5-Aryl-3-(alkylthio)-4*H*-1,2,4-triazoles as Selective Antagonists of Strychnine-Induced Convulsions and Potential Antispastic Agents

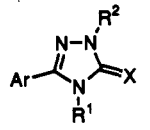
John M. Kane,* Michael A. Staeger, Christopher R. Dalton, Francis P. Miller, Mark W. Dudley, Ann Marie L. Ogden, John H. Kehne, Herbert J. Ketteler, Timothy C. McCloskey, Yaw Senyah, Paula A. Chmielewski, and Jerry A. Miller

Marion Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215

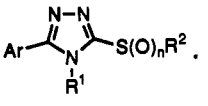
Received September 20, 1993•

Selected examples from three series of isomeric (alkylthio)-1,2,4-triazoles were prepared and examined for anticonvulsant activity versus strychnine-, maximal-electroshock-, pentylenetetrazole-, and 3-mercaptopropionic-acid-induced seizures in mice. A number of 5-aryl-3-(alkylthio)-4*H*-1,2,4-triazoles were selective antagonists of strychnine-induced convulsions. The isomeric 3-aryl-5-(alkylthio)- and 5-aryl-3-(alkylthio)-1*H*-1,2,4-triazoles were essentially inactive as anticonvulsants. The most potent antagonist of strychnine-induced convulsions was 5-(2-fluorophenyl)-4-methyl-3-(methylthio)-4*H*-1,2,4-triazole (**3s**), while the most selective antagonist was 5-(3-fluorophenyl)-4-methyl-3-(methylsulfonyl)-4*H*-1,2,4-triazole (**3aa**). The anticonvulsant profiles of these 4*H*-1,2,4-triazoles suggested that they were acting functionally like glycine receptor agonists. Since it has recently been postulated that compounds possessing glycine-agonist-like properties might be useful in the treatment of spasticity, we examined 5-phenyl-4-methyl-3-(methylsulfonyl)-4*H*-1,2,4-triazole (**3c**) in an *in vivo* model of spasticity. In this regard, **3c** reduced the occurrence of hyperreflexia in rats that had received spinal transections 5-10 weeks previously. While triazole **3c** appeared to possess glycine-agonist-like properties *in vivo*, it did not displace [^{3}H]strychnine binding from rat brain stem/spinal cord membranes *in vitro*. On the other hand, **3c** enhanced muscimol-stimulated ^{36}Cl influx in a rat cerebellar membrane preparation, indicating a possible interaction of these triazoles with the $GABA_A$ receptor.

We have recently described the antidepressant-like activity associated with a series of 2,4-dihydro-3*H*-1,2,4-triazol-3-thiones **1**.[1] More recently, 2,4-dimethyl-5-(3-fluorophenyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (**1**, Ar = 3-FC_6H_4, $R^1 = R^2 = CH_3$; MDL 26,479) has shown congnition enhancing potential in several test systems.[2,3] During the development of this series, we also prepared a number of 2,4-dihydro-3*H*-1,2,4-triazol-3-ones **2** as potential metabolites of **1**. Interestingly, derivatives of **2** were completely devoid of antidepressant-like activity, instead exhibiting anticonvulsant activity against a variety of convulsant stimuli.[4] We have subsequently extended our investigations into the central nervous system (CNS) actions of 1,2,4-triazoles and now report that certain 5-aryl-3-(alkylthio)-4*H*-1,2,4-triazoles **3** are selective antagonists of strychnine-induced convulsions which may be useful in the treatment of spasticity.



1, X = S
2, X = O

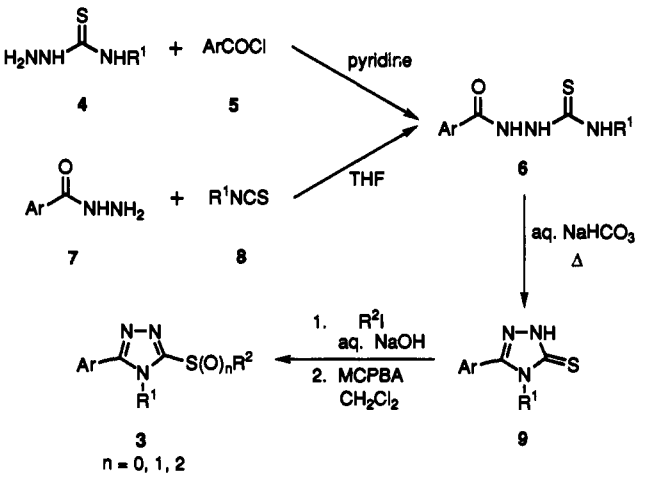


3
n = 0, 1, 2

Chemistry

The 4*H*-1,2,4-triazoles employed in this study were prepared according to the methods of Kubota and Uda[5] (Scheme 1). Thus, acylation of thiosemicarbazides **4** with aroyl chlorides **5** afforded 1-aroylthiosemicarbazides **6** (Table 1). Alternatively, derivatives of **6** could be prepared by the reaction of carboxylic acid hydrazides **7** and alkyl isothiocyanates **8**. Alkaline ring closure of **6** gave 5-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **9** (Table 2) which were readily S-alkylated yielding 5-aryl-3-(alkylthio)-4*H*-1,2,4-triazoles **3** ($n = 0$) (Table 3). Oxidation to the corresponding sulfoxides **3** ($n = 1$) and sulfones **3** ($n = 2$) was then easily accomplished using either 1 or 2 equiv of *m*-chloroperoxybenzoic acid (MCPBA).

Scheme 1

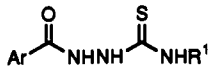


The isomeric 3-aryl-5-(alkylthio)-1*H*-1,2,4-triazoles **10** were prepared as depicted in Scheme 2. More specifically, condensation of aroyl isothiocyanates **11** and alkyl hydrazines **12** in toluene at 80–85 °C gave a mixture of the desired 2-alkyl-5-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **13**[6] and what was presumed to be the intermediate 4-aroyl-2-alkylthiosemicarbazides **14**. Heating this mixture in aqueous sodium bicarbonate completed the cyclization of **14** yielding essentially pure **13**. Alternatively, reaction of aroyl chlorides **5** and 2-alkylthiosemicarbazides **15**[7] afforded 1-aroyl-2-alkylthiosemicarbazides **16** which,

• Abstract published in *Advance ACS Abstracts*, December 1, 1993.

0022-2623/94/1837-0125$04.50/0

Table 1. 1-Aroylthiosemicarbazides

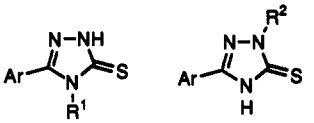


6

compd	Ar	R^1	mp, °C	crystn solvent[a]	method	% yield	formula[b]
6a	C_6H_5	CH_3	199–200[c]	A	A	69	$C_9H_{11}N_3OS$
6b	2-$C_{10}H_7$	CH_3	211–212 dec	B	B	49	$C_{13}H_{13}N_3OS$
6c	4-$CH_3C_6H_4$	CH_3	183–185	A	A	54	$C_{10}H_{13}N_3OS$
6d	4-$CH_3OC_6H_4$	CH_3	205–206[d]	A	A	64	$C_{10}H_{13}N_3O_2S$
6e	2-ClC_6H_4	CH_3	196–197	A	A	73	$C_9H_{10}ClN_3OS$
6f	4-ClC_6H_4	CH_3	201–202 dec[e]	A	A	75	$C_9H_{10}ClN_3OS$
6g	4-ClC_6H_4	C_2H_5	192–195 dec[f]	A	B	52	$C_{10}H_{12}ClN_3OS$
6h	2-FC_6H_4	CH_3	216–217 dec	C	A	79	$C_9H_{10}FN_3OS$
6i	2-FC_6H_4	C_2H_5	171–173	A	B	49	$C_{10}H_{12}FN_3OS$
6j	3-FC_6H_4	CH_3	199–201 dec	A	B	46	$C_9H_{10}FN_3OS$
6k	4-FC_6H_4	H	187–189[g]	A	B	25	$C_8H_8FN_3OS$
6l	4-FC_6H_4	CH_3	169–170	A	A	67	$C_9H_{10}FN_3OS$
6m	2-C_4H_3S	CH_3	201–202	A	A	57	$C_7H_9N_3OS_2$

[a] A = ethanol, B = ethanol/acetone, C = aqueous ethanol. [b] Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds except **6k** and **6l**. [c] Literature[9] mp 195 °C. [d] Literature[9] mp 210 °C. [e] Literature[10] mp 196–197 °C. [f] Literature[11] mp 203–204 °C. [g] Literature[12] mp 150 °C.

Table 2. 1,2,4-Triazole-3-thiones



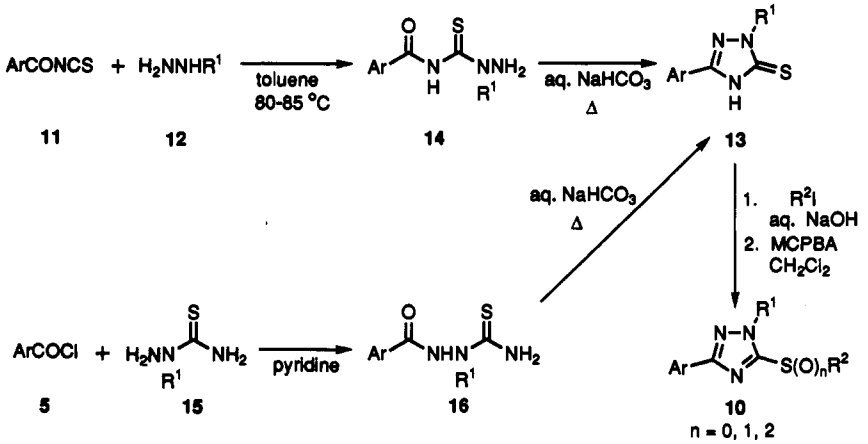
9 13

compd[a]	Ar	R^1	mp, °C	crystn solvent[b]	method	% yield	formula[c]
9a	C_6H_5	CH_3	164–166[d]	A	C	60	$C_9H_9N_3S$
9b	$C_{10}H_7$	CH_3	223–225	A	C	63	$C_{13}H_{11}N_3S$
9c	4-$CH_3C_6H_4$	CH_3	201–203	A	C	49	$C_{10}H_{11}N_3S$
9d	4-$CH_3OC_6H_4$	CH_3	172–174[e]	A	C	62	$C_{10}H_{11}N_3OS$
9e	2-ClC_6H_4	CH_3	142–144	B	C	77	$C_9H_8ClN_3S$
9f	4-ClC_6H_4	CH_3	210–212[f]	C	C	31	$C_9H_8ClN_3S$
9g	4-ClC_6H_4	C_2H_5	204–206[g]	C	C	68	$C_{10}H_8ClN_3S$
9h	2-FC_6H_4	CH_3	137–139[h]	B	C	40	$C_9H_8FN_3S$
9i	2-FC_6H_4	C_2H_5	138–140	B	C	48	$C_{10}H_{10}FN_3S$
9j	3-FC_6H_4	CH_3	150–151	D	C	47	$C_9H_8FN_3S$
9k	4-FC_6H_4	H	269–272 dec[i]	E	C	54	$C_8H_6FN_3S$
9l	4-FC_6H_4	CH_3	207–209	A	C	68	$C_9H_8FN_3S$
9m	2-C_4H_3S	CH_3	155–157	C	C	77	$C_7H_7N_3S_2$
13a	4-ClC_6H_4	CH_3	269–271[j]	A	G	55	$C_9H_8ClN_3S$
13b	3-FC_6H_4	CH_3	237–239	F	H	40	$C_9H_8FN_3S$

[a] Triazolethiones **13** (Ar = C_6H_5, R^1 = CH_3) and **21** (Ar = C_0H_5, R^1 = CH_3) were prepared according to ref 13. [b] A = ethanol, B = ethyl acetate/hexane, C = isopropyl alcohol, D = sublimation 130–135 °C (0.005 mm), E = water, F = aqueous ethanol. [c] Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds. [d] Literature[14] mp 163–164 °C. [e] Literature[9] mp 176–178 °C. [f] Literature[10] mp 212–213 °C. [g] Literature[10] mp 203–204 °C. [h] Literature[1] mp 137–139 °C. [i] Literature[1] mp 269–272 °C. [j] Literature[1] mp 269–271 °C.

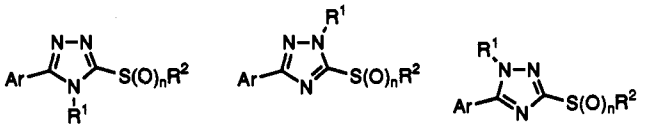
without purification, were cyclized to **13** in refluxing aqueous sodium bicarbonate. S-Alkylation[5] and subsequent oxidation with MCPBA proceeded in an analogous fashion to that presented in Scheme 1 to yield **10**.

Scheme 2



11 12 14 13 5 15 16 10

Table 3. *S*-Alkyl-1,2,4-triazoles



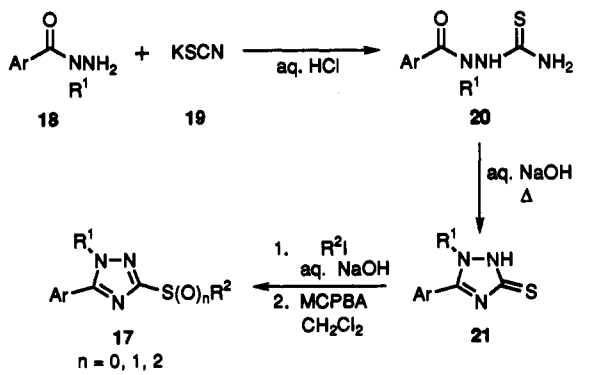
3 10 17

compd	Ar	R^1	R^2	n	mp, °C	crystn solvent[a]	method	% yield	formula[b]
3a	C_6H_5	CH_3	CH_3	0	134–136[c]	A	D	72	$C_{10}H_{11}N_3S$
3b	C_6H_5	CH_3	CH_3	1	144–146	B	E	73	$C_{10}H_{11}N_3OS$
3c	C_6H_5	CH_3	CH_3	2	158–160	C	F	72	$C_{10}H_{11}N_3O_2S$
3d	C_6H_5	CH_3	C_2H_5	0	94–99	C	D	68	$C_{11}H_{13}N_3S$
3e	C_6H_5	CH_3	C_2H_5	1	131–133	B	E	69	$C_{11}H_{13}N_3OS$
3f	C_6H_5	CH_3	C_2H_5	2	141–143	C	F	75	$C_{11}H_{13}N_3O_2S$
3g	$C_{10}H_7$	CH_3	CH_3	0	177–179	B	D	74	$C_{14}H_{13}N_3S$
3h	$C_{10}H_7$	CH_3	CH_3	1	224–226	D	E	59	$C_{14}H_{13}N_3OS$
3i	$C_{10}H_7$	CH_3	CH_3	2	204–206	E	F	75	$C_{14}H_{13}N_3O_2S$
3j	4-$CH_3C_6H_4$	CH_3	CH_3	0	140–142	C	D	61	$C_{11}H_{13}N_3S$
3k	4-$CH_3C_6H_4$	CH_3	CH_3	1	161–163	B	E	76	$C_{11}H_{13}N_3OS$
3l	4-$CH_3C_6H_4$	CH_3	CH_3	2	170–172	C	F	70	$C_{11}H_{13}N_3O_2S$
3m	4-$CH_3OC_6H_4$	CH_3	CH_3	0	149–151[d]	B	D	69	$C_{11}H_{13}N_3OS$
3n	4-$CH_3OC_6H_4$	CH_3	CH_3	1	168–170	B	E	75	$C_{11}H_{13}N_3O_2S$
3o	4-$CH_3OC_6H_4$	CH_3	CH_3	2	187–189	B	F	76	$C_{11}H_{13}N_3O_3S$
3p	2-ClC_6H_4	CH_3	CH_3	0	oil	H	D	77	$C_{10}H_{10}ClN_3S$
3q	4-ClC_6H_4	CH_3	CH_3	0	105–107	E	D	74	$C_{10}H_{10}ClN_3S$
3r	4-ClC_6H_4	C_2H_5	CH_3	0	113–115[e]	F	D	82	$C_{11}H_{12}ClN_3S$
3s	2-FC_6H_4	CH_3	CH_3	0	oil	H	D	73	$C_{10}H_{10}FN_3S$
3t	2-FC_6H_4	CH_3	CH_3	1	95–97	C	E	68	$C_{10}H_{10}FN_3OS$
3u	2-FC_6H_4	CH_3	CH_3	2	128–130	C	F	77	$C_{10}H_{10}FN_3O_2S$
3v	2-FC_6H_4	CH_3	C_2H_5	0	95–97	G	D	58	$C_{11}H_{12}FN_3S$
3w	2-FC_6H_4	CH_3	C_2H_5	1	63–67	H	E	84	$C_{11}H_{12}FN_3OS$
3x	2-FC_6H_4	CH_3	C_2H_5	2	145–147	C	F	83	$C_{11}H_{12}FN_3O_2S$
3y	2-FC_6H_4	C_2H_5	CH_3	0	oil	I	D	74	$C_{11}H_{12}FN_3S$
3z	3-FC_6H_4	CH_3	CH_3	0	151–153	B	D	72	$C_{10}H_{10}FN_3S$
3aa	3-FC_6H_4	CH_3	CH_3	2	175–177	C	F	72	$C_{10}H_{10}FN_3O_2S$
3ab	4-FC_6H_4	H	CH_3	0	145–146	F	D	78	$C_9H_8FN_3S$
3ac	4-FC_6H_4	CH_3	CH_3	0	193–195	A	D	82	$C_{10}H_{10}FN_3S$
3ad	2-C_4H_3S	CH_3	CH_3	0	83–85	B	D	63	$C_9H_9N_3S_2$
3ae	2-C_4H_3S	CH_3	CH_3	1	105–107	B	E	53	$C_9H_9N_3OS_2$
3af	2-C_4H_3S	CH_3	CH_3	2	157–159	C	F	76	$C_9H_9N_3O_2S_2$
10a	C_6H_5	CH_3	CH_3	0	oil[f]	J	D	78	$C_{10}H_{11}N_3S$
10b	C_6H_5	CH_3	CH_3	1	107–108	C	E	78	$C_{10}H_{11}N_3OS$
10c	C_6H_5	CH_3	CH_3	2	112–114[g]	C	F	73	$C_{10}H_{11}N_3O_2S$
10d	4-ClC_6H_4	CH_3	CH_3	0	84–86[h]	G	D	61	$C_{10}H_{10}ClN_3S$
10e	4-ClC_6H_4	CH_3	CH_3	2	122–123	A	F	81	$C_{10}H_{10}ClN_3O_2S$
10f	3-FC_6H_4	CH_3	CH_3	2	104–105	G	F	83	$C_{10}H_{10}FN_3O_2S$
17a	C_6H_5	CH_3	CH_3	0	49–50[i]	K	D	45	$C_{10}H_{11}N_3S$
17b	C_6H_5	CH_3	CH_3	1	89–91	C	E	77	$C_{10}H_{11}N_3OS$
17c	C_6H_5	CH_3	CH_3	2	69–71	C	F	74	$C_{10}H_{11}N_3O_2S$

[a] A = isopropyl alcohol, B = ethyl acetate, C = ethyl acetate/hexane, D = toluene, E = ethanol, F = aqueous ethanol, G = cyclohexane, H = isolated directly following flash chromatography, I = Kugelrohr distilled 225–235 °C (0.35 mm), J = Kugelrohr distilled 160–170 °C (0.3 mm), K = ether/pentane. [b] Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds. [c] Literature[15] mp 138 °C. [d] Literature[5] mp 153–155 °C. [e] Literature[16] mp 118 °C. [f] Literature[13] mp 24–25 °C. [g] Literature[4] mp 112–114 °C. [h] Literature mp 84–85 °C. [i] Literature[13] mp 48–49 °C.

The isomeric 5-aryl-3-(alkylthio)-1*H*-1,2,4-triazoles **17** were prepared as depicted in Scheme 3. Thus, reaction of hydrazides **18**[8] with potassium thiocyanate (**19**) afforded 1-aroyl-1-alkylthiosemicarbazides **20** which were cyclized to triazole-3-thiones **21**[5] in refluxing aqueous sodium hydroxide. S-Alkylation and subsequent oxidation with MCPBA proceeded in an analogous fashion to that presented in Scheme 1 to yield **17**.

Scheme 3



Results and Discussion

The anticonvulsant activities for the triazoles listed in Table 3 were evaluated against strychnine-, maximal-electroshock-, pentylenetetrazole-, and 3-mercaptopropionic-acid-induced seizures in mice. These results are presented in Table 4 along with LD_{50} estimates for the individual examples. As can be seen, the acute toxicity of these triazoles was quite low. All of the compounds exhibited an LD_{50} in excess of 400 mg/kg, and approximately three-quarters of the compounds exhibited an LD_{50} in excess of 800 mg/kg. In regard to anticonvulsant activity, approximately 40% of the triazoles in Table 4 displayed significant activity against strychnine-induced

Table 4. Activities for 5-Aryl-3-(alkylthio)-1,2,4-triazoles

compd	estmd LD_{50}, mg/kg ip	ED_{50}, mg/kg ip			
		STR[a]	MES[b]	PTZ[c]	MPA[d]
3a	>800	37.8	>200	100–200	100–200
3b	400–800	18.6	>100	>100	>100
3c	>800	12.8	>200	55	>200
3d	400–800	>200	>200	100–200	>200
3e	>800	14.4	>200	100–200	>200
3f	>800	19.3	>200	50–100	100–200
3g	>800	>200	>200	>200	>200
3h	>800	>200	>200	>200	>200
3i	>800	>200	>200	>200	>200
3j	400–800	50–100	>100	>100	>100
3k	>800	50–100	>200	>200	>200
3l	>800	>200	>200	>200	>200
3m	400–800	>100	>100	>100	>100
3n	400–800	50–100	>100	>100	>100
3o	>800	>200	>200	>200	>200
3p	400–800	>50	>50	>50	>50
3q	>800	28.1	>200	>200	
3r	>800	26.7	>50	25–50	50–100
3s	400–800	8.8	>200	61.3	
3t	>800	20.0	>200	57.9	>200
3u	>800	17.6	>100	>100	>100
3v	400–800	18.6	100–200	50–100	>200
3w	>800	32.7	100–200	>200	>200
3x	>800	85.8	>200	100–200	>200
3y	>800	75.0	100–200	25–50	100–200
3z	>800	13.8	>200	>200	>200
3aa	>800	12.1	>200	>200	>200
3ab	>800	>200	>200	>200	>200
3ac	>800	33.4	>200	>200	>200
3ad	400–800	34.1	>100	>100	>100
3ae	>800	32–50	>200	>200	>200
3af	>800	>200	>200	>200	>200
10a	>800	>200	100–200	50–100	>200
10b	400–800	>200	50–100	50–100	>200
10c	>800	>200	>200	>200	>200
10d	>800	>200	>200	50–100	>200
10e	>800	>200	>200	>200	>200
10f	400–800	>100	>100	>100	>100
17a	>800	>100	>100	>100	>100
17b	>800	>200	>200	>200	>200
17c	>800	>200	>200	>200	>200
diazepam		40.6	14.8	1.5	0.9
valproate		>200	>200	100–200	183
phenyltoin		>200	25.9	>32	89

[a] Antagonism of strychnine-induced seizures in mice. [b] Antagonism of maximal-electroshock-induced seizures in mice. [c] Antagonism of pentylenetetrazole-induced seizures in mice. [d] Antagonism of 3-mercaptopropionic-acid-induced seizures in mice.

seizures (ED_{50} < 50 mg/kg). In contrast, only two compounds exhibited an ED_{50} below 50 mg/kg versus pentylenetetrazole-induced seizures and no compound displayed an ED_{50} below 50 mg/kg versus either maximal-electroshock- or 3-mercaptopropionic-acid-induces seizures. The selectivity of these triazoles for antagonizing strychnine-induced seizures differs from the selectivity observed for more well-known anticonvulsants, e.g., diazepam, sodium valproate, and phenytoin (Table 4).

In regard to structure, the most important variable affecting the antistrychnine activity was the position of the alkyl group on the 1,2,4-triazole nucleus. Examination of Table 4 clearly showed that this activity resided solely with the 4*H*-1,2,4-triazoles **3**. Varying the structural features within **3** had a less defined impact on the antistrychnine activity, although some trends were noted. In general, the compounds which effectively antagonized strychnine-induced convulsions contained aromatic rings which were either unsubstituted or halogenated. Increasing the size of the aromatic group to naphthyl or the addition of electron-donating substituents to the aromatic ring tended to either decrease or abolish activity. The time-honored interchange of phenyl and thienyl substituents generally had a negative effect on the antistrychnine activity. Interestingly, fluorine substitution almost always resulted in compounds with good antistrychnine activity irrespective of the relative position of the fluorine substituent. A similar observation was noted relative to fluorine substitution and antidepressant-like activity in triazole-3-thiones **1**.[1] Increasing the size of the N_4-alkyl group was not beneficial, resulting in essentially no change in activity in one instance (**3q** versus **3r**) and significantly decreasing activity in another instance (**3s** versus **3y**). Variation of the size of the *S*-alkyl group generally decreased antistrychnine activity, although in one case, a slight increase was observed (**3b** versus **3e**). Increasing the oxidation state of the sulfur atom gave totally variable results, producing a steady increase in activity in one instance (**3a** versus **3b** versus **3c**), a steady decrease in activity in another instance (**3v** versus **3w** versus **3x**), and essentially no change in antistrychnine activity in a third instance (**3z** versus **3aa**). Of the compounds which we have studied, 2-fluorophenyl derivative **3s** was the most potent while 3-fluorophenyl sulfone **3aa** was the most selective, being more than 16 times more potent at antagonizing strychnine-induced convulsions than it was at antagonizing the seizures produced by the other convulsant stimuli.

Immunohistochemical studies in human and nonhuman tissues[17,18] have demonstrated that the strychnine-sensitive glycine receptor is localized primarily in caudal regions of the CNS, particularly in the brain stem and spinal cord. At this receptor, glycine produces hyperpolarizing effects[19] on motor neurons and interneurons by gating a chloride ion channel complex[20] which is closely related to the γ-aminobutyric $acid_A$ ($GABA_A$) receptor complex.[21] These hyperpolarizing effects are selectively blocked by strychnine.[22,23] In this regard, a number of the 4*H*-1,2,4-triazoles appeared to act functionally like glycine-receptor agonists. Recently, it has been suggested that compounds possessing glycine-receptor-agonist-like properties might be useful in the treatment of spasticity.[24,25] In order to examine this hypothesis, triazole **3c** (MDL 27,531), which had been identified as a selective antagonist of strychnine-induced convulsions very early in the development of this series, was chosen for further evaluation in an *in vivo* model of spasticity.

To elaborate, spinal transection in rats results in immediate paralysis and loss of spinal reflexes (spinal shock) followed by the gradual development of some manifestations of spasticity, i.e., hyperresponsiveness to light touch and spontaneous hind-limb movements (hyperreflexia).[26–28] Clonidine, an α_2-adrenergic agonist with demonstrated utility in treating various symptoms of spinal trauma-induced spasticity,[29–32] decreases elevated hind-limb muscle activity as measured electromyographically in the chronic spinal transected rat.[28] It was therefore decided to evaluate both **3c** and clonidine in this model of spasticity. In addition, both agents were also assessed for potential ataxic effects using the rotarod test.[33]

As can be seen in Table 5, triazole **3c** reduced the occurrence of hyperreflexia in rats that had received spinal transections 5–10 weeks previously. This suppression was statistically significant for the 10, 20, and 40 mg/kg doses of **3c**. In contrast, **3c** did not affect the amplitude of the reflex responses to an applied mechanical stimulus (stretching of the hind limb, reflex function) at any of the doses tested. Clonidine exhibited a pattern of effects on

Table 5. Effect of Either **3c** or Clonidine on Hyperreflexia and Reflux Function in Spinal Transected Rats

compd	dose[a]	integrated activity, % of control: spontaneous (hyperreflexia)	integrated activity, % of control: stretch-evoked (reflux function)
3c	5	137 ± 10	101 ± 8
	10	46 ± 17[b]	116 ± 26
	20	78 ± 22[b]	114 ± 33
	40	43 ± 9[b]	87 ± 29
clonidine	3.1	130 ± 44	112 ± 19
	12.5	50 ± 10[c]	115 ± 16
	50	30 ± 6[b]	86 ± 19

[a] Dose = mg/kg for **3c** and μg/kg for clonidine. [b] Significantly different from vehicle-injected controls, $p < 0.05$. [c] $p = 0.056$ versus controls.

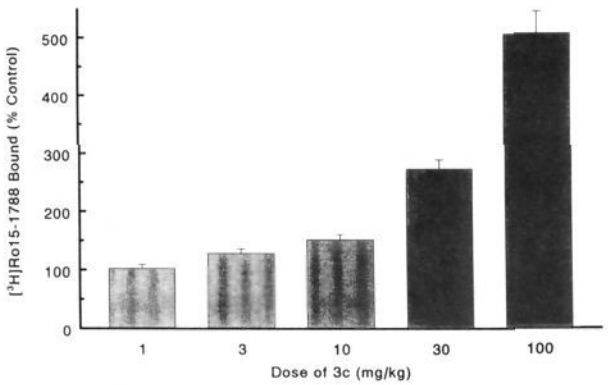


Figure 1. Effects of triazole **3c** on *in vivo* binding of [3H]Ro15-1788 in mouse cerebral cortex. Average total bound [3H]Ro15-1788 in control (saline-pretreated) animals was 37.3 ± 4.5 cpm/mg ($n = 6$). Values are expressed as percent of control with error bars representing SEM.

hyperreflexia and reflex function that was similar to that seen with **3c**. The effect of the 50 μg/kg dose was statistically significant, and the 12.5 μg/kg dose approached statistical significance ($p = 0.056$). In contrast, clonidine produced ataxia as measured by disruption of rotarod performance with an ED_{50} = 7.3 μg/kg, whereas triazole **3c** exhibited no apparent ataxic effects at doses as high as 800 mg/kg.

Compound **3c**, while effective *in vivo* against strychnine-induced seizures, *in vitro* at concentrations up to 100 μM did not displace [3H]strychnine binding from rat brain stem/spinal cord membranes. Additionally, this compound was also inactive *in vitro* at concentrations up to 100 μM at displacing [3H]muscimol ($GABA_A$ receptor), [3H]flunitrazepam ($GABA_A$–benzodiazepine site), [35S]-TBPS ($GABA_A$–picrotoxin convulsant site), and [3H]-baclofen ($GABA_B$ receptor) binding in rat cortical membranes.

While compound **3c** was inactive in binding assays *in vitro*, it was active *in vivo* in that it stimulated the binding of [3H]Ro15-1788 in mouse cerebral cortex in a dose-dependent manner (Figure 1).[34] Doses of **3c** in the range of 3–100 mg/kg ip, given 1 h prior to the radioligand, resulted in increases of 25–500% of control binding.

Muscimol (1 μM)-stimulated ^{36}Cl flux was enhanced 55% by the addition of compound **3c** (10 μM) in a rat cerebellar vesicle preparation (Table 6). Compound **3c** had no effect on ^{36}Cl uptake in the absence of agonists (data not shown). In the muscimol-stimulated ^{36}Cl flux assay, compound **3c** enhanced the uptake of ^{36}Cl by the agonists. This suggests a positive modulatory effect similar to that seen with benzodiazepine agonists.

Table 6. Enhancement of Muscimol-Stimulated ^{36}Cl Uptake into Rat Cerebellar Membrane Preparations by Triazole **3c**[a]

treatment	muscimol-stimulated ^{36}Cl uptake (nmol/mg of protein/3 s)	% of vehicle
vehicle	2.47 ± 0.33	100
3c	3.82 ± 0.62[b]	155

[a] Cerebellar membranes were exposed to the drugs as described in the Experimental Section. Muscimol was present at a concentration of 1 μM. Uptake is expressed as the mean ± SEM, $n = 3$. [b] Significantly different from vehicle ($p < 0.05$, student's t test).

In conclusion, three series of isomeric *S*-alkyl-1,2,4-triazoles were prepared and evaluated for anticonvulsant activity. Of the three series, certain 5-aryl-3-(alkylthio)-4*H*-1,2,4-triazoles were selective antagonists of strychnine-induced convulsions in mice. From this series, 4-methyl-3-(methylsulfonyl)-5-phenyl-4*H*-1,2,4-triazole (**3c**) was evaluated in an *in vivo* model of spasticity where it was shown to reduce the occurrence of hyperreflexia in rats that had received spinal transections 5–10 weeks previously. Mechanistically, the activity profile of **3c** suggested an interaction with the strychnine-sensitive glycine receptor. When tested, however, **3c** was found not to be a competitive antagonist at the strychnine-binding site since it did not inhibit the *in vitro* binding of [3H]strychnine in rat brain stem/spinal cord membranes. On the other hand, the strychnine-sensitive glycine receptor and the $GABA_A$ receptor show a high degree of sequence homology. Consequently, the ability of **3c** to influence the *in vivo* binding of the benzodiazepine antagonist [3H]Ro15-1788 may suggest that it is allosterically regulating both receptors. A positive modulatory effect of **3c** on the $GABA_A$ receptor was indicated by the ability of **3c** to enhance muscimol-stimulated ^{36}Cl flux in a rat cerebellar vesicle preparation. Previously, we have reported that certain 5-aryl-3*H*-1,2,4-triazole-3-thiones had activity which mimicked that of benzodiazepine inverse agonists *in vivo*.[35] The current results suggest that 1,2,4-triazoles may modulate the $GABA_A$ receptor in either a positive or negative manner. Further communications defining the nature of these interactions will be forthcoming.

Experimental Section

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

Representative Procedures for the Preparation of 1-Aroylthiosemicarbazides. 1-(2-Chlorobenzoyl)-4-methylthiosemicarbazide (6e). Method A. To a stirred, room-temperature solution of CH_3NCS (1.65 g, 22.6 mmol) in dry THF (50 mL) was added a solution of 2-chlorobenzoic acid hydrazide (4.20 g, 24.6 mmol) in dry THF (80 mL). The reaction was refluxed for 2 h and then stirred at room temperature overnight. The solvent was evaporated at reduced pressure, and the resulting solid was crystallized from EtOH affording a colorless solid: 3.99 g (73%); mp 196–197 °C; ^1H NMR (DMSO-d_6) δ 2.91 (d, 3 H, J = 3.8 Hz), 7.39–7.55 (m, 3 H), 7.76–7.87 (m, 2 H), 9.50 (br s, 1 H), 10.24 (br s, 1 H). Anal. ($C_9H_{10}ClN_3OS$) C, H, N.

1-(3-Fluorobenzoyl)-4-methylthiosemicarbazide (6j). Method B. To a stirred, room-temperature solution of 4-methylthiosemicarbazide (8.48 g, 80.6 mmol) and pyridine (100 mL) was added dropwise 3-fluorobenzoyl chloride (9.8 mL, 80 mmol). After the solution was stirred for 17 h, the excess pyridine was evaporated at reduced pressure. The resulting solid was washed with H_2O and collected by filtration. Crystallization from EtOH afforded a colorless solid: 8.43 g (46%); mp 199–201 °C dec; ^1H

NMR (DMSO-d_6) δ 2.86 (d, 3 H, J = 4.2 Hz), 7.40–7.59 (m, 2 H), 7.69–7.78 (m, 2 H), 8.09 (br s, 1 H), 9.38 (br s, 1 H), 10.44 (br s, 1 H). Anal. ($C_9H_{10}FN_3OS$) C, H, N.

Representative Procedure for the Preparation of 5-Aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones. 5-(2-Chlorophenyl)-4-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (9e). Method C. 1-(2-Chlorobenzoyl)-4-methylthiosemicarbazide (9.00 g, 36.9 mmol) and 1 M aqueous $NaHCO_3$ (370 mL) were stirred and heated to reflux. After being refluxed for 24 h, the reaction mixture was filtered. When the filtrate had cooled to room temperature, it was acidified by the careful addition of concentrated aqueous HCl (31.0 mL). The resulting mixture was extracted three times with EtOAc. The EtOAc 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 a solid which crystallized from EtOAc/hexane affording small, off-white needles: 6.4 g (77%); mp 142–144 °C; ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 3 H), 7.44–7.48 (m, 2 H), 7.55–7.60 (m, 2 H), 12.32 (s, 1 H). Anal. ($C_9H_8ClN_3S$) C, H, N.

Representative Procedure for the Preparation of 3-(Alkylthio)-5-aryl-1,2,4-triazoles 3, 10, and 17 (n = 0). 5-(3-Fluorophenyl)-4-methyl-5-(methylthio)-4*H*-1,2,4-triazole (3z). Method D. 5-(3-Fluorophenyl)-4-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6.55 g, 31.3 mmol) was dissolved in 1 M aqueous NaOH (70 mL). The solution was stirred, and a solution of CH_3I (3.2 mL, 51 mmol) in EtOH (16 mL) was added. After being stirred overnight at room temperature, the reaction mixture was diluted with H_2O and the precipitate was collected by filtration. Crystallization from EtOAc afforded colorless needles: 5.03 g (72%); mp 151–153 °C; ^{1}H NMR ($CDCl_3$) δ 2.79 (s, 3 H), 3.61 (s, 3 H), 7.17–7.25 (m, 1 H), 7.35–7.54 (m, 3 H). Anal. ($C_{10}H_{10}FN_3S$) C, H, N.

Representative Procedure for the Preparation of 3-(Alkylsulfinyl)-5-aryl-1,2,4-triazoles 3, 10, and 17 (n = 1). 5-(4-Methoxyphenyl)-4-methyl-3-(methylsulfinyl)-4*H*-1,2,4-triazole (3n). Method E. To a stirred, 0 °C solution of 5-(4-methoxyphenyl)-4-methyl-3-(methylthio)-4*H*-1,2,4-triazole (5.00 g, 21.2 mmol) and CH_2Cl_2 (150 mL) was added portionwise 80% MCPBA (4.59 g, 21.3 mmol). After being stirred for 17 h, the reaction mixture was washed twice with saturated aqueous $NaHCO_3$ and once with saturated aqueous NaCl. After the solution was dried over anhydrous Na_2SO_4, the CH_2Cl_2 was evaporated at reduced pressure leaving a solid. Purification by flash chromatography[36] (50% acetone/EtOAc) and crystallization (EtOAc) afforded colorless needles: 4.0 g (75%); mp 168–170 °C; ^{1}H NMR ($CDCl_3$) δ 3.33 (s, 3 H), 3.89 (s, 3 H), 3.96 (s, 3 H), 7.03–7.09 (m, 2 H), 7.57–7.62 (m, 2 H). Anal. ($C_{11}H_{13}N_3O_2S$) C, H, N.

Representative Procedure for the Preparation of 3-(Alkylsulfonyl)-5-aryl-1,2,4-triazoles 3, 10, and 17 (n = 2). 5-(3-Fluorophenyl)-4-methyl-3-(methylsulfonyl)-4*H*-1,2,4-triazole (3aa). Method F. To a stirred, 0 °C solution of 5-(3-fluorophenyl)-4-methyl-3-(methylthio)-4*H*-1,2,4-triazole (3.00 g, 13.4 mmol) and CH_2Cl_2 (100 mL) was added portionwise 80% MCPBA (11.6 g, 33.6 mmol). After being stirred for 17 h, the reaction mixture was diluted with CH_2Cl_2 (150 mL) and then washed twice with saturated aqueous $NaHCO_3$ and once with saturated aqueous NaCl. After the solution was dried over anhydrous Na_2SO_4, the CH_2Cl_2 was evaporated at reduced pressure leaving a solid. Purification by flash chromatography (20% EtOAc/CH_2Cl_2) and crystallization (EtOAc/hexane) afforded colorless, matted needles: 2.47 g (72%); mp 175–177 °C; ^{1}H NMR ($CDCl_3$) δ 3.61 (s, 3 H), 3.98 (s, 3 H), 7.26–7.47 (m, 3 H), 7.52–7.61 (m, 1 H). Anal. ($C_{10}H_{10}FN_3O_2S$) C, H, N.

Representative Procedure for the Preparation of 2-Alkyl-5-aryl-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones 13. 5-(4-Chlorophenyl)-2-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (13a). Method G. To a stirred mixture of 4-chlorobenzoyl isothiocyanate (4.5 g, 23 mmol) and dry toluene (20 mL) was added dropwise methylhydrazine (1.1 mL, 21 mmol). The reaction mixture was heated at 80–85 °C for 30 min, and it was then allowed to cool to room temperature. The reaction was diluted with toluene, and the precipitate was collected by filtration affording an off-white solid. This was treated with 1 M aqueous $NaHCO_3$ (150 mL), and the resultant mixture was heated to reflux. After 17 h, the reaction mixture was filtered and the filtrate was allowed to cool to room temperature. With stirring, the filtrate was acidified by the careful addition of 1 M aqueous HCl (155 mL). The precipitate was collected by filtration and crystallized from EtOH affording colorless, matted needles: 2.6 g (55%); mp 269–271 °C; ^{1}H NMR (DMSO-d_6) δ 3.68 (s, 3 H), 7.57–7.63 (m, 2 H), 7.88–7.93 (m, 2 H), 14.07 (b s, 1 H). Anal. ($C_9H_8ClN_3S$) C, H, N.

5-(3-Fluorophenyl)-2-methyl-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (13b). Method H. To a stirred, room-temperature solution of 2-methylthiosemicarbazide (4.17 g, 39.7 mmol) and pyridine (150 mL) was added dropwise 3-fluorobenzoyl chloride (5.0 mL, 41 mmol). After the solution was stirred for 17 h, the pyridine was evaporated at reduced pressure leaving a yellow oil which was heated to reflux in 1 M aqueous $NaHCO_3$ (500 mL). After being refluxed for 17 h, the reaction mixture was filtered and the filtrate was allowed to cool to room temperature. With stirring, the filtrate was carefully acidified by the addition of concentrated aqueous HCl (42 mL). The precipitate was collected by filtration and crystallized from aqueous EtOH affording a colorless solid: 3.34 g (40%); mp 237–239 °C; ^{1}H NMR ($CDCl_3$) δ 3.88 (s, 3 H), 7.17–7.26 (m, 1 H), 7.45–7.54 (m, 1 H), 7.62–7.71 (m, 2 H), 13.35 (br s, 1 H). Anal. ($C_9H_8FN_3S$) C, H, N.

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. The dose range encompassing the 50% lethal effect was recorded as the estimated LD_{50}.

Anticonvulsant Activity. Groups of 5 or 10 CD-1 male mice (18–26 g) were housed individually before being administered the test compound ip as a solution is 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) strychnine sulfate (STR, 2.7 mg/kg ip), (b) maximal electroshock (MES, 50 mA, 0.2 s, corneal electrodes), (c) pentylenetetrazole (PTZ, 60 mg/kg iv), or (d) 3-mercaptopropionic acid (MPA, 100 mg/kg iv). In each of these tests, mice were considered protected according to the following criteria: (a) STR, absence of tonic extension for more than 15 min after STR administration, (b) MES, absence of tonic hind-limb extension, (c) PTZ, absence of clonic seizures for 2 min after PTZ administration, and (d) MPA, absence of seizures for 5 min after MPA 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.

Antispastic Activity. Adult male Charles River CD rats (188–440 g) were spinal transected at the midthoracic level. Over a period of weeks following transection, the rats gradually developed a condition in which the paralyzed hind limbs became hyperreflexic, i.e., they exhibited dramatic bouts of rapid flexions and extensions either spontaneously or in response to tactile stimuli that would otherwise have been innocuous in a normal animal. A computer-controlled test apparatus was designed to record spontaneous movements of the paralyzed hind limbs (spasticity) as well as reflexive movements produced by an externally applied mechanical stimulation (a brief, rapid stretching of the legs produced by a solenoid driver device). The limbs were affixed to a force-displacement transducer, the output of which was amplified and integrated. A counterbalanced repeated measures design was used in which each rat served as its own control, receiving each treatment at 4-day intervals. A test session started with a 20-min acclimatization period followed by a 15-min test segment which was further divided into 60-s periods. The limb-extender device was activated for 100 ms at the beginning of each 60-s period. The transducer output was integrated twice: the first integration occurred over the initial 3 s after leg pull in order to measure the elicited reflex response, and the second integration occurred over the following 57 s in order to measure ongoing spontaneous hind-limb activity. A test session was run before and after ip dosing with the test

compounds (triazole 3c, prepared as a suspension in distilled H_2O/1% Tween 80, and clonidine, prepared as a solution in distilled H_2O) or vehicle. Data was analyzed by paired t test or by individual comparisons using the least-squared-means test.

Rotarod Measurement.[33] Adult male Charles River CD rats were pretested by placement on a horizontal rod which rotated at 7 rpm and observed for their ability to remain on this rod for 120 s, allowing only 1 falloff. Rats having more than 1 falloff were not used. The rats were again placed on the rotarod 30 min after ip administration of the test compounds (triazole 3c and clonidine, both prepared as described above) or vehicle. The rats were observed for 120 s. Those that fell off the rotarod two times were considered significantly affected. The ED_{50} was defined as the dose that impaired motor ability in 50% of the rats, and it was calculated using a method for the analysis of quantal data.[37]

Receptor Binding (*in Vivo*). *In vivo* binding of the benzodiazepine antagonist [^{3}H]Ro15-1788 to the $GABA_A$ receptor of the mouse cerebral cortex was performed as previously described.[38] Mice (Swiss-Webster, 25–30 g) were obtained from Charles River. Compound 3c was given ip 1 h before the ligand. Twenty minutes prior to killing the mice, 3 μCi of [^{3}H]Ro15-1788 (71.8 Ci/nmol, DuPont NEN) was given by iv administration. The mice were killed by decapitation, and the cortex was removed and tissue-solubilized in Protosol at 3 °C overnight. Beckman Ready Protein scintillation fluid (10 mL) was added to the vials and radioactivity determined by liquid scintillation spectrophotometry. Nonspecific binding of [^{3}H]Ro15-1788 was defined as the binding in the presence of clonazepine pretreatment (5 mg/kg ip) 30 min prior to the [^{3}H]Ro15-1788. Specific binding was routinely 85–95% of total bound counts.

Receptor Binding (*in Vitro*). Brain tissue from young adult male Sprague–Dawley rats (200–250 g) was used for receptor-binding studies. The methods used were published procedures: [^{3}H]flunitrazepam,[39] [^{3}H]muscimol,[40] [^{35}S]-*tert*-butylbicyclophosphorthionate ([^{32}S]TBPS),[41] [^{3}H]baclofen,[42] and [^{3}H]-strychnine.[43]

Chloride Influx Assay. Adult male Sprague–Dawley rats (175–200 g, Charles River) were sacrificed by decapitation, and the cerebellum was dissected and removed. Membrane vesicles were prepared by a modification of the method of Harris and Allan.[44] For each preparation, two cerebella were pooled and the tissue was homogenized (8 strokes) with a glass Teflon homogenizer in 12 mL of ice-cold buffer [145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM D-glucose, 1 mM $CaCl_2$, and 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) adjusted to pH 7.5 with Tris base]. The homogenate was centrifuged at 1000*g* for 15 min at 4 °C. The supernatant was decanted and the pellet resuspended in 12 mL of buffer and centrifuged again at 1000*g* for 15 min. The final pellet was suspended in 12 mL of buffer, yielding a preparation containing 3–4 mg of protein/mL. Protein content was determined by the method of Lowry.[45]

Membrane vesicle aliquots (200 μL) were preincubated on ice with either vehicle (0.2% DMSO) or 10 μM compound 3c for 15 min. The tissue preparation was then incubated at 34 °C for 5 min in a gently shaking water bath. Following the incubation, chloride uptake was initiated by the addition of 200 μL of buffer solution containing ^{36}Cl (7 μCi/mL) and the solutions were rapidly mixed by vortexing. To some tubes, muscimol (1 μM final concentration) was added to stimulate Cl uptake with either vehicle or 10 μM compound 3c. After 3 s, influx of ^{36}Cl was terminated by the addition of 4 mL of ice-cold buffer containing 100 μM picrotoxin and rapid filtration under vacuum onto a 2.4-cm Whatman GF/B glass filter using a Hoefer Manifold (Hoefer Scientific, San Francisco, CA). The filter was washed twice more with 4 mL of picrotoxin buffer. The amount of radioactivity on the filters was determined by liquid scintillation spectrophotometry. The amount of ^{36}Cl bound to the filters in the absence of tissue was subtracted from all values. Muscimol-stimulated uptake was defined as the amount of ^{36}Cl taken up in the presence of agonist minus the amount of ^{36}Cl taken up in the absence of muscimol.

Muscimol and picrotoxin were purchased from Sigma (St. Louis, MO), while ^{36}Cl was purchased from Amersham (Arlington Heights, IL).

References

(1) Kane, J. M.; Dudley, M. W.; Sorensen, S. M.; Miller, F. P. 2,4-Dihydro-3*H*-1,2,4-triazole-3-thiones as Potential Antidepressant Agents. *J. Med. Chem.* **1988**, *31*, 1253–1258.

(2) Moran, P. M.; Kane, J. M.; Moser, P. C. Enhancement of Working Memory Performance in the Rat by MDL 26,479, A Novel Compound with Activity at the $GABA_A$ Receptor Complex. *Brain Res.* **1992**, *569*, 156–158.

(3) Sorensen, S. M.; Kane, J. M.; Miller, J. A.; Moser, P. C.; Dudley, M. W.; Kehne, J. H.; Palfreyman, M. G. MDL 26,479, A Novel Triazole with Cognition Enhancing Potential. *Drug News and Perspectives* **1991**, *4*, 624–627.

(4) Kane, J. M.; Baron, B. M.; Dudley, M. W.; Sorensen, S. M.; Staeger, M. A.; Miller, F. P. 2,4-Dihydro-3*H*-1,2,4-triazol-3-ones as Anticonvulsant Agents. *J. Med. Chem.* **1990**, *33*, 2772–2777.

(5) Kubota, S.; Uda, M. 1,2,4-Triazoles. IV. Tautomerism of 3,5-Disubstituted 1,2,4-Triazoles. *Chem. Pharm. Bull.* **1975**, *23*, 955–966.

(6) Durant, G. J. The Reaction of Benzoyl Isothiocyanate with Hydrazine Derivatives. Part I. Reaction with Some Alkyl Hydrazines. *J. Chem. Soc. C* **1967**, 92.

(7) Greer, A. H.; Smith, G. B. L. 1-Methyl-1-aminoguanidine. *J. Am. Chem. Soc.* **1950**, *72*, 874–875.

(8) Peet, N. P.; Sunder, S. Synthesis of 3-Methyl-[1,2,4]-triazepino-[6,5,4-jk]carbazol-4(3*H*)one. *J. Heterocycl. Chem.* **1977**, *14*, 1147–1150.

(9) Hoggarth, E. Compounds Related to Thiosemicarbazide. Part II. 1-Benzoylthiosemicarbazides. *J. Chem. Soc.* **1949**, 1163–1167.

(10) Mhasalkar, M. Y.; Shah, M. H.; Nikam, S. T.; Anantanarayanan, K. G.; Deliwala, C. V. 4-Alkyl-5-aryl-4*H*-1,2,4-traizole-3-thiols as Hypoglycemic Agents. *J. Med. Chem.* **1970**, *13*, 672–674.

(11) Shah, M. H.; Mhasalkar, M. Y.; Patki, V. M.; Deliwala, C. V.; Sheth, U. K. New 1,2,4(H)-Triazole Derivatives as Diuretic Agents. *J. Pharm. Sci.* **1969**, *58*, 1398–1401.

(12) Giri, S.; Singh, H. Thiadiazoles. I. Synthesis of Substituted Sulfathiadiazoles and Related Compounds. *J. Indian Chem. Soc.* **1964**, *41*, 295–298.

(13) Kubota, S.; Uda, M. 1,2,4-Triazoles. III. The Tautomerism of 3-Phenyl-1,2,4-triazolin-5-one, 3-Phenyl-1,2,4-triazoline-5-thione and Their N-Methyl Derivatives. *Chem. Pharm. Bull.* **1973**, *21*, 1342–1350.

(14) Young, G.; Oates, J. Contribution to the Chemistry of the Triazoles. 1-Methyl-5-hydroxytriazoles. *J. Chem. Soc.* **1901**, 659–668.

(15) Hoggarth, E. Compounds Related to Thiosemicarbazides. Part III. 1-Benzoyl-S-methylisothiosemicarbazides. *J. Chem. Soc.* **1949**, 1918–1923.

(16) Mhasalkar, M. Y.; Shah, M. H.; Nikam, S. T.; Anantanarayanan, K. G.; Deliwala, C. V. Further Studies in Substituted 4H-1,2,4-Triazoles for Possible Hypoglycemic Activity. *J. Med. Chem.* **1971**, *14*, 260–262.

(17) Triller, A.; Cluzeaud, F.; Seitanidous, T. Immunocytochemical Localization of the Glycine Receptor. In *Glycine Neurotransmission*; Ottersen, O. P., Storm-Mathisen, J., Eds.; John Wiley and Sons: Chichester, 1990; pp 83–109.

(18) Cortes, P.; Palacios, J. M. Autoradiographic Mapping of Glycine Receptors by [^{3}H]Strychnine Binding. In *Glycine Neurotransmission*; Ottersen, O. P., Storm-Mathisen, J., Eds.; John Wiley and Sons: Chichester, 1990; pp 239–263.

(19) Werman, R.; Davidoff, R. A.; Aprison, M. H. Inhibition of Motoneurons by Iontophoresis of Glycine. *Nature (London)* **1967**, *214*, 681–683.

(20) Grenningloh, G.; Rienitz, A.; Schmitt, B.; Methfessel, C.; Zensen, M.; Beyreuther, K.; Gundelfinger, E. D.; Betz, H. The Strychnine-binding Subunit of the Glycine Receptor Shows Homology with Nicotinic Acetylcholine Receptors. *Nature (London)* **1987**, *328*, 215–220.

(21) Schofield, P. R.; Darlison, M. G.; Fujita, N.; Burt, D. R.; Stephenson, F. A.; Rodriguez, H.; Rhee, L. M.; Ramachandran, J.; Reale, V.; Glencorse, T. A.; Seeburg, P. H.; Barnard, E. A. Sequence and Functional Expression of the $GABA_A$ Receptor Shows a Ligand-gated Receptor Super-family. *Nature (London)* **1987**, *328*, 221–227.

(22) Curtis, D. R.; Dugan, A. W.; Johnston, G. A. R. The Specificity of Strychnine as a Glycine Antagonist in the Mammalian Spinal Cord. *Exp. Brain Res.* **1971**, *12*, 547–565.

(23) Becker, C.-M.; Betz, H. Strychnine: Useful Probe or Not? *Trends Pharmacol. Sci.* **1987**, *8*, 379–380.

(24) Drummond, J.; Johnson, G.; Nickell, D. G.; Ortwine, D. F.; Bruns, R. F.; Welbaum, B. Evaluation and Synthesis of Aminohydroxyisoxazoles and Pyrazoles as Potential Glycine Agonists. *J. Med. Chem.* **1989**, *32*, 2116–2128.

(25) Johnson, G.; Drummond, J. T.; Boxer, P. A.; Bruns, R. F. β-Proline Analogues as Agonists at the Strychnine-sensitive Glycine Receptor. *J. Med. Chem.* **1992**, *35*, 233–241.

(26) Cedarbaum, J. M.; Schliefer, L. S. Drugs for Parkinson's Disease, Spasticity, and Acute Muscle Spasms. In *The Pharmaceutical Basis of Therapeutics*, 8th ed.; Goodman, A. G., Rall, T. W., Nies, A. S., Taylor, P., Eds.; Pergamon Press: New York, 1990; pp 463–484.

(27) Ito, T.; Furukawa, K.; Karasawa, T.; Kadokawa, T.; Shimizue, M. Functional Change in the Rat Spinal Cord by Chronic Spinal Transection and Possible Roles of Monoamine Neurons. *Jpn. J. Pharmacol.* **1985**, *38*, 243–251.
(28) Tremblay, L. E.; Bedard, P. J. Effect of Clonidine on Motoneuron Excitability in Spinalized Rats. *Neuropharmacology* **1986**, *25*, 41–46.
(29) Maynard, F. M. Early Clinical Experience with Clonidine in Spinal Spasticity. *Arch. Phys. Med. Rehabil.* **1984**, *65*, 632.
(30) Nance, P. M.; Shears, A. H.; Nance, D. M. Clonidine in Spinal Cord Injury. *Can. Med. Assoc. J.* **1985**, *133*, 41–42.
(31) Nance, P. M.; Shears, A. H.; Nance, D. M. Reflex Changes Induced by Clonidine in Spinal Cord Injured Patients. *Paraplegia* **1989**, *27*, 296–301.
(32) Tuckman, J.; Chu, D. S.; Petrillo, C. R. Clinical Trial of an Alpha Adrenergic Receptor Stimulating Drug (Clonidine) for Treatment of Spasticity in Spinal Cord Injured Patients. In *Spinal Cord Injury*; Naftchi, N. E., Ed.; Spectrum Publishers: Jamaica, NY, 1982; pp 133–137.
(33) Rackhma, A. Opiate-induced Muscle Rigidity in the Rat. Effects of Centrally Acting Agents. *Neuropharmacology* **1980**, *19*, 855–859.
(34) Dudley, M. W.; Ogden, A. M.; Kane, J. M.; Miller, F. P.; Lippert, B. The Enhancement and Inhibition of *In Vivo* Binding of [^{3}H]-Ro15-1788 in Mouse Cerebral Cortex by a Series of 5-Aryltriazoles. *Eur. J. Pharmacol.* **1990**, *183*, 588–589.
(35) Miller, J. A.; Dudley, M. W.; Kehne, J. H.; Sorensen, S. M.; Kane, J. M. MDL 26,479: A Potential Cognition Enhancer with Benzodiazepine Inverse-like Properties. *Br. J. Pharmacol.* **1992**, *107*, 78.
(36) Still, W. C.; Kahn, M.; Mitra, A. Rapid Chromatographic Technique for Preparative Separations with Moderate Resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(37) Finney, D. J. *Statistical Method in Biological Assay*, 3rd Ed; Macmillan: New York, 1978.
(38) Miller, L. G.; Greenblatt, D. J.; Barnhill, J. G.; Summer, W. R.; Shader, R. I. "GABA Shift" *In Vivo*: Enhancement of Benzodiazepine Binding *In Vivo* by Modulation of Endogenous GABA. *Eur. J. Pharmacol.* **1988**, *148*, 123–130.
(39) Braestrup, C.; Squires, R. F. Pharmacological Characterization of Benzodiazepine Receptors in the Brain. *Eur. J. Pharmacol.* **1978**, *48*, 123–130.
(40) De Feudis, F. V.; Ossola, L.; Schmitt, G.; Wolff, P.; Mandel, P. Na^+-Independent Binding of [^{3}H]GABA and [^{3}H]Muscimol to Subcellular Particles of Neural Primary Cultures and Whole Brain. *J. Neurochem.* **1980**, *34*, 216–218.
(41) Trullas, R.; Havoundjian, H.; Skolnick, P. Stress-induced Changes in [^{35}S]t-Butylbicyclophosphorothionate Binding to Gamma-animobutyric Acid-gated Chloride Channels are Mimicked by *In Vitro* Occupatation of Benzodiazepine Receptors. *J. Neurochem.* **1987**, *49*, 968–974.
(42) Bowery, N. G.; Hill, D. R.; Hudson, A. L. [^{3}H](−)Baclofen: An Improved Ligand for GABA B sites. *Neuropharmacology* **1985**, *24*, 207–210.
(43) Young, A. B.; Snyder, S. H. Strychnine Binding Associated with Glycine Receptors of the Central Nervous System. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2832–2841.
(44) Harris, R. A.; Allan, A. M. Functional Coupling of GABA Receptors of Chloride Channels in Brain Membranes. *Science*, **1985**, *228*, 1108–1110.
(45) Lowry, O. H.; Rosenbrough, N. J.; Farr, A. L.; Randall, R. J. Protein Measurement with the Folin Phenol Reagent. *J. Biol. Chem.* **1951**, *193*, 265–275.