Condensation of Muscimol or Thiomuscimol with Aminopyridazines Yields GABA-A Antagonists

Anita Melikian,† Gilbert Schlewer,*,† Jean-Pierre Chambon,‡ and Camille Georges Wermuth†

Laboratoire de Pharmacochimie Moléculaire, Centre de Neurochimie du CNRS, 5, Rue Blaise Pascal, 67084 Strasbourg, France, and Sanofi Recherche, 371, Rue du Professeur J. Blayac, 34184 Montpellier Cedex 04, France

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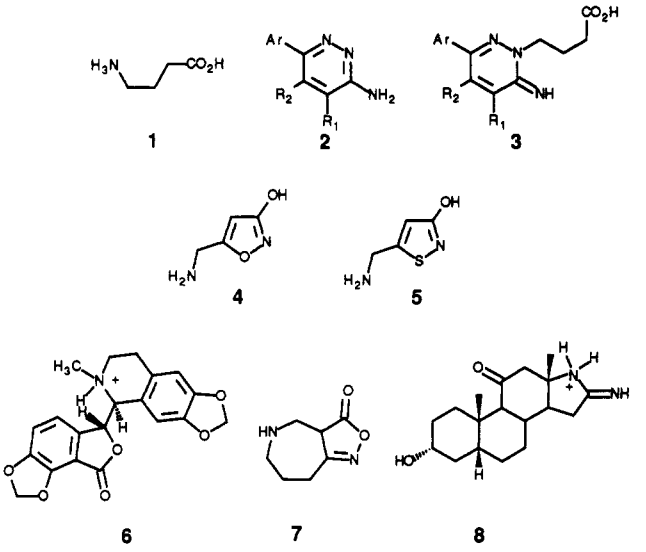
Ten analogs of muscimol and thiomuscimol in which the amino function was delocalized in an amidinic system were prepared by N2 alkylation of 6-aryl-3-aminopyridazines with (chloromethyl)isoxazole or (chloromethyl)isothiazole derivatives. These muscimol and thiomuscimol derivatives show potent binding properties for GABA-A receptors (they displace [^{3}H]GABA and [^{3}H]gabazine) and provoke convulsions after iv injections. They fit well with the model pharmacophore proposed by our group for the GABA-A antagonists and show similar structure–activity profiles to that of the pyridazinyl-GABAs.

In the preceding papers of this series we showed that the association of the elements of γ-aminobutyric acid (GABA, 1) and various 3-amino-6-arylpyridazines 2 in a unique structure in 2-(carboxypropyl)-3-imino-2,3-dihydropyridazines 3 (pyridazinyl-GABA's), yielded potent and selective antagonists against GABA-A receptors.[1-5] Particularly gabazine (3, Ar = *p*-methoxyphenyl, $R_1 = R_2 =$ H; also known as SR 95531) was shown to represent a valuable research tool and was made available both in the cold and the tritiated form.[6,7]

The antagonistic character of the pyridazinyl-GABAs was interpreted[5] in the light of Ariëns theory which stipulates that a given agonist structure is often transformed into an antagonistic one simply by attaching some lipophilic accessory binding sites (usually phenyl rings) to the original agonist.[8] A recent application of this principle is described in the muscarinic series.[9] In the pyridazinyl-GABA's it is easy to recognize the GABA entity since the complete GABA backbone is present, with the slight difference that the primary amine of GABA appears as a delocalized amidine function, the pyridazine and the 6-aryl ring playing the role of the lipophilic groups evoked by Ariëns.



If Ariëns' theory possesses predictive value in this situation, the replacement of the GABA backbone in the structure of the pyridazinyl-GABAs 3 by other recognized GABA agonists should, again, yield new families of GABA antagonists. Therefore we became interested in the replacement of the GABA entity by other highly potent GABA agonistic ligands such as muscimol (4) and thiomuscimol (5) in which the carboxylic function is replaced by the hydroxyisoxazole and the hydroxyisothiazole, respectively. The IC_{50} values for muscimol (4) and thiomuscimol (5) are 6 and 19 nM, compared to 33 nM for GABA.[10-14] We report here the synthesis, the binding assays, and some in vivo studies of these compounds, in comparison to the previously described pyridazinyl-

† Laboratoire de Pharmacochimie.
‡ Sanofi Recherche.

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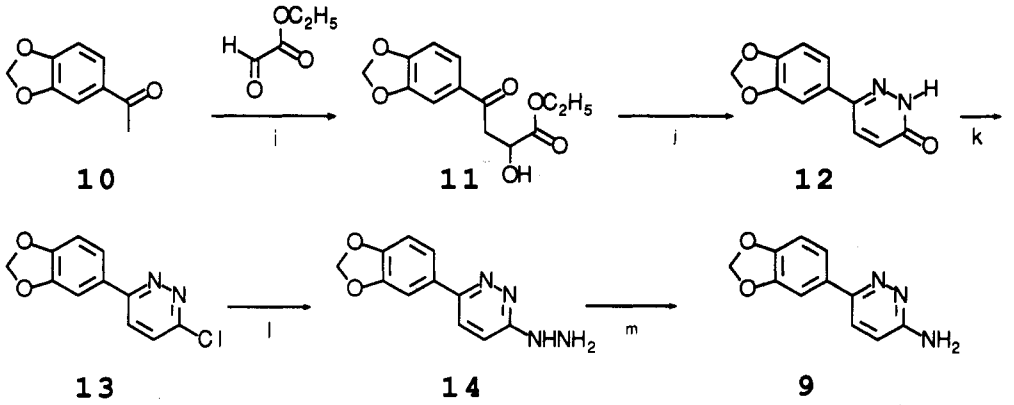
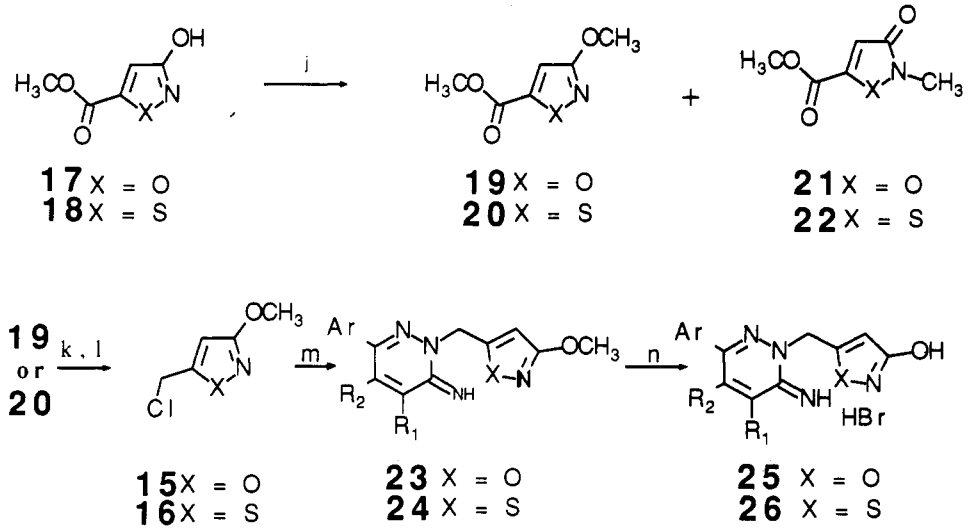
Scheme I. Preparation of 3-Amino-6-[3,4-(methylenedioxy)phenyl]pyridazine.



[a] (i) oil bath 135 °C; (j) excess $H_2NNH_2 \cdot H_2O$; (k) $POCl_3$; (l) $H_2NNH_2 \cdot H_2O$; (m) H_2, Raney Ni.

Scheme II. Synthetic Scheme for the Preparation of Antagonists Derived from Muscimol (X = O) and from Thiomuscimol (X = S).



[a] (j) CH_3I, acetone, K_2CO_3 (**19**/**21** and **20**/**22** in a 1:1 ratio); (k) $NaBH_4$; (l) $SOCl_2$; (m) **9**, DMF, 80 °C; (n) AcOH, HBr, 100 °C, 12 h.

GABAs[5] and to some reference antagonists such as bicuculline (**6**),[15] iso-THAZ (**7**)[10] or the amidinic steroid R-5135 (**8**).[16] A molecular modeling study as well as some structure–activity relationships are also presented.

Chemistry

The key intermediates for the synthesis of the muscimol and thiomuscimol derivatives are 3-amino-6-arylpyridazines, most of which were previously described.[5] As an example, the preparation of 3-amino-6-[3,4-(methylenedioxy)phenyl]pyridazine (**9**) is illustrated in Scheme I. The first step of the synthesis of the aminopyridazine **9** was a thermal condensation of ethyl glyoxylate with 3,4-(methylenedioxy)acetophenone (**10**) to give the hydroxy keto ester **11**. Treatment of **11** with an excess of hydrazine hydrate simultaneously effected cyclization and dehydration yielding the pyridazinone **12**,[17] which was transformed into the imino chloride **13** by reaction with phosphorus oxychloride. The autocatalyzed nucleophilic substitution[18] of the imino chloride **13** with hydrazine hydrate gave the 3-hydrazinopyridazine **14** which was transformed into the expected 3-aminopyridazine **9** by hydrogenolysis.

Compounds **15** and **16**, precursors in the synthesis of muscimol (**4**) and thiomuscimol (**5**), respectively, were prepared according to slightly modified procedures previously published by Krogsgaard-Larsen et al.[11,13] Alkylation of 5-(methoxycarbonyl)-3-hydroxyisoxazole (**17**) and 5-(methoxycarbonyl)-3-hydroxyisothiazole (**18**) with iodomethane afforded a mixture of O- and N-methylated products (**19**–**22**) in the same proportion (1:1) as does alkylation with diazomethane (Scheme II).[13] The final steps for the preparation of **15** and **16** followed the published procedures.[11,13]

Alkylation of the aminopyridazines **2** with the chloromethyl derivatives **15** and **16** yielded the N2-alkylated

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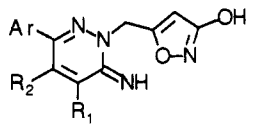
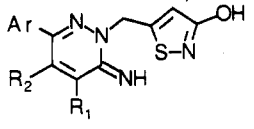
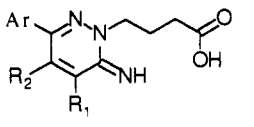
Table I. Binding Data for Muscimol-, Thiomuscimol-, and GABA-derivatives (See Ref. 5 for previously described GABA derivatives)



Muscimol derivatives



Thiomuscimol derivatives



GABA derivatives

no.	derivative of	X	R_1	R_2	Ar	binding GABA: IC_{50} (nM)[a]	binding gabazine: IC_{50} (nM)[a]	mp (°C)	anal.	formula	yield
25a	muscimol	O	CH_3	H	C_6H_5	1000	NT[b]	164	CHN	$C_{15}H_{14}N_4O_2$·HBr	65%
28a	GABA	-	CH_3	H	C_6H_5	2300	800		(5)		
25b	muscimol	O	H	H	C_6H_5	1800	240	207	CHN	$C_{14}H_{12}N_4O_2$·HBr	61%
28b	GABA	-	H	H	C_6H_5	1200	NT		(5)		
25c	muscimol	O	H	H	β-thienyl	1600	88	220	CHN[c]	$C_{12}H_{10}N_4O_2S$·0.5H_2O	25%
28c	GABA	-	H	H	β-thienyl	2370	NT		(5)		
25d	muscimol	O	H	H	p-ClC_6H_4	1200	80	220	CHN	$C_{14}H_{11}ClN_4O_2$·HBr·H_2O	60%
26d	thiomuscimol	S	H	H	p-ClC_6H_4	150	29	255	CHN	$C_{14}H_{11}ClN_4OS$·HBr	85%
28d	GABA	-	H	H	p-ClC_6H_4	280	NT		(5)		
25e	muscimol	O	benzo		C_6H_5	120	190	150	CHN[d]	$C_{18}H_{14}N_4O_2$·HBr·0.75H_2O	60%
26e	thiomuscimol	S	benzo		C_6H_5	160	25	>300	CHN	$C_{18}H_{14}N_4OS$·HBr	74%
25f	muscimol	O	H	H	3,4-$CH_2O_2C_6H_3$	75	6	240	CHN	$C_{14}H_{12}N_4O_2$·HBr	62%
26f	thiomuscimol	S	H	H	3,4-$CH_2O_2C_6H_3$	17	0.76	262	CHN	$C_{15}H_{12}N_4OS$·HBr	79%
28f	GABA	-	H	H	3,4-$CH_2O_2C_6H_3$	50	NT	226	CHN	$C_{15}H_{15}N_3O_4$·HBr·0.67H_2O	96%
26g	thiomuscimol	S	H	C_6H_5	CH_3	6600	1000	178	CHN	$C_{15}H_{14}N_4OS$·HBr	61%
28g	GABA	-	H	C_6H_5	CH_3	10600	NT		(5)		
1 (GABA)						25	1450				
4 (muscimol)						2	135				
5 (thiomuscimol)						19	NT				
6 (bicuculline)						44000	6200				
7 (isoTHAZ)						56000	NT				
8 (R-5135)						43	3				
29 (gabazine)						140	8				

[a] Experiments were run in triplicate; confidence interval was better than 95%. [b] NT = not tested. [c] H: calcd 3.53, found 2.98; N: calcd 15.51, found 14.73. [d] N: calcd 13.42, found 12.52.

products **23** and **24** in accordance with Lund's observations.[19] These compounds are strongly colored (orange-yellow). The final products **25** and **26** were obtained after deprotection of the hydroxyl group in position 3 of the isoxazole or isothiazole ring by treatment with hydrobromic acid in acetic acid (Scheme II). By means of these reactions we prepared six muscimol derivatives (compounds **25a–f**) and four thiomuscimol derivatives (compounds **26d–g**). Similarly the pyridazinyl-GABA analogs **28a–f** were prepared by alkylation of the 3-amino-6-arylpyridazines **2** with ethyl 4-bromobutyrate and subsequent hydrolysis.[5] The structures of the final products are given in Table I.

Binding Assays and in Vivo Studies

The affinity of the prepared compounds for GABA-A receptors was measured by their ability to displace the labeled agonist [^{3}H]GABA and the labeled antagonist [^{3}H]gabazine[7] from rat brain preparations according to Enna and Snyder[20] and to Heaulme et al.[6] (Table I). The convulsive properties were observed after intravenous injection into mice.

Results and Discussion

The results given in Table I show that the muscimol analogs **25a–f**, as well as some of their thiomuscimol counterparts (**26e–g**) are able to displace [^{3}H]GABA with IC_{50} values ranging from 17 to 6600 nM. The antagonistic character of these compounds is suggested by their convulsive properties in mice, occurring at doses ranging from 15 mg/kg iv (compound **25c**) to 200 mg/kg iv (compound **25b**) and (exception made for compound **25e**) by their preferential displacement of the antagonist [^{3}H]gabazine (0.76 to 1000 nM) with respect to that of [^{3}H]GABA.

Molecular modeling using the SYBYL program[21] enabled Wermuth and Rognan to propose a model pharmacophore for GABA-A antagonists.[22] Figure 1a illustrates the carboxypropyl iminopyridazine conformation which fits with this pharmacophore. Our muscimol and thiomuscimol derivatives can also adopt a conformation which fits to the same pharmacophore, and as such, no significant strain energies appeared. Figure 1c shows the minimized conformation of the thiomuscimol derivative **26f** and Figure 1b the superposition of both structures. It appears that the muscimol and thiomuscimol derivatives present conformations which are perfectly able to fit the same pharmacophore; thus all the functional groups: acidic head, amidine function, and also the accessory binding sites, fit well into the proposed pharmacophore.

When compared to the already described[5] corresponding pyridazinyl-GABAs **28**, the muscimol and thiomuscimol analogs showed similar structure–activity relationships. Thus the presence of substituents such as methyl, phenyl, or benzo in the 4- and/or 5-position of the pyridazine ring (R_1 and R_2) is rather detrimental, whereas 4-chloro or, better, 3,4-methylenedioxy substituents on the phenyl ring are favorable. On the other hand the muscimol-derived antagonists show affinities which are more or less com-

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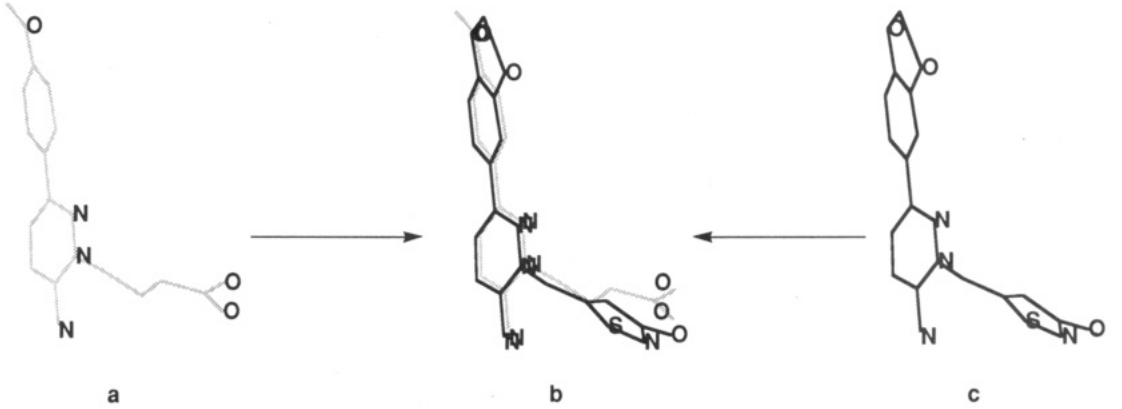

Figure 1. (a) "Active conformation" of gabazine (**29**) according to ref 22; (b) superposition of **29** and **26f**; (c) minimized conformation of **26f**.

parable to that of the corresponding pyridazinyl-GABAs whereas the thiomuscimol derivatives are more potent. Particularly, compound **26f** is 10 times more potent than gabazine and several thousand times more potent than bicuculline, which is currently considered as the reference GABA-A antagonist. One can therefore conclude that Ariëns' theory also applies to the pyridazinylmuscimols **25** and the pyridazinylthiomuscimols **26** as it did for the pyridazinyl-GABAs. The fact that the GABA antagonistic potency of these compounds is mainly governed by the arylpyridazine moiety is also in agreement with Ariëns' theory which states that in antagonists the dominant interactions are provided by the accessory binding sites rather that by the agonist portion of the molecule. However, when similar structural features are present in the accessory binding site elements, alterations on the agonist part of the molecule can provide an additional benefit.[5] Thus it seems highly probable that the improvement observed when passing from the pyridazinylmuscimol **25f** to its thiomuscimol analog **26f** is attributable to its higher lipophilicity and a lesser degree of ionization.[23]

Experimental Section

Melting points were measured on a Mettler PF 62 apparatus and are uncorrected. NMR spectra were recorded on a Bruker AC 200 spectrometer using the δ scale. Coupling constants are given in hertz.

Some 3-aminopyridazines were described earlier: 3-amino-4-methyl-6-phenylpyridazine,[1] 3-amino-6-phenylpyridazine,[5] 3-amino-6β-thienylpyridazine,[5] 3-amino-6-methyl-4-phenylpyridazine,[5] 3-amino-8-phenylbenzo[*d*]pyridazine,[5] and 3-amino-6-(4-chlorophenyl)pyridazine.[5] Also compounds **28a–d,g** were described earlier.[5]

The molecular modeling studies were performed using the SYBYL software package[21] on a micro VAX II and using an Evans and Sutherland PS 390 graphic station. Molecules were generated from existing crystallographic data (except for hydrogen atoms positioned at standard bond distances and angles). The potential energy of each structure was then refined by a molecular mechanics procedure (MAXIMIN). Flexible molecules were submitted to a conformational analysis using the SEARCH option with the following constraints: (i) rotation of all rotatable bonds with a stepwise increment of 1°, (ii) van der Waals radius factor of 0.90, (iii) elimination of all conformations having an energy 15 kcal/mol higher than the global minimum. Superpositions were performed using the SYBYL least squares fitting process.

3-Amino-6-[3,4-(methylenedioxy)phenyl]pyridazine (9). The hydrazinopyridazine **14** (1.0 g, 4.34 mmol) was dissolved in hot methanol, and a catalytic amount of Raney nickel was added for hydrogenolysis (24 h, 5 atm). The nickel was filtered and washed with 250 mL of methanol. The solvents were evaporated, and the residue was recrystallized from methanol. A second recrystallization from 2-propanol furnished 0.5 g (52%) of a greenish powder: mp 189 °C; ^{1}H-NMR ($CDCl_3$) 6.04 (s, 2 H, OCH_2O), 6.8–7.9 (m, 5H, including at 7.35 (AB system, $\Delta\delta = 0.82$, $J_{AB} = 10.0$, 2 H, $C_4N_2H_2$) and 3 H, C_6H_3), 9.80 (broad s, exchangeable with D_2O, 2 H, NH_2).

6-[3,4-(Methylenedioxy)phenyl]-3(2*H*)-pyridazinone (12). A mixture of 75 g (0.45 mol) of 3,4-(methylenedioxy)acetophenone and 68.8 g (0.67 mol) of ethyl glyoxylate was heated at 135 °C for 24 h. After cooling to room temperature, 500 mL of 1-butanol was added, followed by a slow addition of 32.7 g (0.65 mol) of hydrazine hydrate. This mixture was refluxed overnight. The solvents were evaporated, and the crude product was recrystallized in 1 L of methanol yielding 63.7 g (65%) of a yellow powder: mp 281 °C; ^{1}H-NMR (d_6-DMSO) 6.09 (s, 2 H, OCH_2O), 6.9–8.0 (m, 5 H, including at 7.06 (AB system, $\Delta\delta = 1.03$, $J_{AB} = 10.0$, 2 H, $C_4N_2H_2$) and 3 H, C_6H_3), 13.10 (broad s, exchangeable with D_2O, 1 H, N*H*). Anal. ($C_{11}H_8N_2O_3$) C, H, N.

6-Chloro-6-[3,4-(methylenedioxy)phenyl]pyridazine (13). A solution of 6-[3,4-(methylenedioxy)phenyl]-3(2*H*)-pyridazinone (**12**) (60 g, 0.27 mol) in 150 mL of phosphorus oxychloride was heated at 80 °C for 3 h. The excess of phosphorus oxychloride was evaporated under reduced pressure, and 300 g of crushed ice was added to the residue. The mixture was made alkaline with 33% NaOH pH 11. The precipitate was filtered and washed with 1 L of water. Recrystallization from ethyl acetate gave 58.7 g (97%) of white crystals: mp 197.6 °C; ^{1}H-NMR ($CDCl_3$) 6.06 (s, 2 H, OCH_2O), 6.9–7.7 (m, 5 H, containing at 7.34 (AB system, $\Delta\delta = 0.80$, $J_{AB} = 10.0$, 2 H, $C_4N_2H_2$) and 3 H, C_6H_3).

3-Hydrazino-6-[3,4-(methylenedioxy)phenyl]pyridazine (14). A mixture of 3-chloro-6-[3,4-(methylenedioxy)phenyl]pyridazine (**13**) (50.0 g, 0.21 mol) in 100 mL of hydrazine hydrate was heated at 100 °C for 8 h. After cooling to room temperature, the precipitate was filtered and recrystallized from 2-propanol to yield 43.8 g (90%) of orange crystals: mp 199 °C; ^{1}H-NMR ($CDCl_3$) 6.02 (s, 2 H, OCH_2O), 6.6–7.6 (m, 5 H, including at 7.23 (AB system, $\Delta\delta = 0.82$, $J_{AB} = 10.0$, $C_4N_2H_2$) and 3 H, C_6H_3).

3-Methoxy-5-(methoxycarbonyl)isoxazole (19). After dissolution of 3-hydroxy-5-(methoxycarbonyl)isoxazole[11] (**17**) (72 g, 0.5 mol) in 1 L of acetone containing 175 g (0.8 mol) of potassium carbonate, iodomethane (85 g, 0.6 mol) was slowly added under stirring. Stirring was maintained for 6 h. The potassium carbonate was filtered off, and the organic phase was concentrated under reduced pressure. The residue containing the 1:1 mixture of O- and N-methylated compounds **19** and **21**, respectively, was chromatographed on silica gel with a mixture of ether–hexane 1:1. This gave 30 g (37%) of crystalline *O*-methyl derivative **19**: mp 70 °C; ^{1}H-NMR ($CDCl_3$) 3.82 (s, 3 H, OCH_3), 4.05 (s, 3 H, CO_2CH_3), 6.65 (s, 1 H, =C*H*).

3-Methoxy-5-(methoxycarbonyl)isothiazole (20). After dissolution of 3-hydroxy-5-(methoxycarbonyl)isothiazole[13] (**18**) (7.0 g, 0.044 mol) in 350 mL of acetone containing 25.4 g (0.184 mol) of potassium carbonate, iodomethane (24 g, 0.28 mol) was

(23) Chu, K. C., The quantitative analysis of structure-activity relationships. In *The Basis of Medicinal Chemistry*; Wolf, M. E., Ed.; J. Wiley & Sons: New York, 1980; pp 393–418.

added and the mixture was stirred for 24 h at room temperature. The mixture was filtered, and the filtrate was evaporated to dryness. The residue was purified by chromatography on a silica gel column eluted with a mixture of toluene–ethyl acetate 1:1. We obtained 5.2 g (65%) of the expected compound: mp 54 °C; ^{1}H-NMR ($CDCl_3$) 3.75 (s, 3 H, OCH_3), 3.85 (s, 3 H, CO_2CH_3), 6.45 (s, 1 H, =C*H*).

2-[(3-Methoxy-5-isoxazolyl)methyl]-3-imino-6-phenyl-2,3 dihydropyridazine Hydrochloride (23b). A mixture of 3-amino-6-phenylpyridazine[5] (0.5 g, 0.0029 mol), 3-methoxy-5-(chloromethyl) isoxazole (**15**) (0.64 g, 0.0044 mol), and 10 drops of dimethylformamide was heated at 80 °C for 24 h. The traces of dimethylformamide were removed under reduced pressure, and the residue was triturated with acetone. The precipitate was filtered and recrystallized from ethanol to give 0.8 g (85%) of **23b**: mp >300 °C; ^{1}H-NMR ($CDCl_3$) 3.97 (s, 3 H, OCH_3), 5.47 (s, 2 H, CH_2), 6.07 (s, 1 H, OC=C*H*), 6.8–8.2 (m, 7 H, including at 7.38 (AB system, $\Delta\delta = 0.47$, $J = 10.5$, $C_4N_2H_2$) and 5 H, C_6H_5). Anal. ($C_{15}H_{15}N_4O_2Cl$) C, H, N.

Similarly we prepared **2-[(3-methoxy-5-isoxazolyl)methyl]-3-imino-6-(β-thienyl)-2,3-dihydropyridazine hydrochloride (23c).** Starting from 3-amino-6-(β-thienyl)pyridazine:[5] yield 78%; mp >300 °C; ^{1}H-NMR (d_4-methanol) 3.97 (s, 3 H, OCH_3), 5.92 (s, 2 H, CH_2), 6.32 (s, 1 H, OC=C*H*), 7.5–8.5 (m, including at 8.01 (AB system, $\Delta\delta = 0.69$, $J = 9.0$, 2 H, $C_4N_2H_2$) and 3 H, thienyl). Anal. ($C_{13}H_{13}N_4O_2SCl$) C, H, N.

2-[(3-Methoxy-5-isoxazolyl)methyl]-3-imino-6-(*p*-chlorophenyl)-2,3 dihydropyridazine Hydrochloride (23d). Starting from 3-amino-6-(*p*-chlorophenyl)pyridazine:[5] yield 25%; ^{1}H-NMR (d_6-DMSO) 3.72 (s, 3 H, OCH_3), 5.70 (s, 2 H, CH_2), 6.45 (s, 1 H, SC=C*H*), 7.67 ($(AB)_2$ system $\Delta\delta = 0.38$, $J = 9.2$, 4 H, *p*-C_6H_4), 7.93 (AB system, $\Delta\delta = 0.83$, $J = 9.0$, 2 H, $C_4N_2H_2$). Anal. ($C_{15}H_{14}Cl_2N_4O_2$) C, H, N.

2-[(2-Methoxy-5-isoxazolyl)methyl]-3-imino-8-phenyl-2,3-dihydrobenzo[*d*]pyridazine Hydrochloride (23e). Starting from 3-amino-8-phenylbenzo[*d*]pyridazine:[5] yield 45%; mp 223 °C; ^{1}H-NMR (d_6-DMSO) 3.71 (s, 3 H, CH_3), 5.70 (s, 2 H, CH_2), 6.35 (s, 1 H, OC=C*H*), 7.50 (s, 4 H, C_6H_4), 7.7–9.0 (m, 5H, C_6H_5), 10.17 (broad, exchangeable with D_2O, 2H, NH_2^+). Anal. ($C_{19}H_{17}O_2N_4Cl$) C, H, N.

2-[(3-Methoxy-5-isoxazolyl)methyl]-3-imino-6-[3,4-(methylenedioxy)phenyl]-2,3-dihydropyridazine Hydrochloride (23f). Starting from 3-amino-6-[3,4-(methylenedioxy)phenyl]pyridazine (**9**): yield 59%; ^{1}H-NMR (d_6-DMSO) 3.72 (s, 3 H, OCH_3), 5.55 (s, 2 H, CH_2), 5.97 (s, OCH_2O), 6.32 (s, 1 H, OC=C*H*), 6.8–8.3 (m, 5 H, including at 7.55 (AB system, $\Delta\delta = 1.10$, $J = 10.5$, 2 H, $C_4N_2H_2$) and 3 H, C_6H_3). Anal. ($C_{16}H_{15}O_4Cl$) C, H, N.

2-[(3-Methoxy-5-isothiazolyl)methyl]-3-imino-6-(*p*-chlorophenyl)-2,3-dihydropyridazine Hydrochloride (24d). Same procedure as for **23d** starting from 3-amino-6-(*p*-chlorophenyl)pyridazine[5] and 3-methoxy-5-(chloromethyl)isothiazole[13] (**10**): yield 53%; ^{1}H-NMR (d_6-DMSO) 3.72 (s, 3 H, OCH_3), 5.75 (s, 2 H, CH_2), 6.85 (s, 1 H, SC=C*H*), 7.92 (AB system $\Delta\delta = 0.68$, $J = 9.0$, 2 H, $C_4N_2H_2$), 7.67 ($(AB)_2$ system $\Delta\delta = 0.37$, $J = 9.0$, 4 H, *p*-C_6H_4). Anal. ($C_{15}H_{14}Cl_2N_2SO$) C, H, N.

Similarly we prepared **2-[(3-methoxy-5-isothiazolyl)-methyl]-3-imino-8-phenyl-2,3-dihydrobenzo[*d*]pyridazine hydrochloride (24e).** Same procedure as for **23e** using 3-methoxy-5-(chloromethyl)isothiazole[13] (**10**): yield 79%; mp 25.7 °C; ^{1}H-NMR (d_6-DMSO) 3.69 (s, 3 H, OCH_3), 6.04 (s, 2 H, CH_2), 7.04 (s, 1 H, SC=C*H*), 7.66 (s, 4 H, C_6H_4), 7.9–8.2 (m, 5 H, C_6H_5), 8.98 (broad s, exchangeable with D_2O, 2 H, NH_2^+).

2-[(3-Methoxy-5-isothiazolyl)methyl]-3-imino-6-[3,4-(methylenedioxy)phenyl]-2,3-dihydropyridazine Hydrochloride (24f). Starting from 3-amino-6-[3,4-(methylenedioxy)phenyl]pyridazine (**9**): yield 60%; ^{1}H-NMR (d_6-DMSO) 3.90 (s, 3 H, OCH_3), 5.84 (s, 2 H, CH_2), 6.15 (s, 2 H, OCH_2O), 6.98 (s, 1 H, SC=C*H*), 7.1–7.6 (m, 5 H including at 7.36 (AB system, $\Delta\delta = 0.47$, $J = 10.0$, $C_4N_2H_2$) and 3 H, C_6H_3), 8.38 (broad d, 2 H, NH_2^+). Anal. ($C_{16}H_{15}N_4O_3SCl$) C, H, N.

2-[(3-Methoxy-5-isothiazolyl)methyl]-3-imino-5-phenyl-6-methyl-2,3-dihydropyridazine Hydrochloride (24g). Starting from 3-amino-5-phenyl-6-methylpyridazine:[5] yield 40%; mp 240 °C; ^{1}H-NMR (d_4-methanol) 2.45 (s, 3 H, $H_3CC_4N_2H_4$), 4.01 (s, 3 H, OCH_3), 5.82 (s, 1 H, SC=C*H*), 7.37 (s, 1 H, C_4N_2H), 7.55 (s, 5 H, C_6H_5). Anal. ($C_{16}H_{17}N_4OSCl$) C, H, N.

2-[(3-Hydroxy-5-isoxazolyl)methyl]-3-imino-6-phenyl-2,3-dihydropyridazine Hydrobromide (25b). After neutralization of 2-[(3-methoxy-5-isoxazolyl)methyl]-3-iminopyridazine hydrochloride (**23b**) (0.4 g, 1.4 mmol) by means of 1 N sodium hydroxyde, the free base was extracted with dichloromethane. The product obtained after evaporation of the solvent was hydrolyzed in 15 mL of hydrobromic acid in acetic acid (48%) at 100 °C for 12 h. The mixture was evaporated to dryness and the crude product was recrystallized from 2-propanol to give 0.30 g of pink powder: ^{1}H-NMR (d_6-DMSO) 5.55 (s, 2 H, CH_2), 6.15 (s, 1 H, OC=C*H*), 7.3–8.3 (m, 7 H, including at 7.93 (AB system, $\Delta\delta = 0.78$, $J = 9.0$, 2 H, $C_4N_2H_2$) and 5 H, C_6H_5), 9.12 (broad s, exchangeable with D_2O, 2 H, NH_2^+), 11.45 (broad s, exchangeable with D_2O, 1 H, O*H*).

Similarly we prepared **2-[(3-hydroxy-5-isoxazolyl)methyl]-3-imino-6-(β-thienyl)-2,3-dihydropyridazine hydrobromide (25c).** Starting from **23c**: ^{1}H-NMR (d_4-methanol) 5.55 (s, 2 H, CH_2), 6.12 (s, 1 H, OC=C*H*), 7.6–8.6 (m, 5 H, including at 8.10 (AB system, $\Delta\delta = 0.67$, $J = 9.0$, $C_4N_2H_2$) and 3 H, thienyl), 9.07 (broad s, exchangeable with D_2O, 2 H, NH_2^+).

2-[(3-Hydroxy-5-isoxazolyl)methyl]-3-imino-6-(*p*-chlorophenyl)-2,3-dihydropyridazine Hydrobromide (25d). Starting from **23d**: ^{1}H-NMR (d_6-DMSO) 5.70 (s, 2 H, CH_2), 6.67 (s, 1 H, OC=C*H*), 7.70 ($(AB)_2$ system, $\Delta\delta = 0.33$, $J = 9.0$, 4 H, C_6H_4), 7.92 (AB system, $\Delta\delta = 0.77$, $J = 10.5$, 2 H, $C_4N_2H_2$).

2-[(3-Hydroxy-5-isoxazolyl)methyl]-3-imino-8-phenyl-2,3-dihydrobenzo[*d*]pyridazine (25e). Starting from **23e**: yield 60%; ^{1}H-NMR (d_6-DMSO) 5.50 (s, 2 H, CH_2), 6.07 (s, 1 H, OC=C*H*), 7.50 (s, 4 H, C_6H_4), 7.7–8.7 (m, 5 H, C_6H_5), 9.85 (broad s, exchangeable with D_2O, 2 H, NH_2^+), 11.45 (broad s, exchangeable with D_2O, 1 H, O*H*). Anal. ($C_{18}H_{15}N_4O_2Br \cdot {}^3/_4H_2O$) C, H; N: calcd 13.42, found 12.57.

2-[(3-Hydroxy-5-isoxazolyl)methyl]-3-imino-6-[3,4-(methylendioxy)phenyl]-2,3-dihydropyridazine Hydrobromide (25f). Starting from **23f**: ^{1}H-NMR (d_6-DMSO) 5.37 (s, 2 H, CH_2), 5.92 (s, 2 H, OCH_2O), 6.07 (s, 1 H, OC=C*H*), 6.8–8.2 (m, 5 H including at 7.53 (AB system, $\Delta\delta = 1.3$, $J = 9.0$, 2 H, $C_4N_2H_2$) and 3 H, C_6H_3).

2-[(3-Hydroxy-5-isothiazolyl)methyl]-3-imino-6-(*p*-chlorophenyl)-2,3-dihydropyridazine Hydrobromide (26d). Starting from **24d**: ^{1}H-NMR (d_6-DMSO) 5.65 (s, 2 H, CH_2), 6.62 (s, 1 H, SC=C*H*), 7.69 ($(AB)_2$ system, $\Delta\delta = 0.32$, $J = 9.0$, 4 H, C_6H_4), 7.91 (AB system, $\Delta\delta = 0.79$, $J = 10.5$, 2 H, $C_4N_2H_2$), 9.91 (broad s, exchangeable with D_2O, 2 H, NH_2^+).

2-[(3-Hydroxy-5-isoxazolyl)methyl]-3-imino-8-phenyl-2,3-dihydrobenzo[*d*]pyridazine Hydrobromide (26e). Starting from **24e**: ^{1}H-NMR (d_6-DMSO) 5.87 (s, 2 H, CH_2), 6.77 (s, 1 H, SC=C*H*), 7.60 (s, 4 H, C_6H_4), 7.9–8.8 (m, 5 H, C_6H_5), 9.85 (broad s, exchangeable with D_2O, 2 H, NH_2^+), 11.45 (broad s, exchangeable with D_2O, 1 H, O*H*).

2-[(3-Hydroxy-5-isothiazolyl)methyl]-3-imino-6-[3,4-(methylenedioxy)phenyl]-2,3-dihydropyridazine Hydrobromide (26f). Starting from **24f**: ^{1}H-NMR (d_6-DMSO) 5.80 (s, 2 H, CH_2), 6.12 (s, 2 H, OCH_2O), 6.77 (s, exchangeable with D_2O, 1 H, O*H*), 6.9–8.3 (m, 5 H, including at 7.70 (AB system, $\Delta\delta = 1.3$, $J = 9.0$, 2 H, $C_4N_2H_2$), and 3 H, C_6H_3), 9.91 (broad s, exchangeable with D_2O, 2 H, NH_2^+).

2-[(3-Hydroxy-5-isothiazolyl)methyl]-3-imino-5-phenyl-6-methyl-2,3-dihydropyridazine Hydrobromide (26g). Same procedure as for **25a** starting from **24g**: ^{1}H-NMR (d_6-DMSO) 2.37 (s, 3 H, CH_3), 5.70 (s, 2 H, CH_2), 6.62 (s, 1 H, SC=C*H*), 7.51 (s, 5 H, C_6H_5), 9.92 (broad s, exchangeable with D_2O, 2 H, NH_2^+).

2-(3-Carbethoxypropyl)-3-imino-6-[3,4-(methylenedioxy)phenyl]-2,3-dihydropyridazine Hydrobromide (27f). A mixture of 3-amino-6-[3,4-(methylenedioxy)phenyl]pyridazine (**9**) (0.37 g, 0.0017 mol), ethyl 4-bromobutyrate, (0.5 g, 0.0026 mol), and 10 drops of dimethylformamide was heated at 80 °C for 24 h. After drying under reduced pressure, the product was recrystallized from ethanol to give 0.5 g (70%) of a greenish powder: mp 239 °C; ^{1}H-NMR (d_6-DMSO) 1.10 (t, $J = 7.5$, 3 H, OCH_2CH_3), 2.0–2.1 (m, 2 H, $CH_2CH_2CH_2$), 3.95 (q, $J = 7.2$, 2 H, OCH_2CH_3), 4.34 (t, $J = 7.5$, 2 H, NCH_2) 6.14 (s, 2 H, OCH_2O), 7.0–8.3 (m, 5 H, including at 7.74 (AB system, $\Delta\delta = 1.24$, $J = 10.0$, 2 H, $C_4N_2H_2$) and 3 H, C_6H_3), 9.06 (broad s, 2 H, NH_2^+). Anal. ($C_{17}H_{20}N_3O_4Br$) C, H, N.

2-(3-Carboxypropyl)-3-imino-6-[3,4-(methylenedioxy)-phenyl]-2,3-dihydropyridazine Hydrobromide (28f). A solution of 0.45 g (0.0011 mol) of the corresponding ester **27f** in 10 mL of a solution of hydrobromic acid in acetic acid (48%) was heated at 100 °C for 2 h. The solvents were removed under reduced pressure. The crude product was recrystallized from 2-propanol to give 0.40 g of a pink powder: ^{1}H-NMR (d_6-DMSO) 1.9–2.6 (m, 4 H, CH_2CH_2), 2.8–3.7 (broad s, exchangeable with D_2O, O*H*), 4.40 (t, J = 7.5, 2 H, NCH_2), 6.22 (s, 2 H, OCH_2O), 7.1–8.4 (m, 5 H, including at 7.80 (AB system $\Delta\delta$ = 1.3, J = 12.0, 2 H, $C_4N_2H_2$) and 3 H C_6H_3).

Biochemical Assays. Standard [^{3}H]GABA Binding Assay. [^{3}H]GABA binding was performed as previously described by Enna and Snyder.[20] In brief, 200-μL aliquots of synaptic membrane suspensions (1 mg of protein) were transferred to 5-mL plastic tubes containing 1.6 mL of Tris-citrate buffer (0.05 M, pH 7.1) and [^{3}H]GABA (2.9 nM final concentration; 83 Ci/mmol specific activity). Varying concentrations of test compounds dissolved in distilled water were then added to each tube under a volume of 200 μL; the final mixture was incubated for 5 min at 4 °C and the assay terminated by filtration through Whatman GF/C filters.

Standard [^{3}H]SR 95531 ([^{3}H]gabazine) Binding Assay. Aliquots of synaptic membrane suspensions (200 μL, 0.4 mg protein) were transferred to 5-mL plastic tubes containing 1.6 mL of Tris-citrate buffer (0.05 M; pH 7.1) and [^{3}H]SR 95531 (6.5 nM final concentration). Varying concentrations of potential radioligand displacers dissolved in distilled water were then added to each tube under a volume of 200 μL. The final incubation volume was 2.0 mL. The final mixture was incubated for 30 min at 4 °C and the assay terminated by filtration through Whatman GF/C filters. Filters were washed with 10 mL of ice-cold distilled water, placed in scintillation vials, and allowed to dry overnight; ten milliliters of scintillation fluid (Biofluor, NEN) was then added, and radioactivity was determined in a Kontron, Betamatic liquid scintillation counter. Specific binding of [^{3}H]SR 95531 was determined as the difference between total binding in the presence of radioligand alone and nonspecific binding in the presence of 100 μM unlabeled SR 95531.[6] Experiments were run in triplicate, and the results of the confidence interval are better than 95%.

Convulsive Effects. The convulsive effects were examined after intravenous administration in groups of 10 female Swiss albino CD1 mice (Charles River Breeding Laboratories). Test compounds were injected at doses ranging from 5 to 200 mg/mL.

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