Table I. Opioid Antagonist Activity of Bimorphinans in the GPI and MVD

antagonist	agonist IC_{50} ratio[a] ± SEM		
	morphine[b]	ethylketazocine[b]	DADLE[c]
1a	1.6 ± 0.4	50.4 ± 9.9	2.0 ± 0.1
1b	5.4 ± 1.3	128.1 ± 16.6	4.5 ± 0.7
2 (naltrexone)	17.3	2.9	

[a] The IC_{50} value of agonist in presence of antagonist (20 nM) divided by the control IC_{50} value (no antagonist) in the same preparation (N = 6 for **1a** and **1b**). [b] Determined in the GPI preparation. [c] Determined in the MVD preparation.

for his advice and assistance. We also thank Dr. Ian Jardine for determining the FAB-MS of **1a**.

Registry No. **1a**, 105618-26-6; **1b**, 105618-27-7; **2**·HCl, 16676-29-2; NH_2NH_2, 302-01-2; NH_2NHMe, 60-34-4.

[†] Department of Medicinal Chemistry.
[‡] Department of Pharmacology.

P. S. Portoghese,*[†] A. W. Lipkowski,[†] A. E. Takemori[‡]

Department of Medicinal Chemistry
College of Pharmacy
Department of Pharmacology
Medical School
University of Minnesota
Minneapolis, Minnesota 55455

Received September 5, 1986

Articles

Synthesis and Structure–Activity Relationships of a Series of Aminopyridazine Derivatives of γ-Aminobutyric Acid Acting as Selective GABA-A Antagonists

Camille-Georges Wermuth,*[†] Jean-Jacques Bourguignon,[†] Gilbert Schlewer,[†] Jean-Pierre Gies,[†] Angèle Schoenfelder,[†] Anita Melikian,[†] Marie-Jeanne Bouchet,[†] Dominique Chantreux,[‡] Jean-Charles Molimard,[‡] Michel Heaulme,[‡] Jean-Pierre Chambon,[‡] and Kathleen Biziere[‡]

Département de Pharmacochimie Moléculaire, Centre de Neurochimie du CNRS, Université Louis Pasteur, 67084 Strasbourg Cédex, France, and Centre de Recherches CLIN-MIDY, Groupe Sanofi, 34082 Montpellier Cédex, France.
Received May 12, 1986

We have recently shown that an arylaminopyridazine derivative of GABA, SR 95103 [2-(3-carboxypropyl)-3-amino-4-methyl-6-phenylpyridazinium chloride], is a selective and competitive GABA-A receptor antagonist. In order to further explore the structural requirements for GABA receptor affinity, we synthesized a series of 38 compounds by attaching various pyridazinic structures to GABA or GABA-like side chains. Most of the compounds displaced [^{3}H]GABA from rat brain membranes. All the active compounds antagonized the GABA-elicited enhancement of [^{3}H]diazepam binding, strongly suggesting that all these compounds are GABA-A receptor antagonists. None of the compounds that displaced [^{3}H]GABA from rat brain membranes interacted with other GABA recognition sites (GABA-B receptor, GABA uptake binding site, glutamate decarboxylase, GABA-transaminase). They did not interact with the Cl^- ionophore associated with the GABA-A receptor and did not interact with the benzodiazepine, strychnine, and glutamate binding sites. Thus, these compounds appear to be specific GABA-A receptor antagonists. In terms of structure–activity, it can be concluded that a GABA moiety bearing a positive charge is necessary for optimal GABA-A receptor recognition. Additional binding sites are tolerated only if they are part of a charge-delocalized amidinic or guanidinic system. If this delocalization is achieved by linking a butyric acid moiety to the N(2) nitrogen of a 3-aminopyridazine, GABA-antagonistic character is produced. The highest potency (≃250 times bicuculline) was observed when an aromatic π system, bearing electron-donating substituents, was present on the 6-position of the pyridazine ring.

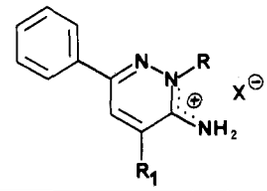
It is well-known that N-alkylation of the central neurotransmitter γ-aminobutyric acid (GABA) leads to an almost complete loss of affinity for the GABA receptor site. This has been observed with monosubstituted derivatives such as *N*-methyl-GABA[1] and *N*-butyl- or *N*-phenethyl-GABA,[2] disubstituted derivatives such as *N,N*-dipropyl-GABA or 4-pyrrolidinobutyric acid (Table I), and quaternary ammonium salts such as *N,N,N*-trimethyl-GABA.[2] A similar detrimental effect has been described for muscimol.[1] However, if a secondary amino function is present in a cyclic structure as in piperidine-4-carboxylic acid, some affinity (IC_{50} = 15 μM[1]) for the GABA-A receptor is recovered. Other cyclic secondary amines derived from flexible GABA agonists such as isoguvacine, piperidine-4-sulfonic acid, and THIP (4,5,6,7-tetrahydroisoxazolo-[5,4-*c*]pyridin-3-ol) even possess high affinities.[3] For these cyclic analogues, again, N-methylation strongly weakens the potency.[3] These observations suggest that ligands for the GABA receptors are sensitive to steric hindrance at the proximity of the cationic moiety of the molecule and that the primary or secondary structure of the amino group is of minor importance. N-Acylation of GABA also led to poorly active compounds in terms of receptor binding as shown for *N*-lauroyl-GABA[4] or for *N*-*t*-BOC-GABA and

[†] Département de Pharmacochimie Moléculaire.
[‡] Centre de Recherches CLIN-MIDY, Groupe Sanofi.

(1) Krogsgaard-Larsen, P.; Johnston, G. A. R. *J. Neurochem.* **1978**, *30*, 1377.
(2) Breckenridge, R. J.; Nicholson, S. H.; Nicol, A. J.; Suckling, C. J.; Leigh, B.; Iversen, L. *J. Neurochem.* **1981**, *37*, 837.
(3) Falch, E.; Krogsgaard-Larsen, P.; Jacobsen, P.; Engesgaard, A.; Braestrup, C.; Curtis, D. R. *Eur. J. Med. Chem.—Chim. Ther.* **1985**, *20*, 447.

0022-2623/87/1830-0239$01.50/0

Table I. Chemical Data and GABA Receptor Affinities of Compound SR 95103 (**10**), Analogues with Modified Side Chains, and Reference Compounds



compd	starting pyridazone lit. ref	R	R_1	crystn solvent	mp, °C	formula (anal.)		K_i, μM
10	11, 60	$(CH_2)_3CO_2H$	CH_3	AcOH–Et_2O	238	$C_{15}H_{18}N_3O_2Cl$	(C, H, N)	2.3
11	11, 60	$(CH_2)_3CO_2Et$	CH_3	EtOH	>260	$C_{17}H_{22}N_3O_2Br$	(C, H, N)	>100
12	11, 60	$(CH_2)_3CONH_2$	CH_3	*i*-PrOH	119	$C_{15}H_{19}N_4O\cdot H_2O$	(C, H, N)	91
13	11, 60	$(CH_2)_3C{\equiv}N$	CH_3	*i*-PrOH	*a*	$C_{15}H_{17}N_4Br$	(C, H, N)	20
14	11, 60	CH_2CO_2H	CH_3	AcOH–Et_2O	240[b]	$C_{13}H_{14}N_3O_2$	(C, H, N)	62
15	11, 60	$(CH_2)_2CO_2H$	CH_3	AcOH	>210[b]	$C_{14}H_{16}N_3O_2Br$	(C, H, N)	7
16	11, 60	$(CH_2)_4CO_2H$	CH_3	*i*-PrOH	214	$C_{16}H_{20}N_3O_2Cl$	(C, H, N, Cl)	10
17	11, 60	$(CH_2)_5CO_2H$	CH_3	*i*-PrOH	197	$C_{17}H_{22}N_3O_2Br$	(C, H, N)	12.7
18	11, 60	$(CH_2)_2CH(CH_3)CO_2H$	CH_3	*i*-PrOH–H_2O	175	$C_{16}H_{20}N_3O_2Br$	(C, H, N)	7
19	11, 60	$CH(CH_3)(CH_2)_2CO_2H$	CH_3	*i*-PrOH–H_2O	180	$C_{16}H_{20}N_3O_2Br$	(C,[c] H, N)	9
20	60	$(CH_2)_3C{\equiv}N$	H	*i*-PrOH	*a*	$C_{14}H_{15}N_4Br$	(C, H, N)	91
21	61, 62	$(CH_2)_3C{\equiv}N$	C_6H_5	*i*-PrOH	*a*	$C_{20}H_{19}N_4Br$	(C, H, N)	91
N-di-*n*-propyl-GABA				*i*-PrOH–Et_2O	125	$C_{10}H_{22}NO_2Cl$	(C, H, N)	>100
4-pyrrolidinobutyric acid				*i*-PrOH	127[d]	$C_8H_{16}NO_2Cl$	(C, H, N)	>100
4-ureidobutyric acid								~100
N-*t*-BOC-GABA								~100
bicuculline methiodide								38
iso-THAZ								50
R 5135								0.047

[a] Hygroscopic. [b] Decomposition. [c] C: calcd, 52.68; found, 53.40. [d] Literature: 127 °C.[67]

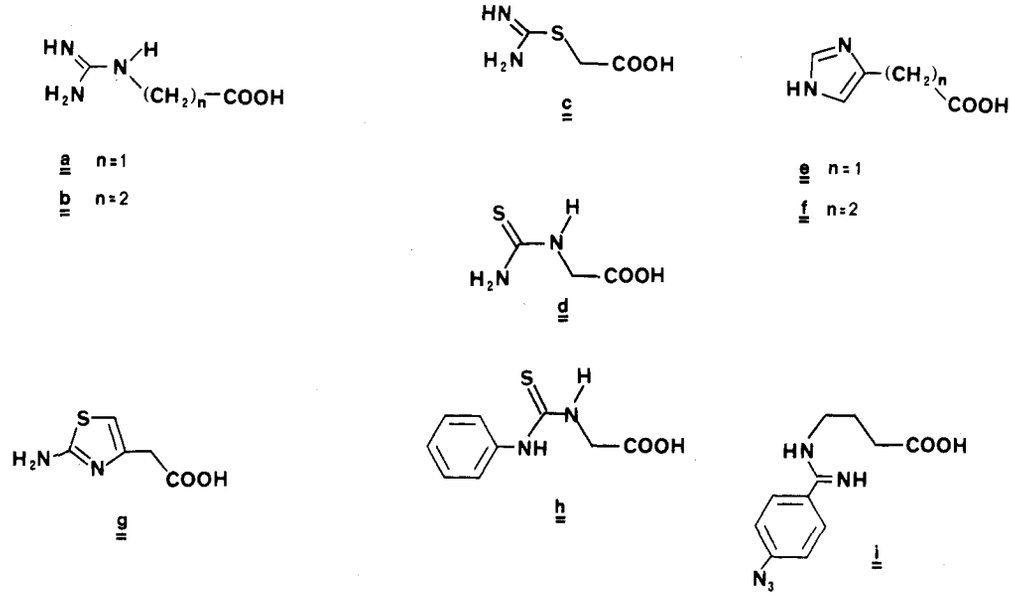


Figure 1. Amidinic and guanidinic GABA-mimetic compounds. IC_{50} values for the inhibition of [^{3}H]GABA binding (μM): a = 2.1;[2] b = 1.8[4] or 26.6;[2] c = 29.0;[2] d = 18.0;[6] e = 0.9[4] or 0.5;[2] f = 290;[2] g = 68.0;[2] i = 9.0[8,9] (K_i value). K_i value for the sodium-dependent synaptosomal uptake (μM): i = 8.[9] K_i value for the inhibition of GABA-transaminase (μM): h = 91.[7]

ureidobutyric acid (Table I). However, *N*-benzoyl- and *N*-pivaloyl-GABA exhibit some anticonvulsant activity in animals[5] presumably because they act as prodrugs of GABA.

Unexpectedly, the incorporation of the amino group of GABA in an amidinic system such as that found in guanidinoacetic acid (a)[6] and guanidinopropionic acid (b)[4] (Figure 1) or in *S*-thioureylacetic acid (c)[2] and *N*-(thiocarbamoyl)glycine (d)[7] or even in cyclic amidines like imidazoleacetic (e) and imidazolepropionic (f) acids[2,4] or (2-aminothiazol-4-yl)acetic acid (g)[2] restores the affinity for the GABA-A receptor site, and reasonable IC_{50} values, in

(4) Olsen, R. W.; Ticku, M. K.; Greenlee, D.; Van Ness, P. In *GABA-Neurotransmitters, Pharmacochemical, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Krüger, J., Kofold, H., Eds.; Munksgaard: Copenhagen, 1979; p 168.
(5) Galzigna, L.; Garbin, L.; Bianchi, M.; Marzotto, A. *Arch. Int. Pharmacodyn. Ther.* **1978**, *235*, 73.
(6) Iversen, L. L.; Bird, E.; Spokes, E.; Nicholson, S. H.; Suckling, C. J. In *GABA-Neurotransmitters, Pharmacochemical, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Krüger, J., Kofold, H., Eds.; Munksgaard: Copenhagen, 1979; p 180.
(7) Tunnicliff, G.; Welborn, K. L.; Ngo, T. T. *Gen. Pharmacol.* **1985**, *16*, 25.

Figure 2. Structure of minaprine (left) and of SR 95103 (right).

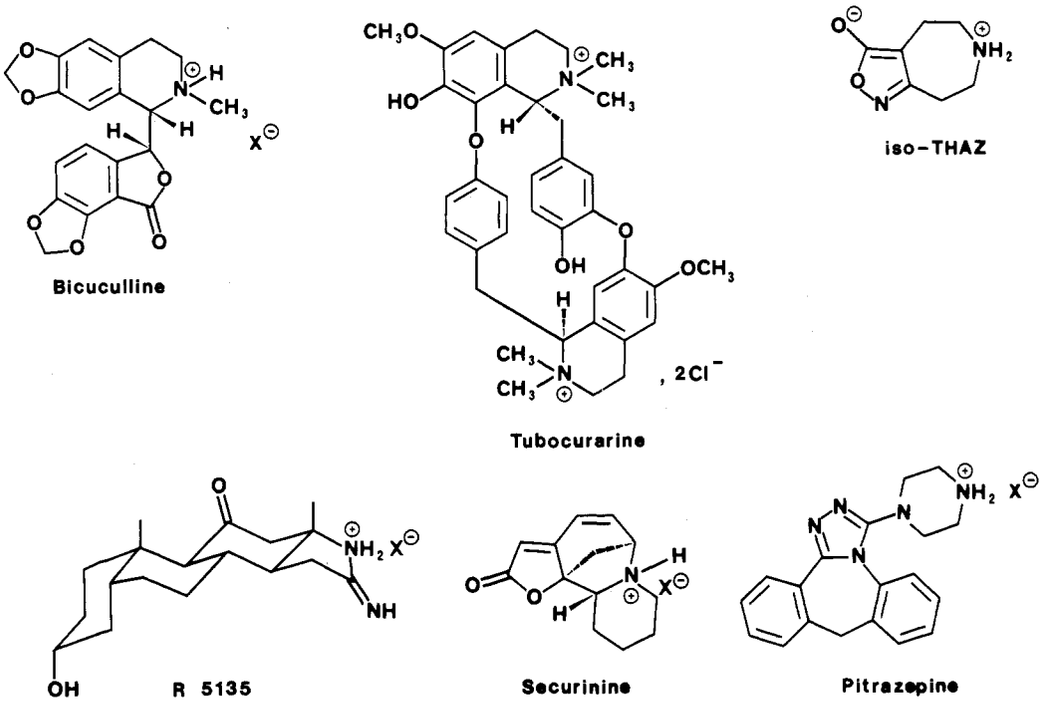
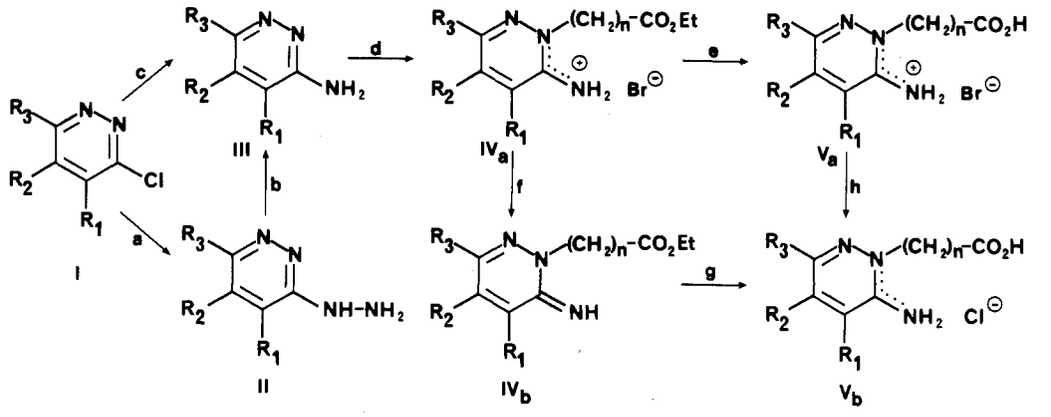


Figure 3. GABA antagonists at the GABA-A receptor site.

Scheme I[a]



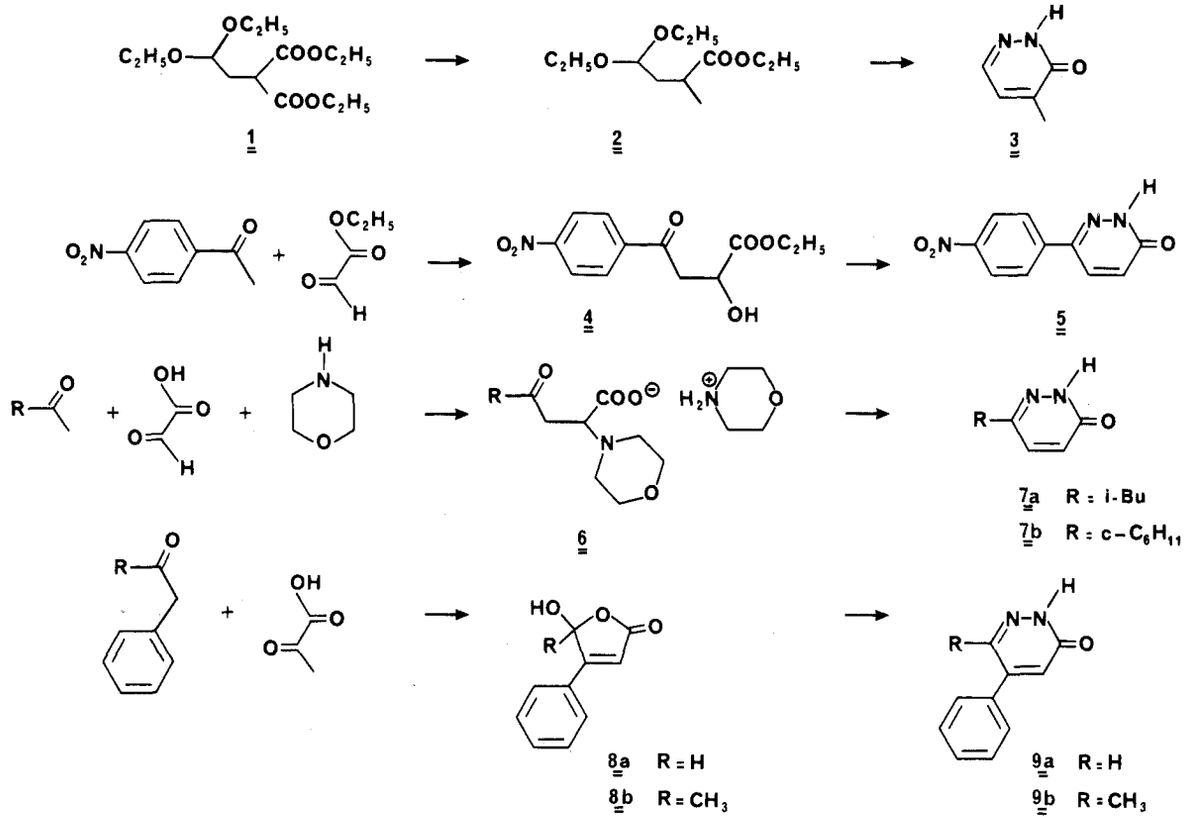
[a] a: H_2NNH_2, BuOH, reflux. b: H_2–Raney Ni–MeOH. c: NH_4OH, 28%; NH_4Cl; autoclave. d: $Br(CH_2)_nCO_2Et$, DMF, 60 °C. e: HBr (48%)–AcOH, 100 °C. f: K_2CO_3–H_2O. g: HCl (36%)– AcOH, 100 °C. h: (1) 2 N NaOH; (2) HCl, 36%.

the micromolar range, have been observed for these compounds. Two other amidinic compounds, phenylthiohydantoic acid (h)[8] and 4-[(4-azidobenzoimidyl)amino]-butanoic acid (i),[9,10] have also been shown to possess notable affinity for GABA recognition sites such as GABA-α-ketoglutarate transaminase (GABA-T) or the GABA uptake system, respectively. Finally, the potent (but not selective) synthetic GABA antagonist R 5135 contains an endo-exocyclic amidinic system carried by a steroid skeleton (Figure 3).[11] Taken together, these observations suggest that N-substitution, even by bulky substituents, is tolerated if it is part of a charge-delocalized amidinic or guanidinic system.

Previous studies from our group on minaprine (Figure 2), an antidepressant containing a 3-aminopyridazinic structure,[12-14] had demonstrated that this compound and

(8) Tunnicliff, G. 14th Annual Meeting of the Society of Neuroscience, Anaheim, CA, October 10–15, 1984; Abstracts, Vol. 10, Part 2, 286.10, p 974.
(9) Tunnicliff, G.; Smith, J. A. *Neurochem. Int.* **1981**, *3*, 371.
(10) Tunnicliff, G.; Ngo, T. T. *J. Neurochem.* **1982**, *39*, 998.
(11) Hunt, P.; Clements-Jewery, S. *Neuropharmacology* **1981**, *20*, 357.
(12) Wermuth, C. G.; Exinger, A. *Agressologie* **1972**, *13*(5), 285.
(13) Biziere, K.; Kan, J. P.; Souilhac, J.; Muyard, J. P.; Roncucci, R. *Arzneim.-Forsch. (Drug Res.)* **1982**, *32*II, 8, 824.

Scheme II. Pyridazinone Syntheses



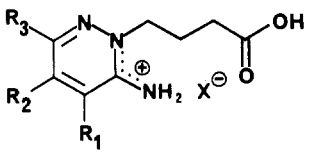
its analogues also presented an endo–exo amidinic charge delocalization[15] and prompted us to link the butyric chain of GABA to a 6-phenyl-4-methyl-3-aminopyridazine. The resulting GABA derivative, SR 95103 (Figure 2, compound **10**, Table I), displaced [^{3}H]GABA from rat brain membranes with an apparent K_i of 2.2 μM and was shown, on the basis of biochemical, electrophysiological, and pharmacological results, to be a selective and competitive antagonist of GABA at the GABA-A receptor site.[16] The object of the present study is to describe the synthesis and to examine the structure–activity relationships of a series of compounds structurally related to SR 95103.

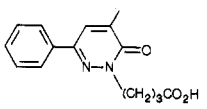
Chemistry

Direct alkylation of the exo–endo amidinic system of the 3-aminopyridazines III with ethyl 4-bromobutyrate or its homologues followed by hydrolysis in acidic medium yielded the endo-substituted isomers V (Scheme I). The acetic acid derivative **14** (Table I) was obtained by reacting the triethylammonium salt of bromoacetic acid in acetone with 3-amino-4-methyl-6-phenylpyridazine. The same reaction performed with 4-bromobutyronitrile yielded the cyano analogues **13**, **20**, and **21** (Table I) and, after partial hydrolysis, the primary amide **12**. The starting 3-aminopyridazines III were obtained from the corresponding 3-chloropyridazines I either by direct substitution with ammonia or by catalytic hydrogenolysis of the 3-hydrazinopyridazines II.[17] Quaternizations of 3-aminopyridazines have been examined by Lund and Lunde, with use of ^{1}H NMR.[18] Alkylation generally led to a mixture of N(1)- and N(2)-alkyl derivatives in a ratio of 1:1, and no alkylation on the exo amino group was observed. However, when a phenyl group was present at the 6-position (III, $R_1 = R_2 = H$, $R_3 = C_6H_5$), the amount of the N(1)-alkylated derivative was considerably decreased (<8%). This result could be attributed to a steric effect, the phenyl ring hindering the approach of the electrophilic agent toward the N(1) nitrogen. In addition, the electron-attracting effect of the phenyl ring renders the N(1) nitrogen less nucleophilic. In our series, we obtained almost exclusively N(2)-alkylated derivatives when we alkylated 3-amino-6-arylpyridazines with ω-bromoalkanoic esters. Some N-(1)-alkylation occurred when the 6-aryl ring was replaced by an aliphatic (e.g., isobutyl) group. However, a careful crystallization afforded the pure N(2) isomer, as observed in similar cases by Barlin.[19] In IR spectroscopy, the N(2)-alkylated ester bromides IVa showed a characteristic pyridazinium band at 1650 cm^{-1}, and in ^{1}H NMR they presented a broad singlet around 9 ppm assigned to the first methylene group attached to the N(2) nitrogen. The endo substitution was also confirmed by a radiocrystallographic study performed on compound **22**.[20] For purification purposes, as well as for the obtention of pyridazinium chloride salts, it was necessary in some instances to liberate the neutral form IVb from the bromide salts IVa. This neutral form exists exclusively as an exo-imino tautomer as suggested by the disappearance of the pyridazinium band at 1650 cm^{-1} in IR spectroscopy and the presence, in ^{1}H NMR, of an excocyclic imino hydrogen singlet at 5 ppm accompanied by a triplet at 4.2 ppm assigned to the methylene group beared by the uncharges N(2) nitrogen.

(14) Wermuth, C. G. *Actual. Chim. Ther.* **1985**, *12*, 3.
(15) Michel, A.; Gustin, R.; Evrard, G.; Durant, F. *Bull. Soc. Chim. Belg.* **1982**, *91*, 49.
(16) Chambon, J. P.; Feltz, P.; Heaulme, M.; Restle, S.; Schlichter, R.; Biziere, K.; Wermuth, C. G. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1832.
(17) Murakami, H.; Castle, R. N. *J. Heterocycl. Chem.* **1967**, *4*, 555.
(18) Lund, H.; Lunde, P. *Acta Chem. Scand.* **1966**, *21*, 1067.
(19) Barlin, G. B. *J. Chem. Soc., Perkin Trans. 1* **1976**, 1424.
(20) Van der Brempt, C.; Evrard, G.; Durant, F. *Acta Crystallogr.*, in press.

Table II. Chemical Data and GABA Receptor Affinities of Analogues of SR 95103 (**10**) Bearing Various Substituents on the Pyridazine Ring



compd	starting pyridazone lit. ref	R_1	R_2	R_3	crystn solvent	mp, °C	formula (anal.)		K_i, μM
22	60	H	H	C_6H_5	*i*-PrOH	240	$C_{14}H_{16}N_3O_2Br$	(C, H, N)	1.22
23	61, 62	C_6H_5	H	C_6H_5	*i*-PrOH	204	$C_{20}H_{20}N_3O_2Cl$	(C, H, N)	100
24	63	H	CH_3	C_6H_5	*i*-PrOH–*i*-Pr_2O		$C_{15}H_{18}N_3O_2Br$	(C, H, N, Br)	10.4
25	24	H	C_6H_5	H	EtOH	238	$C_{14}H_{16}N_3O_2Cl \cdot {}^3/_2H_2O$	(C, H, N)	100
26	*a*	H	C_6H_5	CH_3	*i*-PrOH–Et_2O	186	$C_{15}H_{18}N_3O_2Br$	(C, H, N)	10.6
27	*a*	CH_3	H	H	*i*-PrOH–Et_2O	146	$C_9H_{14}N_3O_2Br$	(C, H, N)	31.9
28	*a*	H	H	$(CH_3)_2CHCH_2$	*i*-PrOH	195	$C_{12}H_{20}N_3O_2Br$	(C, H, N)	22.3
29	*a*	H	H	c-C_6H_{11}	*i*-PrOH–Et_2O	170	$C_{14}H_{22}N_3O_2Br$	(C, H, N)	3.74
30	*b*	H	H	Cl	*i*-PrOH	182	$C_8H_{11}Cl_2N_3O_2 \cdot H_2O$	(C, H, N, Cl)	91.2
31	64	H	H	α-thienyl	*i*-Pr_2O	228	$C_{12}H_{14}N_3O_2SBr$	(C, H, N)	0.58
32	*a*	H	H	β-thienyl	EtOH–Et_2O	252	$C_{12}H_{14}N_3O_2SBr$	(C, H, N)	2.37
33	65	CH_3	H	α-naphthyl	*i*-PrOH	260	$C_{19}H_{20}N_3O_2Cl$	(C,[c] H, N, Cl)	2.64
34	66	H	H	2-Cl-C_6H_4	*i*-PrOH	226	$C_{14}H_{15}N_3O_2BrCl$	(C, H, N)	1.37
35	65	CH_3	H	2-Cl-C_6H_4	EtOH–Et_2O	221	$C_{15}H_{17}N_3O_2Cl_2 \cdot {}^1/_2H_2O$	(C, H, N)	2.14
36	65	CH_3	H	3-Cl-C_6H_4	*i*-PrOH	235	$C_{15}H_{17}N_3O_2BrCl$	(C, H, N)	0.45
37	66	H	H	4-Cl-C_6H_4	*i*-PrOH–Et_2O	232	$C_{14}H_{15}N_3O_2Cl_2$	(C, H, N, Cl)	0.28
38	66	H	H	2,4-Cl_2-C_6H_3	*i*-PrOH–Et_2O	>250	$C_{14}N_3O_2Cl_3$	(C, H, N, Cl)	0.56
39	66	H	H	4-F-C_6H_4	H_2O	230	$C_{14}H_{15}N_3O_2ClF$	(C, H, N, Cl)	1.27
40	*a*	H	H	4-NO_2-C_6H_4	EtOH–Et_2O	204	$C_{14}H_{15}N_4O_4Br$	(C, H, N)	1.37
41	*a*	CH_3	H	4-NO_2-C_6H_4	EtOH–Et_2O	258 dec	$C_{15}H_{17}N_4O_4Cl \cdot {}^3/_2H_2O$	(C, H, N)	22.8
42	66	H	H	4-CH_3O-C_6H_4	EtOH	200	$C_{15}H_{18}N_3O_3Br$	(C,[d] H, N)	0.15
43	65	CH_3	H	4-CH_3O-C_6H_4	H_2O	165	$C_{16}H_{20}N_3O_3Br \cdot {}^1/_2H_2O$	(C, H, N)	0.31
44	65	CH_3	H	4-OH-C_6H_4	HCl, 36%	>250	$C_{15}H_{18}N_3O_3Cl$	(C, H, N)	0.43
45	65	CH_3	H	4-CH_3-C_6H_4	*i*-PrOH–H_2O	>250	$C_{16}H_{20}N_3O_2Br$	(C, H, N, Br)	0.55
46	11, 60				*i*-PrOH–Et_2O	124	$C_{15}H_{16}N_2O_3$	(C, H, N)	>100

[a] See Experimental Section. [b] Commercially available. [c] C: calcd, 63.75; found, 62.77. [d] C: calcd, 48.92; found, 50.01.

The starting 3-chloropyridazines I, properly substituted in the 4-, 5-, and 6-positions, were prepared by the action of phosphorus oxychloride on the corresponding pyridazinones, most of which are described in the literature. Specific syntheses have been developed for particular pyridazinones (Scheme II). The previously described[21] 4-methyl-3(2*H*)-pyridazinone (**3**) was prepared in an unequivocal manner by a malonic synthesis using bromoacetaldehyde diethyl acetal. The initially obtained diester **1** was then alkylated and submitted to a decarboxylation according to Krapcho et al.[22] After hydrolysis of the ester and acetal functions, the γ-aldehydo acid was condensed with hydrazine and the double bond was created in the usual manner by bromination and elimination of hydrobromic acid. This synthetic approach may constitute a general mode of preparation of selectively 4-substituted 3(2*H*)-pyridazinones. 6-(*p*-Nitrophenyl)-3(2*H*)-pyridazinone (**5**) resulted from a cyclocondensation of hydrazine with ethyl β-(*p*-nitrobenzoyl)lactate (**4**), the latter being obtained through aldol reaction between *p*-nitroacetophenone and ethyl glyoxylate. Attempts to prepare pyridazinone **5** by other methods involving aminoalkylations[23] or a modified Strecker synthesis[24] were unsuccessful. The 4-methyl-6-(*p*-nitrophenyl)pyridazinone necessary for the synthesis of compound **41** was prepared according to a general method published earlier.[12,65]

(21) Linholter, S.; Kristensen, A. B.; Rosenorn, R.; Nielsen, S. E.; Kaaber, H. *Acta Chem. Scand.* **1961**, *15*, 1660.
(22) Krapcho, A. P.; Weismaster, J. F.; Eldridge, S. M.; Jahngen, E., Jr.; Lovey, A. J.; Stephens, W. P. *J. Org. Chem.* **1978**, *43*, 138.
(23) Schreiber, J.; Wermuth, C. G.; Meyer, A. *Bull. Soc. Chim. Fr.* **1973**, 625.
(24) McEvoy, F. J.; Albright, J. D. *J. Org. Chem.* **1979**, *44*, 4597.
(25) Bourguignon, J. J.; Wermuth, C. G. *J. Org. Chem.* **1981**, *46*, 4889.
(26) Fujimoto, M.; Okabayashi, T. *Life Sci.* **1981**, *28*, 895.
(27) Karobath, M.; Sperk, G. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 1004.
(28) Buchi, J. *Grundlagen der Arzneimittelforschung und der synthetischen Arzneimittel*; Birkhaüser Verlag: Basel, 1963; pp 116–122.
(29) Schlewer, G.; Wermuth, C. G.; Chambon, J. P. *Eur. J. Med. Chem.—Chim. Ther.* **1984**, *19*, 181.
(30) Nicholson, S. H.; Suckling, C. J.; Iversen, L. L. *J. Neurochem.* **1979**, *32*, 249.
(31) Galli, A.; Ziletti, L.; Scotton, M.; Adembri, G.; Giotti, A. *Pharmacol. Res. Commun.* **1980**, *12*, 266.
(32) Humblet, C.; Marshall, G. R. *Drug Dev. Res.* **1981**, *1*, 409.
(33) Ariëns, E. J.; Beld, A. D.; Miranda, J. F. R.; Simonis, A. M. In *The Receptors*; O'Brien, R. D., Ed.; Plenum: New York, 1979; Vol. 1, pp 33–91.
(34) Curtis, D. R.; Duggan, A. W.; Felix, D.; Johnston, G. A. R. *Nature (London)* **1970**, *226*, 1222.

Aminoalkylation of isobutyl methyl ketone with glyoxylic acid in basic medium according to a method we described previously[25] led to 6-isobutyl-3(2*H*)-pyridazinone (**7a**) via the intermediate Mannich base **6**. The same approach was used for the synthesis of 6-cyclohexyl-3(2*H*)-pyridazinone (**7b**) (see Experimental Section). A similar aminoalkylation procedure applied in acidic medium to phenylacetaldehyde led to 4-phenyl-5-hydroxy-2(5*H*)-furanone (**8a**), which was easily converted to 5-phenyl-3-pyridazinone (**9a**) by treatment with hydrazine.[25] Thermic condensation of benzyl methyl ketone with glyoxylic acid hydrate leads to 4-phenyl-5-hydroxy-2(5*H*)-furanone (**8b**), and further treatment with hydrazine yielded 5,6-disubstituted pyridazinone **9b**.

The synthesis of the reference compound, *N*,*N*-di-*n*-propyl-GABA, is described in the Experimental Section. All the synthesized compounds and their affinity for the GABA recognition sites are listed in Tables I and II.

Results and Discussion

The compounds listed in Tables I and II were first tested at a concentration of 100 μM for their ability to displace [^{3}H]GABA from its receptor site. Most of the compounds were found active at this concentration. If, at 100 μM, the compounds inhibited [^{3}H]GABA binding by more than 50%, dose-inhibition curves were generated with four to eight concentrations of inhibitor in triplicate incubations and IC_{50} values were calculated by log-probit analysis. As shown in Tables I and II, some of the compounds in this series were found to be potent GABA-A receptor antagonists and revealed an approximately 250-fold-greater affinity for the GABA-A receptor than bicuculline.

It has previously been reported that GABA (10 μM) enhances [^{3}H]diazepam binding in rat synaptosomal membranes and that this effect can be counteracted by GABA antagonists.[26,27] All the compounds examined in this study that displaced [^{3}H]GABA from rat brain membranes also dose-dependently antagonized the GABA-elicited enhancement of [^{3}H]diazepam binding. This strongly suggests that, like the lead structure **10**, all these compounds are GABA receptor antagonists.

As most of the compounds examined in this study contain a GABA moiety, we also examined whether these compounds interacted with other GABA-related recognition sites. At 100 μM, none of the compounds significantly interacted with the GABA-B receptor, the chloride ionophore associated with the GABA-A receptor, or the benzodiazepine receptor, nor did any of these compounds affect sodium-dependent, synaptosomal GABA uptake. Finally, the most potent compounds in this series (compounds **36**, **37**, **42**, **43**, and **44**) were also shown not to interact, at 100 μM, with [^{3}H]strychnine and [^{3}H]-L-glutamate binding sites and did not affect GABA-transaminase and glutamate-decarboxylase activities. Thus, the compounds described in this stuby all share with the lead-structure **10** the property of being specific GABA-A antagonists.

The *structure–activity relationships* showed that, compared to the free acid **10**, functional derivatives of the carboxylic group, such as the ester **11**, the carboxamide **12**, or the nitriles **13**, **20**, and **21**, presented decreased affinities for the GABA receptor site (Table I). Modifying the length of the side chain led to compounds that were all weaker displacers of specific [^{3}H]GABA binding than compound **10**. The acetic acid derivative **14** was practically devoid of affinity for the GABA receptor, whereas the propionic acid analogue **15** was only slightly less active than compound **10**. Lengthening the butyryl side chain led to a more gradual loss of affinity, compounds **16** and **17** being ca. 5 and 6 times less potent than compound **10**. Such a profile of structure–activity relationship has often been described in homologous series.[28] Methyl-substituted analogues in the position α or γ to the carboxylic group (**18** and **19**) were slightly less active. This finding was also described for the α- and γ-methylated analogues of GABA itself.[6] These observations clearly indicate that an intact GABA sequence is optimal for receptor recognition and are in keeping with previous reports showing that for GABA agonists an ideal distance of ca. 5 Å between the acidic and the basic function is necessary to ensure maximal affinity.[1,29-32]

We then examined the structural requirements at the level of the pyridazine ring (Table II). Replacing the

(35) Kardo, J.; Blasko, G.; Kerekes, P.; Kovacs, I.; Simonyi, M. *Biochem. Pharmacol.* **1984**, *33*, 3537.
(36) Hill, R. G.; Simmonds, M. A.; Straughan, D. W. *Nature (London)* **1972**, *240*, 51.
(37) Curtis, D. R.; Johnston, G. A. R. *Ergeb. Physiol., Biol. Chem. Exp. Pharmakol.* **1974**, *69*, 98.
(38) Arnt, J.; Krogsgaard-Larsen, P. *Brain Res.* **1979**, *177*, 395.
(39) Krogsgaard-Larsen, P.; Hjeds, H.; Curtis, D. R.; Leah, J. D.; Peet, M. J. *J. Neurochem.* **1982**, *39*, 1319.
(40) Gähwiller, B. H.; Maurer, R.; Wuthrich, H. J. *Neurosci. Lett.* **1984**, *45*, 311.
(41) Beutler, J. A.; Karbon, E. W.; Brubaker, A. N.; Malik, R.; Curtin, D. R.; Enna, S. J. *Brain Res.* **1985**, *330*, 135.
(42) Andrews, P. R.; Johnston, G. A. R. *Biochem. Pharmacol.* **1979**, *28*, 2697.
(43) Triggle, D. J.; Triggle, C. R. *Chemical Pharmacology of the Synapse*; Academic: London, 1976.
(44) Honoré, T.; Brehm, L. *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.* **1978**, *B34*, 3417.
(45) Krogsgaard-Larsen, P.; Honoré, T.; Thyssen, K. In *GABA-Neurotransmitters, Pharmacochemical, Biochemical and Pharmacological Aspects*; Krogsgaard-Larsen, P., Scheel-Krüger, J., Kofold, H., Eds.; Munksgaard: Copenhagen, 1979; p 201.
(46) Lloyd, K. G.; Arbilla, S.; Beaumont, K.; Briley, M.; De Montis, G.; Scatton, B.; Langer, S. Z.; Bartholini, G. *J. Pharmacol. Exp. Ther.* **1982**, *220*, 672.
(47) Fleury, M. B.; Letellier, S.; Maupas, B.; Mompon, B.; Poirier, P.; Raizon, B. *Analusis* **1985**, *13*, 170.
(48) Heaulme, M.; Chambon, J. P.; Leyris, R.; Molimard, J. C.; Wermuth, C. G.; Biziere, K. *Brain Res.* **1986**, *384*, 224.
(49) Slobbe, J. *Aust. J. Chem.* **1976**, *29*, 2553.
(50) Leclerc, G.; Wermuth, C. G. *Bull. Soc. Chim. Fr.* **1967**, 1307.
(51) Enna, S. J.; Snyder, S. H. *Brain Res.* **1975**, *100*, 81.
(52) Hill, D. R.; Bowery, N. G. *Nature (London)* **1981**, *290*, 149.
(53) Wong, D. T.; Threlkeld, P. G.; Bymaster, F. P.; Squires, R. F. *Life Sci.* **1983**, *34*, 853.
(54) Squires, R. F.; Braestrup, C. *Nature (London)* **1977**, *266*, 723.
(55) Young, A. B.; Snyder, S. H. *Proc. Natl. Acad. Sci. U.S.A.* **1973**, *70*, 2832.
(56) Biziere, K.; Thompson, H.; Coyle, J. T. *Brain Res.* **1979**, *183*, 421.
(57) Ramsay, P. B.; Krigman, M. R.; Morell, P. *Brain Res.* **1980**, *187*, 383.
(58) Tappaz, M. L.; Brownstein, M. J.; Palkovits, M. *Brain Res.* **1976**, *108*, 371.
(59) Jung, M. J.; Lippert, B.; Metcalf, B. W.; Schechter, P. J.; Bohlen, P.; Sjoerdsma, A. *J. Neurochem.* **1977**, *28*, 717.
(60) Lespagnol, A.; Deprey, J. *Bull. Soc. Chim. Fr.* **1962**, 1117.
(61) Steck, E. A.; Brundage, R. P. *J. Am. Chem. Soc.* **1953**, *75*, 1117.
(62) Jucker, E.; Suess, R. *Helv. Chim. Acta*, **1959**, *42*, 2506.
(63) Albright, J. D.; McEvoy, F. J.; Moran, D. B. *J. Heterocycl. Chem.* **1978**, *15*, 881.
(64) Steck, E.; Nachod, F. *J. Am. Chem. Soc.* **1957**, *79*, 4408.
(65) Laborit, H. Belg. Pat. 740582, (21 Oct 1969), to CEPBEPE.
(66) Albright, J. D.; Moran, D. B.; Wright, W. B.; Collins, J. B.; Beer, B.; Lippa, A. S.; Greenblatt, E. N. *J. Med. Chem.* **1981**, *24*, 592.
(67) Razdan, R. K.; Zitko Terris, B.; Pars, H. G.; Plotnikoff, N. P.; Dodge, P. W.; Oren, A. T.; Kyncl, J.; Somani, P. *J. Med. Chem.* **1976**, *19*, 454.

methyl group in the 4-position with a hydrogen was always beneficial (compare **10** and **22**, **34** and **35**, **40** and **41**, **42** and **43**). Replacement of the 4-methyl group by a phenyl group was detrimental (**23**). This also seemed to be true when the 4-methyl group was displaced to the 5-position (**24**) and more generally for 5-substituted analogues (**25**, **26**). A very important role is, however, played by the substituent in the 6-position as shown by the weak affinity of the unsubstituted compound **27** or the 6-alkyl and 6-chloro derivatives **28–30** in contrast to the strong affinity of compounds bearing a cyclohexyl (**29**) or an aromatic ring in this position (**31–45**). An aromatic plane in this region of space probably accounts for additional binding interactions involving π electrons. Substituent effects on the 6-phenyl ring can notably increase the potency; thus, the *p*-chloro and the *p*-methoxy derivatives **37** and **42** are, respectively, 8 and 15 times more potent than the lead structure **10** and, respectively, 135 and 250 times more potent than bicuculline.

Finally, the presence of a positively charged delocalized system appears to be essential for receptor recognition. Thus, the replacement of the endo–exo amidinic system by the isosteric but neutral amido group, as in the pyridazinone **46**, leads to a total inactivation of the compound.

In conclusion, linking a butyric side chain to the N(2) nitrogen of a 6-aryl-3-aminopyridazine leads to compounds that are potent and selective antagonists of GABA at the GABA-A receptor sites. The *antagonistic character* of these compounds can be discussed in terms of the theory of *accessory binding sites* developed by Ariëns et al.[33] Considering structural modifications of several classes of cholinergic, histaminergic, and α-adrenergic ligands, Ariëns observed that the modification of the polar agonists by adding hydrophobic ring systems turns the compounds into antagonists. These hydrophobic rings probably bind to adjacent hydrophobic accessory binding sites of the receptors. According to Ariëns, stereoisomerism and affinity data indicate that, for the binding of these antagonists, the specific polar groups and the chiral centers, which are so critical for the agonists, become of secondary importance and can be deeply modified or even omitted. As a result, such antagonists, especially if they are flexible, can antagonize other systems and thus possess diverse pharmacological activities.

Applied to other known GABA-A antagonists such as (+)-bicuculline[34] and its analogues,[35] (+)-tubocurarine,[36,37] iso-THAZ (5,6,7,8-tetrahydro-4*H*-isoxazolo[3,4-*d*]azepin-3-ol),[38,39] the steroid derivative R 5135,[10] pitrazepine,[40] or securinine[41] (Figure 3), this hypothesis also seems to hold: (a) In all the compounds, a slight structural resemblance to GABA can be found insofar that a rather electron rich area (enolic, phenolic, or lactonic function, ketonic carbonyl, aminotriazole ring) is located at a distance of about 4.8 Å (Dreiding models) from a positively charged center. (b) All these antagonists contain several (at least two) aromatic or alicyclic ring systems, which can be located either in the proximity of the electron-rich ("negative") side of the molecule (R 5135, pitrazepine) or in the proximity of the cationic side (securinine) or even on both sides (bicuculline, tubocurarine). (c) In all the GABA antagonists considered, the moiety of the molecule that is assumed to be bioisosteric with GABA shows only an approximate resemblance to this agonist. As pointed out by Ariëns et al.,[33] this may induce a partial loss of specificity. Thus, (+)-bicuculline has been reported to antagonize inhibition elicited by compounds other than GABA,[42] (+)-tubocurarine interferes with the nicotinic ACh receptor in the neuromuscular junction,[43] iso-THAZ also blocks

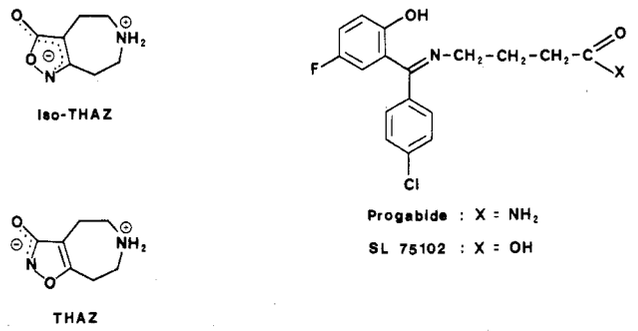


Figure 4. Charge delocalization in iso-THAZ and THAZ (left) and structures of progabide and SL 75102 (right).

neuronal inhibition elicited by glycine,[39] R 5135 strongly interacts with benzodiazepine and glycine receptors,[10] and pitrazepine interacts with benzodiazepine receptors.[40] In contrast, securinine appears to be a selective antagonist; however, it is somewhat weaker than bicuculline.[41] Some observations, however, are apparently not in agreement with Ariëns's theory. It is difficult, for example, to see how one can employ the accessory-binding-site argument to account for the antagonist property of iso THAZ while THAZ is an agonist (Figure 4). Obviously, electronic factors are responsible in this case. In iso-THAZ, the negative charge is delocalized over five atoms whereas it is delocalized over only three atoms in the isomeric THAZ.[38] A similar observation has been reported for muscimol and isomuscimol.[44,45] Ariëns's theory seems also to fail when applied to the compound SL 75102 (Figure 4), which is the partial hydrolysis product of the GABA-mimetic agent progabide and whose in vivo GABA-A agonist properties have been clearly demonstrated.[46] According to Ariëns's predictions, this compound should be a GABA receptor *antagonist*. A possible explanation of this discrepancy could be that SL 75102 possesses GABA-A antagonist properties but that, due to its very rapid hydrolysis at physiological pH values,[47] it liberates free GABA in vivo before its antagonistic properties can be detected.

Applied to the aminopyridazinylbutyric acids and their analogues described in this paper, Ariëns's hypothesis holds true, insofar as the active compounds contain a GABA sequence linked to hydrophobic rings. Again, a strict resemblance to GABA is not an absolute prerequisite, as illustrated by the still-reasonable affinity found for the nitrile **13** or the propionic, valeric, or caproic acid analogues **15**, **16**, and **17**. However, the presence of a positive charge seems to be essential for receptor recognition as illustrated by the inactivity of the pyridazinone **46** in the antagonist series or for *N*-acyl derivatives of GABA in the agonist series (ref 4, ureidobutyric acid and *N*-*t*-BOC-GABA). It should be noted that the localized or delocalized nature of the positive charge is unrelated to the agonist–antagonist character of the compounds since in both series the positive charge can be delocalized or not (Figures 1 and 3).

Concerning *the potency and the selectivity* of GABA antagonists, our results allow some interpretations. As discussed above, the potency is clearly linked to the presence of an aromatic π system on the 6-position of the pyridazine ring. Substituent effects on this system can account for additional binding, which may be due to an increase of the ring electron density (*p*-methyl derivative **45**) or to an extension of the aromatic system through the participation of heteroatom lone pairs (*p*-hydroxy, *p*-methoxy, and *p*-chloro derivatives **44**, **42**, **43**, and **37**). Electron-withdrawing substituents, in contrast, induce a lowering of the affinity (compounds **40** and **41**).

The unique combination of GABA and 3-aminopyridazines is characterized by a high selectivity both toward the different GABA recognition sites and toward other neuronal receptor preparations.[15] This specificity may be due to the fact that, unlike other GABA antagonists, our molecules still contain an almost unaltered GABA sequence. A detailed biochemical study of compounds **37** and **42** is published elsewhere.[48]

Experimental Section

Melting points were determined with a Kofler hot-stage apparatus and are uncorrected. ^{1}H NMR spectra were recorded on Perkin-Elmer R24 (60 MHz) and Bruker WP 80 (80 MHz) instruments, and chemical shifts are reported in parts per million (δ) relative to Me_4Si for $CDCl_3$ and Me_2SO-d_6 solutions or 2,2,3,3-tetradeuterio-3-(trimethylsilyl)propionic acid sodium salt for D_2O solutions as internal standards. IR spectra were taken with a Pye Unicam SP300 spectrophotometer using KBr disks. Glyoxylic acid hydrate and ethyl glyoxylate were generous gifts from the Société Française Hoechst, Paris, France. Peroxide-free diisopropyl oxide was used.

I. Pyridazinones. Most of the starting pyridazinones are already known and were prepared by literature procedures as indicated in column 2 of Tables I and II.

1. 4-Methyl-3(2*H*)-pyridazinone (3). a. Diethyl (β,β-Diethoxyethyl)malonate (1). To 70 mL of absolute EtOH was added 5.2 g (0.22 mol) of Na portionwise under nitrogen, followed after complete reaction of Na by 70 mL (0.42 mol) of diethyl malonate. A white suspension was formed, which disappeared after stirring. The ethanol was then removed under vacuum, and 30 mL (0.19 mol) of bromoacetaldehyde diethyl acetal was added under nitrogen. The mixture was heated in an oil bath for 40 h (external temp. 170 °C). After cooling, the reaction mixture was poured into water and extracted with ether. The combined organic layers were washed with brine and dried over $MgSO_4$. After evaporation of the ether, the residue was distilled. After recovery of unreacted diethyl malonate (50–70 °C (15 mmHg)), the diester 1 distilled at 127 °C (3–4 mmHg), affording 32 g (60%) of a viscous oil: ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 6 H), 1.22 (t, 6 H), 2.1–2.3 (m, 2 H), 3.3–3.7 (m, 5 H), 4.13 (q, 4 H), 4.50 (t, 1 H).

b. Ethyl 2-Methyl-4,4-diethoxybutanoate (2). A solution of 8 g (0.029 mol) of the diester 1 in 120 mL of dry THF was treated with 1.53 g (0.032 mol) of NaH (oil dispersed) at room temperature for 20 min. Then 5.4 mL (0.87 mol) of MeI was added, and the reaction mixture was left to stand at room temperature for 60 h. After the same workup as above, the recovered crude oil (7.8 g, 92%) was used for the next step without further purification: ^{1}H NMR ($CDCl_3$) δ 1.12 (t, 6 H), 1.21 (t, 6 H), 1.43 (s, 3 H), 1.66 (d, 2 H), 3.1–3.8 (m, 4 H), 4.10 (q, 4 H), 4.50 (t, 1 H).

Decarboxylation of the above diester (7.77 g, 0.027 mol) following Krapcho's procedure[22] afforded 4.5 g (77%) of a light yellow oil after distillation with a Kugelrohr apparatus (170–180 °C (15 mmHg)): ^{1}H NMR ($CDCl_3$) δ 1.0–1.5 (m, 12 H), 1.5–2.8 (m, 3 H), 3.0–3.8 (m, 4 H), 4.03 (q, 2 H), 4.46 (t, 1 H).

c. 4-Methyl-3(2*H*)-pyridazinone (3). The ester acetal **2** (7.0 g, 0.032 mol) was saponified by warming it at 50 °C in a solution of 8.0 g of NaOH pellets in 16 mL of H_2O and 100 mL of EtOH. After a careful acidification, 3.8 g of crude α-methyl-γ-hydroxybutyrolactone was recovered as described in ref 49. This lactol was used without purification for the next step. Refluxing during 4 h in a solution of 2.33 mL (0.048 mol) of hydrazine hydrate in 200 mL of absolute EtOH followed by removal of the solvent under reduced pressure yielded the crude 4-methyl-4,5-dihydro-3(2*H*)-pyridazinone. This compound was dissolved in 120 mL of AcOH, and 3.92 mL (0.076 mol) of bromine in 24 mL of AcOH was added dropwise to the mixture held at 50 °C. The solution was then heated at 80 °C for 2 h. After removal of AcOH, the residue was triturated with ether and filtered, and the resulting crystalline powder was washed with ether. The dry solid was taken up in 250 mL of AcOH containing 7.86 g (0.096 mol) of anhydrous NaOAc, and the mixture was refluxed for 1 h. After removal of the solvent, the residue was triturated several times with CH_2Cl_2 and filtered. The combined organic layers were dried, and the solvent was evaporated, leaving a crude solid, which was chromatographed on a short silica gel column, with EtOAc as eluent. Pure 4-methyl-3(2*H*)-pyridazinone (**3**) was obtained (3.05 g, 75% overall yield from diester acetal **1**): mp 167 °C (lit.[21] mp 161 °C); ^{1}H NMR ($CDCl_3$) δ 2.23 (d, 3 H), 7.09 (dq, 1 H), 7.72 (d, 1 H), 11.88 (br s, exch/D_2O, 1 H).

2. 6-(*p*-Nitrophenyl)-3(2*H*)-pyridazinone (5). A mixture of 3.06 g (0.03 mol) of ethyl glyoxylate and of 4.95 g (0.03 mol) of *p*-nitroacetophenone was heated in an oil bath at 135 °C (external temperature) for 16 h. The crude residue was chromatographed (silica gel; solvent system: EtOAc–hexane, 40:60). Pure ethyl β-(*p*-nitrobenzoyl)lactate (**4**; 4 g, 50%) was obtained: ^{1}H NMR ($CDCl_3$) δ 1.25 (t, 3 H), 3.55 (d, 2 H), 3.50 (s, 1 H), 4.30 (q, 2 H), 4.6–4.9 (m, 1 H), 8.22 (AB, J_{AB} = 9.0, $\Delta\delta$ = 0.22, 4 H). A solution of **4** (2.67 g, 0.01 mol) in 20 mL of 1-butanol was treated with 0.5 mL (0.0097 mol) of hydrazine hydrate, and the reaction mixture was refluxed for 8 h. After cooling, the pyridazinone **5** crystallized and was purified by recrystallization in diisopropyl ether. A yellow crystalline powder, mp 260 °C, yield 1.9 g (88%), was obtained: ^{1}H NMR (TFA) δ 7.98 (AB, J_{AB} = 10, $\Delta\delta$ = 0.87, 2 H), 8.25 (AB, J_{AB} = 9.0, $\Delta\delta$ = 0.45, 4 H).

3. 4-Methyl-6-(*p*-nitrophenyl)-3(2*H*)-pyridazinone. A solution of 100 g of *p*-nitroacetophenone (0.6 mol) in 5 L of methanol was added to a chilled solution (0 °C) of 25.2 g (0.2 mol) of potassium pyruvate in water. The solution was kept at 0 °C, and a solution of 14 g of potassium hydroxide in 400 mL of methanol was added. The mixture was allowed to stand at 4 °C overnight and for 2 days at room temperature. The methanol was evaporated after acidification to pH 4 using 10 N sulfuric acid. Water was added to the residue, and this solution was acidified to pH 1 and extracted with ether. The organic phase was extracted with 10% potassium carbonate. The cooled aqueous solution was acidified again to pH 1 and extracted with ethyl acetate. After drying over sodium sulfate and solvent evaporation, 12 g (24%) of (*p*-nitrobenzoyl)lactic acid was obtained: mp 160 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.35 (s, 3 H), 3.49 (s, 2 H), 8.27 (AB, J_{AB} = 8.8, $\Delta\delta$ = 0.15, 4 H).

Hydrazine hydrate (2.96 g, 0.059 mol) was added to a suspension of 10 g (0.039 mol) of (*p*-nitrobenzoyl)lactic acid in 130 mL of 1-butanol. The mixture was refluxed for 20 min. The solution was cooled after azeotropic distillation of the formed water. The expected pyridazinone was filtered and dried (7.6 g, 84%): mp 285 °C; ^{1}H NMR (Me_2SO-d_6) δ 2.17 (d, J = 1.1, 3 H), 8.4–8.7 (m, 5 H).

4. 6-Isobutyl-3(2*H*)-pyridazinone (7a). To a chilled (4 °C) solution of 27.6 g (0.3 mol) of glyoxylic acid hydrate in 60 mL of EtOH 95° was added slowly, with vigorous stirring, 52.3 mL (0.6 mol) of morpholine, followed by 37.6 mL (0.3 mol) of methyl isobutyl ketone. The mixture was allowed to stand at room temperature for 1 h and then heated to 60 °C (external temperature) for 6 h. The reaction mixture was then cooled and stored in a refrigerator for 2 days. The crystalline morpholinium salt **6a** was collected by filtration and recrystallized in 2-propanol (by means of addition of diisopropyl ether). The yield of the morpholinium salt **6a** of 2-morpholino-4-oxo-6,6-dimethylhexanoic acid was 39.6 g (40%): mp 117 °C; ^{1}H NMR (Me_2SO-d_6) δ 0.85 (d, 6 H), 1.9–3.0 (m, 14 H), 3.3–3.8 (m, 8 H).

To 37.9 g (0.115 mol) of the salt **6a** in 350 mL of 1-butanol was added 5.34 mL (0.11 mol) of hydrazine hydrate with stirring. The reaction mixture was maintained at room temperature for 1 h and then refluxed overnight. After removal of the solvent, the residue was taken up in $CHCl_3$. The chloroform solution was washed twice with 10% HCl and twice with 10% $KHCO_3$ and dried over $MgSO_4$. Evaporation of the solvent afforded the crude 6-isobutyl-3-(2*H*)-pyridazinone (**7a**), which was crystallized by trituration with a 1:1 (v/v) diisopropyl ether–hexane mixture. Recrystallization in the same solvent system yielded 10.6 g (61%) of shiny white crystals: mp 63 °C; ^{1}H NMR ($CDCl_3$) δ 0.90 (d, 6 H), 1.6–2.1 (m, 1 H), 2.00 (s, exch/D_2O, 1 H), 2.4–2.5 (m, 2 H), 7.05 (AB, J_{AB} = 9.6, $\Delta\delta$ = 0.30, 2 H).

5. 6-Cyclohexyl-3(2*H*)-pyridazinone (7b). The same procedure as above was used to prepare the cyclohexylpyridazinone **7b**, with methylcyclohexyl ketone as starting material. The yield of the morpholinium salt **6b** of 2-morpholino-4-oxo-cyclohexylbutyric acid was 30%: mp 158 °C. Condensation with hydrazine yielded, after recrystallization, 6-cyclohexyl-3(2*H*)-pyridazinone (**7b**; yield 78%): mp 156 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.0–2.0 (m,

10 H), 7.13 (AB, J_{AB} = 9.4, $\Delta\delta$ = 0.31, 2 H), 12.65 (br s, 1 H).

6. 5-Phenyl-6-methyl-3(2*H*)-pyridazinone (9b). a. 4-Phenyl-5-methyl-5-hydroxy-2(5*H*)-furanone (8b). Glyoxylic acid (9.2 g, 0.1 mol) was heated at 135 °C (external temperature) in the presence of 13.4 g (0.1 mol) of phenylacetone for 17 h. The flask was then fitted with an Hickmann distillation head, and water was gently distilled for a 10-h period (117 °C external temperature). The crude residue crystallized and was used for the next step without any purification: ^{1}H NMR ($CDCl_3$) δ 1.76 (s, 3 H), 5.70 (s, 1 H), 6.13 (s, 1 H), 6.9–7.9 (m, 5 H).

b. 5-Phenyl-6-methyl-3(2*H*)-pyridazinone (9b). Unpurified compound **8b** (6.03 g, 0.12 mol) was refluxed for 12 h in 150 mL of BuOH with 5.8 mL of hydrazine hydrate. The azeotropic BuOH–water mixture was slowly distilled. After removal of BuOH under reduced pressure, the crude pyridazinone was crystallized from EtOH, affording 16.7 g (89%) of crystals: ^{1}H NMR ($CDCl_3$) δ 2.20 (s, 3 H), 6.70 (s, 1 H), 6.8–7.3 (m, 6 H).

II. 3-Chloropyridazines. The appropriate substituted 3-(2*H*)-pyridazinone was treated with an excess of phosphorus oxychloride (10 mL for 1 g of compound). The mixture was heated at 90 ± 5 °C for 4 h, then carefully poured onto ice (200 g for 1 g of compound), and finally rendered alkaline with 20% NaOH. The crude 3-chloropyridazine was collected by filtration, washed with water, and dried under vacuum. The crude 3-chloropyridazine was purified by crystallization in ethanol or 2-propanol or by chromatography on silica gel using a mixture of hexane–ethyl acetate (1:).

III. 3-Aminopyridazines. Method A. The appropriate 3-chloropyridazine I (0.01 mol) was heated at 100 °C during 6 h with 10 mL of hydrazine hydrate. Excess hydrazine was removed under reduced pressure. The crude residue was triturated with the minimal quantity of water, and the solid 3-hydrazinopyridazine was collected by filtration. After being dried, it was used without further purification.[50] The crude hydrazine was dissolved in 40 mL of MeOH and, after addition of 0.8 g of commercial Raney nickel catalyst, was hydrogenated (20 °C (760 mmHg)). The reaction was almost complete after 1–3 days (followed by TLC). The crude 3-aminopyridazines, obtained after filtration and removal of the MeOH, were generally used without purification. If necessary, they can be purified as follows: The crude 3-aminopyridazine is dissolved in CH_2Cl_2, and the organic solution is extracted with 5% aqueous HCl. The separated acidic phase is then carefully neutralized (pH 6–7) with 33% NaOH solution. The resulting white precipitate is recovered by filtration, washed several times with water, and dried. Recrystallization is possible in EtOH or *i*-PrOH.

6-(*p*-Nitrophenyl)-3-aminopyridazine (Method B). A mixture of 1.28 g (5.4×10^{-3} mol) of 6-(*p*-nitrophenyl)-3-chloropyridazine, 0.577 g (10.8×10^{-3} mol) of NH_4Cl, and 100 mL of a saturated solution of ammonia in water at 0 °C was placed in an autoclave and heated at 200 °C for 12 h. The solution was then concentrated under reduced pressure, and the residue was taken up in 40 mL of warm 20% HCl. After filtration of some insoluble solid, the solution was rendered alkaline with 33% NaOH solution. The crude 6-(*p*-nitrophenyl)-3-aminopyridazine precipitated and was collected by filtration. The yield was 0.87 g (74%) of a product sufficiently pure to be used as such for the next step: ^{1}H NMR (Me_2SO-d_6) δ 6.63 (br s, 2 H, 2 H, exch/D_2O), 6.78 (d, 1 H), 7.83 (d, 1 H), 8.14 (br s, 4 H).

IV. Pyridazinium Bromides and Chlorides. 1. Substituted 2-(ω-Carbethoxyalkyl)-3-aminopyridazinium Bromides (IVa). General Procedure. To a solution of the appropriate 3-aminopyridazine III (7×10^{-3} mol) in DMF (10 mL) at 80 °C was added 10.5×10^{-3} mol of ethyl 4-bromobutyrate or any other convenient ω-bromo ester. The temperature was maintained at 80 °C for 8 h. The hot solution was either poured with stirring into ethyl acetate (200 mL), affording a crystalline compound, or concentrated under reduced pressure and the residue triturated with a mixture of Et_2O–*i*-PrOH. In the case of the *p*-nitro derivative **40**, the crude residue was dissolved in hot MeOH, solid impurities were removed by filtration, the MeOH was evaporated, and the residue was crystallized from a mixture of Et_2O and EtOH.

Properties and spectroscopic data for a typical representative, 2-(3-carbethoxypropyl)-3-amino-4-methyl-6-phenylpyridazinium bromide (**11**), include the following: mp 260 °C; IR (KBr) 1735 cm^{-1} (C=O ester); ^{1}H NMR (Me_2SO-d_6) δ 1.05 (t, 3 H), 1.8–2.7 (m, 4 H), 2.20 (s, 3 H), 3.90 (q, 2 H), 4.40 (t, 2 H), 7.3–7.6 (m, 3 H), 7.8–8.1 (m, 2 H), 8.40 (s, 1 H), 8.80 (br s, 2 H).

2. Substituted 2-(3-Cyanopropyl)- and 2-(3-Carboxamidopropyl)-3-aminopyridazinium Salts. The cyanopropyl derivatives **13**, **20**, and **21** were prepared from the corresponding 3-aminopyridazine and 4-bromobutyronitrile in DMF at 80 °C in the same manner as indicated above for the carbethoxy compounds.

The primary amide **12** was prepared by bubbling HCl gas for 4 h through a solution of 0.253 g (10^{-3} mol) of the nitrile **13** in 10 mL of formic acid. After removal of the solvent under reduced pressure, the crude residue was taken up in the minimum quantity of MeOH and the obtained methanolic solution was poured into an excess of dry diethyl ether. The precipitate was collected, dried, and recrystallized from 2-propanol (0.124 g, 45%): IR (KBr) 1530 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.8–2.1 (m, 4 H), 2.15 (s, 3 H), 4.38 (t, 2 H), 5.60 (br s, 2 H), 7.3–8.1 (m, 5 H), 8.20 (s, 1 H).

3. Conversion of the Bromides IVa to the Free Bases IVb. 2-(3-Carbethoxypropyl)-3-imino-4-methyl-6-phenyl-2,3-dihydropyridazine (Free Base Corresponding to Compound 11). The pyridazinium salt was dissolved in the minimal quantity of water, and the solution was made alkaline with K_2CO_3. The aqueous phase was extracted with a 1:1 (v/v) mixture of Et_2O and EtOAc. The organic layer was washed with some water and dried over $MgSO_4$. After evaporation of the solvent, 0.330 g (84%) of **11** (free base) was obtained as a yellow oil: IR (KBr) 1730 (C=O), 1635 cm^{-1} (pyridazine ring); ^{1}H NMR ($CDCl_3$) δ 1.15 (t, 3 H), 2.05 (s, 3 H), 2.0–2.4 (m, 4 H), 4.15 (q, 2 H), 4.25 (t, 2 H), 5.00 (br s, 1 H), 7.10 (s, 1 H), 7.3–7.4 (m, 3 H), 7.6–7.8 (m, 2 H).

4. Acid Hydrolysis of the Esters IVa and IVb. a. 2-(5-Carboxypentyl)-3-amino-4-methyl-6-phenylpyridazinium Bromide (17). A solution of 4.1 g (0.01 mol) of the ethyl ester of 2-(5-carboxypentyl)-3-amino-4-methyl-6-phenylpyridazinium bromide in 10 mL of 48% HBr and 60 mL of glacial acetic acid was heated at 100 °C for 10 h. The solvents were removed under reduced pressure, and the residue as crystallized with THF. After filtration, the precipitate was recrystallized from 2-propanol (1.56 g, 73%): mp 197 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.1–2.0 (m, 6 H), 2.20 (t, 2 H), 2.40 (s, 3 H), 4.40 (t, 2 H), 7.5–8.0 (m, 5 H), 8.40 (s, 1 H).

b. 2-(3-Carboxypropyl)-3-amino-6-(*p*-chlorophenyl)-pyridazinium Chloride (37). With 3.20 g (0.01 mol) of 2-(3-carbethoxypropyl)-3-imino-6-(*p*-chlorophenyl)-2,3-dihydropyridazine as starting material and with use of the procedure indicated above in (a), the only modification being the replacement of 10 mL of 48% HBr by 10 mL of 36% HCl, compound **37** (2.03 g, 62%; *i*-PrOH–Et_2O) was obtained: ^{1}H NMR (Me_2SO-d_6) δ 2.1–2.6 (m, 4 H), 4.35 (t, 2 H), 7.80 ($(AB)_2$, J_{AB} = 8.0, $\Delta\delta$ = 0.4, 4 H), 8.35 (AB, J_{AB} = 9, $\Delta\delta$ = 1.0, 2 H), 9.50 (br s, 2 H).

c. 2-(3-Carboxypropyl)-3-amino-4-methyl-6-(*p*-hydroxyphenyl)pyridazinium Chloride (44). A solution of 0.7 g (2.3×10^{-3} mol) of acid **43** in 7 mL of 48% HBr solution was refluxed overnight. The resulting suspension was collected by filtration, washed with water and acetone, and dried, yielding 0.58 g (68%) of bromide salt: mp 260 °C; IR (KBr). The chloride salt **44** was obtained from the bromide salt by dissolution in a small amount of water and carefully neutralizing the medium with a 2 N NaOH solution until a consistent precipitate was obtained. The solid was collected and recrystallized from concentrated HCl, affording the awaited salt: IR (KBr) 3400, 1705 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.8–2.6 (m, 4 H), 2.35 (s, 3 H), 4.40 (t, 2 H), 7.35 ($(AB)_2$, J_{AB} = 9.0, $\Delta\delta$ = 0.73, 4 H), 8.30 (s, 1 H), 10.50 (br s, 2 H).

d. 2-(Carboxymethyl)-3-amino-4-methyl-6-phenylpyridazinium Acetate (14). The triethylammonium salt of bromoacetic acid was prepared in acetone (40 mL) by adding dropwise 2.8 mL (0.02 mol) of triethylamine to 2.49 g (0.012 mol) of bromoacetic acid dissolved in acetone. To the mixture was then added 1.85 g (0.01 mol) of 3-amino-4-methyl-6-phenylpyridazine. The reaction medium became homogeneous. After 15 min, a precipitate was formed and the mixture was refluxed for 24 h. After cooling, the solid was collected and triturated with 50 mL of MeOH, affording, after filtration and drying, 2.0 g (83%) of solid, which was taken up in AcOH (50 mL) and crystallized with ethyl ether. The solid was collected and dried, yielding 1.71 g (58%) of white powder: mp 240 °C dec; ^{1}H NMR (TFA) δ 2.20 (s, 3 H), 2.55 (s, 3 H), 5.45 (s, 2 H), 7.30 (br s, 2 H), 7.4–7.6 (m,

3 H), 7.7–8.0 (m, 2 H), 8.10 (s, 1 H).

V. Miscellaneous and Reference Compounds. 1. 2-(3-Carboxypropyl)-4-methyl-6-phenyl-3(2*H*)-pyridazinone (46). To a solution of sodium ethanolate prepared from 0.135 g (5.9 × 10^{-3} mol) of Na and 25 mL of absolute EtOH was added 1.1 g (5.9 × 10^{-3} mol) of 4-methyl-6-phenyl-3(2*H*)-pyridazinone in one portion. After the mixture was stirred for 15 min at room temperature, 1.05 g (5.4 × 10^{-3} mol) of ethyl 4-bromobutyrate was added and the mixture was left at room temperature for 16 h. After removal of the solvent under reduced pressure, the crude ester was chromatographed (silica gel, EtOAc–hexane, 1:3), affording 0.8 g (45%) of pure ester, which was hydrolyzed by heating for 3 h at 100 °C in a mixture of 5 mL of concentrated HCl and 45 mL of AcOH. After vacuum evaporation of the solvent, the residue was recrystallized in *i*-PrOH. The yield of **46** was 0.32 g (45%): mp 124 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.6–2.6 (m, 4 H), 2.15 (s, 3 H), 4.53 (t, 2 H), 7.5–8.3 (m, 6 H), 9.03 (br s, 1 H).

2. *N,N*-Di-*n*-propyl-4-aminobutyric Acid Hydrochloride. Ethyl 4-bromobutyrate (7.8 g, 0.04 mol) and di-*n*-propylamine (8.08 g, 0.080 mol) were refluxed overnight in 100 mL of dry benzene. After cooling, the solution was diluted with 100 mL of Et_2O and washed successively with 40 mL of 10% NH_4OH and 40 mL of water. After drying ($MgSO_4$) and evaporation of the solvents, the crude ester was distilled under reduced pressure (120 °C (15 mmHg)), affording 6.1 g (70%) of a colorless oil. This ester was hydrolyzed by refluxing it in 60 mL of 5 N HCl for 8 h. After evaporation of the solvent, the residue was dried by addition of *i*-PrOH and evaporation. The dry residue was then triturated with dry Et_2O, and the collected crystalline powder was recrystallized twice in a mixture of *i*-PrOH–Et_2O. The yield of compound **52** was 3 g (43%): mp 125 °C.

VI. Biochemical Assays. Tritiated derivatives were purchased from New England Nuclear. GABA and muscimol were purchased from Fluka. Bicuculline methiodide was purchased from Pierce Eurochemie B.V. Strychnine sulfate and L-glutamic acid were purchased from Sigma. Diazepam and (−)-baclofen were provided by Hoffman-La Roche. Iso-THAZ was kindly provided by Dr. P. Krogsgaard-Larsen (Royal Danish School of Pharmacy, Copenhagen, Denmark) and R 5135 by Dr. P. Hunt (Roussel-Uclaf). Male Wistar rats weighing 180–220 g (Charles Rivers Breeding Laboratories) were used.

[^{3}H]GABA binding (2.9 nM; 83 Ci/10^{-3} mol) was performed with Triton-treated rat whole brain membranes prepared according to Enna and Snyder.[51] The effects of various compounds on the GABA-elicited enhancement of [^{3}H]diazepam (1.9 nM; 72 Ci/10^{-3} mol) binding in rat brain membranes was evaluated according to Fujimoto and Okabayashi.[26] The possible effects of various compounds on the specific binding of [^{3}H]baclofen (30 nM; 37 Ci/10^{-3} mol) to GABA-B rat whole brain receptors,[52] (^{35}S)S-TPBS (11 nM; 67 Ci/10^{-3} mol) to the Cl^- channels associated with the GABA receptor in rat frontal cortex membranes,[53] [^{3}H]diazepam (1.9 nM; 72 Ci/10^{-3} mol) to rat whole brain benzodiazepine receptors,[54] [^{3}H]strychnine (6 nM; 15 Ci/10^{-3} mol) to glycine rat pons medulla receptors,[55] and [^{3}H]glutamate (3 nM; 53 Ci/10^{-3} mol) to rat L-glutamate frontal cortex receptors[56] were also examined. In vitro sodium-dependent high-affinity synaptosomal GABA uptake was measured as described by Ramsey et al.[57] Rat brain L-glutamic acid decarboxylase (E.C. 4.1.1.15) activity was measured according to Tappaz et al.[58] GABA-α-ketoglutarate transaminase (E.C. 2.6.1.19) activity was measured according to Jung et al.[59]

Acknowledgment. The exploratory phase of this work was supported by a grant from the Direction des Recherches Etudes et Techniques (DRET, contrat "Jeune Chercheur" No. 80/1275/DRET/SIGS/IS5) and by funds from the Institut National de la Santé et de la Recherche Médicale (INSERM, Programme de Recherches Coordonnées, No. 121032). We thank Véronique Zerr and Marlyse Wernert for the excellent technical and secretarial assistance.

Registry No. **1**, 21339-47-9; **1** (diester deriv), 3494-85-7; **2**, 105537-47-1; **3**, 33471-40-8; **3** (3-chloro deriv), 1120-95-2; **3** (4,5-dihydro deriv), 105537-87-9; **4**, 105537-48-2; **5**, 105537-49-3; **5** (3-chloro deriv), 99708-47-1; **6a**, 105537-51-7; **6b**, 105537-89-1; **7a**, 105537-52-8; **7a** (3-chloro deriv), 105538-77-0; **7b**, 105537-90-4; **7b** (3-chloro deriv), 105538-78-1; **8a**, 78920-11-3; **8b**, 105537-53-9; **9a**, 78920-21-5; **9a** (3-chloro deriv), 86663-08-3; **9b**, 63795-91-5; **9b** (3-chloro deriv), 105538-79-2; **10**, 105538-42-9; **10**·Hcl, 96440-63-0; **11**, 105538-43-0; **11**·HBr, 105537-54-0; **12**, 105562-24-1; **13**, 105538-44-1; **13**·HBr, 105537-55-1; **14**, 105537-56-2; **15**, 105538-45-2; **15**·HBr, 105537-57-3; **16**, 105538-46-3; **16**·HBr, 105537-58-4; **17**, 105538-47-4; **17**·HBr, 105537-59-5; **18**, 105538-48-5; **18**·HBr, 105537-60-8; **19**, 105538-49-6; **19**·HBr, 105537-61-9; **20**, 105538-50-9; **20**·HBr, 105537-62-0; **21**, 105538-51-0; **21**·HBr, 105537-63-1; **22**, 105538-53-2; **22**·HBr, 104855-44-9; **23**, 105538-54-3; **23**·HCl, 105537-64-2; **24**, 105538-55-4; **24**·HBr, 105537-65-3; **25**, 105538-56-5; **25**·HCl, 105537-66-4; **26**, 105538-57-6; **26**·HBr, 105537-67-5; **27**, 105538-58-7; **27**·HBr, 105537-68-6; **28**, 105538-59-8; **28**·HBr, 105537-69-7; **29**, 105538-60-1; **29**·HBr, 105537-70-0; **30**, 105538-61-2; **30**·HCl, 105537-71-1; **31**, 105538-62-3; **31**·HBr, 105537-72-2; **32**, 105538-63-4; **32**·HBr, 105537-73-3; **33**, 105538-64-5; **33**·HCl, 105537-74-4; **34**, 105538-65-6; **34**·HBr, 105537-75-5; **35**, 105538-66-7; **35**·HCl, 105537-76-6; **36**, 105538-67-8; **36**·HBr, 105537-77-7; **37**, 105538-68-9; **37**·HCl, 105537-78-8; **38**, 105538-69-0; **38**·HCl, 105537-79-9; **39**, 105538-70-3; **39**·HCl, 105537-80-2; **40**, 105538-71-4; **40**·HBr, 105537-81-3; **41**, 105538-72-5; **41**·HCl, 105537-82-4; **42**, 105538-73-6; **42**·HBr, 104104-50-9; **43**, 105538-74-7; **43**·HBr, 105537-83-5; **44**, 105538-75-8; **44**·HCl, 105537-84-6; **45**, 105538-76-9; **45**·HBr, 105537-85-7; **46**, 105537-86-8; **46** (ethyl ester), 105538-34-9; **52**, 105538-52-1; **52** (ethyl ester), 91689-20-2; **52**·HCl, 91343-21-4; **III** ($R_1 = R_2 = H$, R_3 = 4-$O_2NC_6H_4$), 105537-91-5; **III** ($R_1 = CH_3$, $R_2 = H$, $R_3 = C_6H_5$), 81819-90-1; **III** ($R_1 = R_2 = H$, $R_3 = C_6H_5$), 14966-91-7; **III** ($R_1 = R_3 = C_6H_5$, $R_2 = H$), 17258-23-0; **III** ($R_1 = H$, $R_2 = CH_3$, $R_3 = C_6H_5$), 105537-96-0; **III** ($R_1 = R_3 = H$, $R_2 = C_6H_5$), 105537-97-1; **III** ($R_1 = H$, $R_2 = C_6H_5$, $R_3 = CH_3$), 105537-98-2; **III** ($R_2 = R_3 = H$, $R_1 = CH_3$), 90568-15-3; **III** ($R_1 = R_2 = H$, $R_3 = (CH_3)_2CHCH_2$), 105537-99-3; **III** ($R_1 = R_2 = H$, R_3 = cyclohexyl), 105538-00-9; **III** ($R_1 = R_2 = H$, $R_3 = Cl$), 5469-69-2; **III** ($R_1 = R_2 = H$, $R_3 = \alpha$-thienyl), 105538-01-0; **III** ($R_1 = R_2 = H$, $R_3 = \beta$-thienyl), 105538-02-1; **III** ($R_1 = CH_3$, $R_2 = H$, R_3 = naphthyl), 94477-31-3; **III** ($R_1 = R_2 = H$, R_3 = 2-ClC_6H_4), 105538-03-2; **III** ($R_1 = CH_3$, $R_2 = H$, R_3 = 2-ClC_6H4), 105538-04-3; **III** ($R_1 = CH_3$, $R_2 = H$, R_3 = 3-ClC_6H_4), 105538-05-4; **III** ($R_1 = R_3 = H$, R_3 = 4-ClC_6H4), 58059-47-5; **III** ($R_1 = R_2 = H$, R_3 = 2,4-$Cl_2C_6H_3$), 105538-06-5; **III** ($R_1 = R_2 = H$, R_3 = 4-FC_6H_4), 105538-07-6; **III** ($R_1 = CH_3$, $R_2 = H$, R_3 = 4-$O_2NC_6H_4$), 105538-08-7; **III** ($R_1 = R_2 = H$, R_3 = 4-CH_3OC_6H[4), 4776-87-8; **III** ($R_1 = CH_3$, $R_2 = H$, $R_3 = CH_3OC_6H_4$-4), 86663-21-0; **III** ($R_1 = CH_3$, $R_2 = H$, $R_3 = CH_3C_6H_4$-4), 105538-09-8; **IVa** ($R_1 = CH_3$, $R_2 = H$, $R_3 = C_6H_5$, $n = 2$), 105538-10-1; **IVa** ($R_1 = CH_3$, $R_2 = H$, $R_3 = C_6H_5$, $n = 4$), 105537-92-6; **IVa** ($R_1 = CH_3$, $R_2 = H$, $R_3 = C_6H_5$, $n = 5$), 105537-93-7; **IVa** ($R_1 = CH_3$, $R_2 = H$, $R_3 = C_6H_5$, n = 2,CH(CH_3)), 105537-94-8; **IVa** ($R_1 = CH_3$, $R_2 = H$, $R_3 = C_6H_5$, CH(CH_3), $n = 2$), 105537-95-9; **IVa** ($R_1 = R_2 = H$, $R_3 = C_6H_5$, $n = 3$,, 105538-11-2; **IVa** ($R_1 = R_3 = C_6H_5$, $R_2 = H$, $n = 3$), 105538-12-3; **IVa** ($R_1 = H$, $R_2 = CH_3$, $R_3 = C_9H_5$, $n = 3$), 105538-13-4; **IVa** ($R_1 = R_3 = H$, $R_2 = C_6H_5$, $n = 3$), 105538-14-5; **IVa** ($R_1 = H$, $R_2 = C_6H_5$, $R_3 = CH_3$, $n = 3$), 105538-15-6; **IVa** ($R_1 = CH_3$, $R_2 = R_3 = H$, $n = 3$), 105538-16-7; **IVa** ($R_1 = R_2 = H$, $R_3 = (CH_3)_2CHCH_2$, $n = 3$), 105538-17-8; **IVa** ($R_1 = R_2 = H$, R_3 = cyclohexyl, $n = 3$), 105538-18-9; **IVa** ($R_1 = R_2 = H$, $R_3 = Cl$, n =3), 105538-19-0; **IVa** ($R_1 = R_2 = H$, $R_3 = \alpha$–thienyl, $n = 3$), 105538-20-3; **IVa** ($R_1 = R_2 = H$, $R_3 = \beta$–thienyl, $n = 3$), 105538-21-4; **IVa** ($R_1 = CH_3$, $R_2 = H$, R_3 = naphthyl, $n = 3$), 105538-22-5; **IVa** ($R_1 = R_2 = H$, R_3 = 2-ClC_6H_4, $n = 3$), 105538-23-6; **IVa** ($R_1 = CH_3$, $R_2 = H$, R_3 = 4-ClC_6H_4, $n = 3$), 105538-24-7; **IVa** ($R_1 = CH_3$, $R_2 = H$, R_3 = 3-ClC_6H4, $n = 3$), 105538-25-8; **IVa** ($R_1 = R_2 = H$, R_3 = 4-ClC_6H4, $n = 3$), 105538-26-9; **IVa** ($R_1 = R_2 = H$, R_3 = 2,4-$Cl_2C_6H_3$, $n = 3$), 105538-27-0; **IVa** ($R_1 = R_2 = H$, R_3 = 4-FC_6H_4, $n = 3$), 105538-28-1; **IVa** ($R_1 = R_2 = H$, R_3 = 4-$O_2NC_6H_4$, $n = 3$), 105538-29-2; **IVa** ($R_1 = CH_3$, $R_2 = H$, R_3 = 4-$O_2NC_6H_4$, $n = 3$), 105538-30-5; **IVa** ($R_1 = R_2 = H$, R_3 = 4-$CH_3OC_6H_4$, $n = 3$), 105538-31-6; **IVa** ($R_1 = CH_3$, $R_2 = H$, R_3 = 4-$CH_3OC_6H_4$, $n = 3$), 105538-32-7; **IVa** ($R_1 = CH_3$, $R_2 = H$, R_3 = 4-CH_3C6H_4, $n = 3$), 105538-33-8; **IVa** ($R_1 = CH_3$, $R_2 = H$, $R_3 = C_6H_5$, $n = 3$), 105538-35-0; **IVa** ($R_1 = CH_3$, $R_2 = H$, $R_3 = C_6H_5$, $n = 4$), 105538-36-1; **IVa** ($R_1 = R_3 = C_6H_5$, $R_2 = H$, $n = 3$), 105538-37-2; **IVa** ($R_1 = R_3 = H$, $R_2 = C_6H_5$, n = 3), 105538-38-3; **IVb** ($R_1 = CH_3$, $R_2 = H$, R_3 = naphthyl, n =

3), 105538-39-4; **IVb** (R_1 = CH_3, R_2 = H, R_3 = 4-ClC_6H_4, n = 3), 105538-40-7; **IVb** (R_1 = R_2 = H, R_3 = 4-ClC_6H_4, n = 3), 105538-41-8; $H_2NCONH(CH_2)_3CO_2H$, 2609-10-1; $BOCNH(CH_2)_3CO_2H$, 57294-38-9; 4-$O_2NC_6H_4COCH_2CH(OH)CO_2H$, 81008-13-1; $Br(CH_2)_3CO_2Et$, 2969-81-5; $Br(CH_2)_3CN$, 5332-06-9; $BrCH_2CO_2H$, 79-08-3; $H_3CCHBr(CH_2)_2CO_2Et$, 27126-42-7; $BrCH_2CH(OEt)_2$, 2032-35-1; $EtO_2CCH_2CO_2Et$, 105-53-3; CH_3I, 74-88-4; H_2NNH_2, 302-01-2; $OHCCO_2Et$, 924-44-7; 4-$O_2NC_6H_4Ac$, 100-19-6; $OHCCo_2H$, 298-12-4; $(CH_3)_2CHCH_2COCH_3$, 108-10-1; $H_3CCOCH_2C_6H_5$, 103-79-7; $Br(CH_2)_2CO_2Et$, 539-74-2; $Br(CH_2)_4CO_2Et$, 14660-52-7; $Br(CH_2)_5CO_2Et$, 25542-62-5; $Br(CH_2)_2CH(CH_3)CO_2Et$, 2213-09-4; Pr_2NH, 142-84-7; potassium pyruvate, 4151-33-1; morpholine, 110-91-8; methylcyclohexylketone, 823-76-7; α-methyl-γ-hydroxybutyrolactone, 53561-62-9; 4-methyl-6-(*p*-nitrophenyl)-3(2*H*)-pyridazinone, 105537-88-0; 4-methyl-6-phenyl-3(2*H*)-pyridazinone, 13300-09-9; 4-pyrrolidinobutyric acid, 85614-44-4; 4-pyrrolidinobutyric acid hydrochloride, 49637-21-0; (+)-bicuculline methiodide, 40709-69-1.

Stereochemical Studies on the Cytochrome P-450 Catalyzed Oxidation of (*S*)-Nicotine to the (*S*)-Nicotine $\Delta^{1'(5')}$-Iminium Species

Lisa A. Peterson, Anthony Trevor, and Neal Castagnoli, Jr.*

Department of Pharmaceutical Chemistry, School of Pharmacy, University of California, San Francisco, San Francisco, California 94143. *Received March 20, 1986*

Mammals metabolize the tobacco alkaloid (*S*)-nicotine primarily to the lactam (*S*)-cotinine by a pathway involving an initial cytochrome P-450 catalyzed two-electron oxidation at the prochiral 5′-carbon atom. The stereochemical course of this oxidation was examined with human microsomal preparations and the *E* and *Z* diastereomers of (*S*)-nicotine-5′-d_1. The metabolically generated $\Delta^{1'(5')}$-iminium ion intermediate was trapped and analyzed as the corresponding diastereomeric 5′-cyano derivatives by a capillary column GC–EIMS selected ion monitoring assay. The results of these studies established that this biotransformation proceeds with the stereoselective abstraction of the 5′-*pro-E* proton, that is, the C-5′ proton trans to the bulky pyridyl group. The observed stereoselectivity was independent of proton vs. deuteron abstraction. Additionally, the extent of (*S*)-cotinine formation was minor and did not influence the stereochemical composition of the metabolically derived α-cyano amines. Studies with male Dutch rabbit liver microsomal preparations gave similar results. These findings suggest that the structure of the complex formed between (*S*)-nicotine and the active site of cytochrome P-450 is highly ordered and dictates the stereochemical course of the reaction pathway.

The cytochrome P-450 catalyzed[1] overall two-electron oxidation of (*S*)-nicotine (**1**) at the prochiral C-5′ position to form the iminium intermediate **2** is the initial step in the biotransformation of this alkaloid to (*S*)-cotinine (**3**), the principal metabolite of (*S*)-nicotine in humans.[2] Since the two methylene protons at the C-5′ position are diastereotopic, this α-carbon oxidation may proceed by a process that could result in the selective abstraction of the 5′-*pro-E* (Ha) or 5′-*pro-Z* (Hb) proton. In order to understand better the detailed molecular mechanisms associated with the cytochrome P-450 catalyzed α-carbon oxidation of this and other aliphatic tertiary amines, we have undertaken studies to characterize the stereochemical course followed in this biotransformation.

The available experimental evidence relating to this type of transformation supports a stepwise reaction pathway involving an initial transfer of one electron from the lone pair of the pyrrolidine nitrogen atom to a heme-bound, electron-deficient oxygen atom.[3] The subsequent one-electron oxidation of the resulting radical cation **4** is the step that is accompanied by the net loss of hydrogen from the C-5′ position. This step may proceed directly to the iminium species **2** (loss of a hydrogen atom) or via the carbon-centered radical **5** (loss of a proton) and the carbinolamine **6**, which will be in equilibrium with **2**. The iminium intermediate, which in vivo is oxidized further to (*S*)-cotinine (**3**) in a reaction catalyzed by cytosolic enzymes,[4] may be trapped as the corresponding diastereomeric α-cyano adducts **7** and **8**.[1,5]

Several studies concerned with substrate diastereoselective biooxidations at prochiral methylene carbon atoms have been reported. For example, using cholesterol-7α-t_1 and -7β-t_1, Bergstrom et al. established that the 7α-hydroxylation of cholesterol in the rat proceeds with greater than 90% loss of the 7α-hydrogen atom.[6] A somewhat similar experiment showed that the 11β-hydroxylation of pregnanedione-*11α*, *12α*-t_2 by perfused bovine adrenal glands proceeds with complete retention of label.[7] On the other hand, the cytochrome $P\text{-}450_{cam}$ catalyzed oxidations of both camphor-*5-exo*- and *5-endo*-d_1 yield principally 5-*exo*-hydroxycamphor-*5-endo*-d_1.[8] According to our knowledge, analogous studies on aliphatic tertiary amines have not been reported.

This paper summarizes the results of our stereochemical investigations on the α-carbon oxidation of (*S*)-nicotine by human and male Dutch rabbit liver microsomal preparations with use of the specifically labeled (*E*)- and (*Z*)-5′-monodeuterio diastereomers **9** and **10**, respectively, of (*S*)-nicotine. The synthesis of **9** was achieved by the stereoselective[9] catalytic deuteriation of the corresponding $\Delta^{1'(5')}$-iminium species **2**. Similarly, catalytic hydrogenation of **2**-d_1 provided the corresponding *Z* diastereomer **10**.

(1) Murphy, P. J. *J. Biol. Chem.* **1975**, *248*, 2796.
(2) Gorrod, J. W.; Jenner, P. *Essays Toxicol.* **1975**, *6*, 35.
(3) Shono, J.; Toda, J.; Oshino, N. *J. Am. Chem. Soc.* **1982**, *104*, 2639. Miwa, G. T.; Walsh, J. S.; Kedderis, G. L.; Hollenberg, P. F. *J. Biol. Chem.* **1983**, *258*, 14445. Hanzlik, R. P.; Tullman, R. H. *J. Am. Chem. Soc.* **1982**, *104*, 2048. Augusto, O.; Beilan, H. S.; Ortiz de Montellano, P. R. *J. Biol. Chem.* **1982**, *257*, 11288.
(4) Gorrod, J. W.; Hibberd, A. R. *Eur. J. Drug Metab. Pharmacokinet.* **1982**, *7*, 293.
(5) Nguyen, T. L.; Gruenke, L. D.; Castagnoli, Jr., N. *J. Med. Chem.* **1979**, *22*, 259.
(6) Bergstrom, S.; Lindstredt, S.; Samuelson, B.; Corey, E. J.; Gregoriou, G. A. *J. Am. Chem. Soc.* **1958**, *80*, 2337.
(7) Hayano, M.; Gut, M.; Dorfman, R. I.; Sebek, O. K.; Peterson, D. H. *J. Am. Chem. Soc.* **1958**, *80*, 2336.
(8) Gelb, M. H.; Heimbrook, D. C.; Malkonen, P.; Sligar, S. G. *Biochemistry* **1982**, *21*, 370.
(9) Freifelder, M. *Catalytic Hydrogenation in Organic Synthesis: Procedures and Commentary*; Wiley: New York, 1978; Chapters 2 and 8.

0022-2623/87/1830-0249$01.50/0