawley rats (Charles River, 150–200 g) were used as the source of the cortical or hippocampal membrane preparations used in the in vitro receptor binding assays. 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid ([^{3}H]CPP, 30.7 Ci/mmol) binding was performed as previously described.[22] Frozen membranes were thawed at room temperature and washed with ice cold assay buffer (3 × 30 mL). Assays (1 mL) were conducted in scintillation minivials containing 10 nM [^{3}H]CPP, 200 μg of membrane protein (Bio-Rad protein assay), 50 mM Tris-HCl buffer (pH 7.4 at 25 °C), various concentrations of test compounds (pH adjusted to 7.4) or 1 mM unlabeled glutamate (nonspecific binding). Following a 15-min incubation at 25 °C, vials were centrifuged at 46000*g* for 10 min. Supernatants were decanted and the pellets were solubilized overnight in Ready Protein$^+$ (4 mL, Beckman). Specific binding accounted for 76% of the total binding, which was 11 000 to 20 000 dpm/assay vial. The [^{3}H]flunitrazepam (81.8 Ci/mmol) binding assay was performed as previously described.[23]

[^{3}H]-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ([^{3}H]AMPA) binding was performed as previously described.[24] Assay vials contained 0.5 mg of membrane protein, 5 nM [^{3}H]-AMPA (Du Pont NEN, 27.6 Ci/mmol), 100 mM KSCN, various concentrations of test compounds, 2.5 mM $CaCl_2$, 30 mM Tris-HCl (pH 7.4 at 4 °C) in a final volume of 0.5 mL. Nonspecific binding was defined using 1 mM L-glutamate. Specific binding accounted for >85% of total binding. Following incubation on ice for 60 min, bound ligand was collected by centrifugation.

[^{3}H]Kainate binding was performed as previously described.[25] Assay vials contained 0.5 mg of membrane protein (prepared as above for [^{3}H]AMPA binding), 5 nM [^{3}H]kainate (Du Pont NEN, 58 Ci/mmol), various concentrations of test compounds, 50 mM Tris-HCl (pH 7.4 at 4 °C) in a final volume of 1 mL. Nonspecific binding was defined using 100 μM unlabeled kainic acid. Specific binding accounted for >80% of the total binding. Following incubation on ice for 60 min, bound ligand was collected by centrifugation.

Neuroprotective Activity. Male gerbils (Tumblebrook Farms, 60–80 g) were treated with either water (n = 24), phencyclidine hydrochloride (15 mg/kg ip, n = 12), **28** (40 mg/kg ip, n = 12), **30** (40 mg/kg ip, n = 11), or **45** (40 mg/kg ip, n = 9) 20 min prior to a 5-min hypoxic episode. The gerbils were anesthetized with 3–4% halothane, a midline incision was made, and both carotid arteries were exposed. Hypoxia was induced

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by occluding the carotid arteries with 7-mm Mayfield aneurysm clips for 5 min. Interruption of the carotid blood flow was confirmed by observation of the blanching of radial arteries of the retina using an opthalmoscope. Following removal of the clips, the incision was closed and the animal was allowed to recover. In sham animals, the carotids were exposed, and each animal was maintained under anesthesia for 5 min. After complete recovery from surgery, animals were returned to home cages. Seven days after surgery, the gerbils were killed and their brains were prepared for histological evaluation. Left and right hemispheres were assessed separately for hippocampal damage by three independent investigators who were blind to the treatments. Damage was quantified by using a scoring system which has previously been reported.[14] Damage scores for each group were averaged to obtain the reported values. Comparisons were made by using the Student's t test with the significance level set at $p < 0.05$.

Binding of Indolylalkylamines at 5-HT$_2$ Serotonin Receptors: Examination of a Hydrophobic Binding Region

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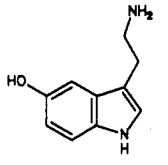
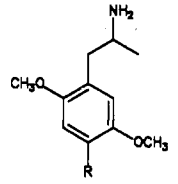
Taking advantage of a proposed hydrophobic region on 5-HT$_2$ receptors previously identified by radioligand-binding studies utilizing various phenylisopropylamine derivatives, we prepared and evaluated several N_1 - and/or C_7-alkyl-substituted derivatives of α-methyltryptamine in order to improve its affinity and selectivity. It was determined that substitution of an n-propyl or amyl group has similar effect on affinity regardless of location (i.e., N_1 or C_7). The low affinity of several N_1-alkylpyrroleethylamines suggests that the benzene portion of the α-methyltryptamines is necessary for significant affinity. Whereas tryptamine derivatives generally display little selectivity for the various populations of 5-HT receptors, N_1-n-propyl-5-methoxy-α-methyltryptamine (**3h**) binds with significant affinity (K_i = 12 nM) and selectivity at 5-HT$_2$ receptors relative to 5-HT$_{1A}$ (K_i = 7100 nM), 5-HT$_{1B}$ (K_i = 5000 nM), 5-HT$_{1C}$ (K_i = 120 nM), and 5-HT$_{1D}$ (K_i > 10000 nM) receptors. As a consequence, this is the most 5-HT$_2$-selective indolylalkylamine derivative reported to date.

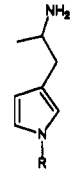
The 5-HT$_2$ population of serotonin (5-hydroxytryptamine; 5-HT) receptors is currently of clinical interest because of its potential role in cardiovascular function and possible involvement in various mental disorders such as schizophrenia, depression, hallucinations, and anxiety (see ref 1 for a review). To date, there are two major classes of 5-HT$_2$ agonists: indolylalkylamines, such as 5-HT (1)



1



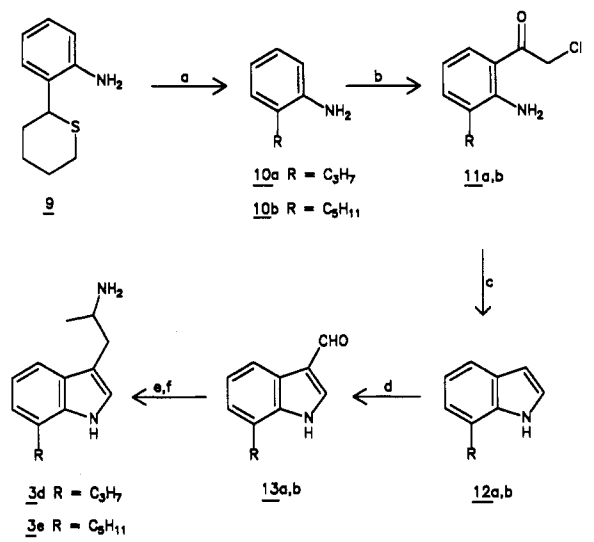
2



4a R = H
4b R = C_3H_7
4c R = C_5H_{11}

itself, and phenylisopropylamines, such as certain 4-substituted derivatives of 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA; **2**, R = H) (e.g. **2**, R = methyl, n-propyl, and bromo). In general, indolylalkylamines are nonselective agents that bind at multiple populations of 5-HT receptors.[2,3] The phenylisopropylamines, on the other hand, are considerably more selective and bind primarily at 5-HT$_2$ sites (with a significant, though lower, affinity for 5-HT$_{1C}$ sites);[4] however, certain of these agents may only be partial agonists.[5] The selectivity and affinity of the phenylisopropylamines for 5-HT$_2$ sites is related to the nature of the 4-position substituent; specifically, we have found that high affinity is associated with increased lipo-

Scheme I[a]



9

10a R = C_3H_7
10b R = C_5H_{11}

11a,b

3d R = C_3H_7
3e R = C_5H_{11}

13a,b

12a,b

[a] (a) Raney Ni; (b) $BCl_3/AlCl_3$, $ClCH_2CN$; (c) $NaBH_4$; (d) $POCl_3$/DMF; (e) $EtNO_2$; (f) $LiAlH_4$.

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