Structure and Molecular Modeling of $GABA_A$ Receptor Antagonists

Didier Rognan,[†,‡] Thierry Boulanger,[§] Rémy Hoffmann,[†] Daniel P. Vercauteren,[§] Jean-Marie Andre,[§] Francois Durant,[§] and Camille-Georges Wermuth*[,†]

Laboratoire de Pharmacochimie Moléculaire, Centre de Neurochimie et Université Louis Pasteur, 67084 Strasbourg Cedex, France, and Laboratoire de Chimie Moléculaire Structurale, Facultés Universitaires Notre-Dame de la Paix, B-5000 Namur, Belgium. *Received March 11, 1991*

The recently described potent and selective $GABA_A$ antagonist SR 95531 (gabazine) is compared to six other $GABA_A$ antagonists: (+)-bicuculline, (−)-securinine, (+)-tubocurarine, iso-THAZ, R-5135, and pitrazepine. Starting from ab initio molecular orbital calculations performed on crystal atomic coordinates, attempts were made to identify in each structure the functional groups that are involved in receptor recognition and binding. A molecular modeling study revealed that (a) all compounds possess accessible cationic and anionic sites separated by an 4.6–5.2 Å intercharge distance, (b) the antagonistic nature of the compounds can be explained by the presence of additional binding sites, (c) the correct spatial orientation of the additional binding sites is crucial for $GABA_A$ selectivity, and (d) the criteria determining the potency of the antagonist effect are an accurate intercharge distance (>5 Å) and the existence of hydrogen-bonding functionalities on one of the additional ring system. The presented pharmacophore accounts also for the inactivity of closely related compounds such as (−)-bicuculline, adlumidine, virosecurinine, allosecurinine, and the 4,6-diphenyl analogue of gabazine.

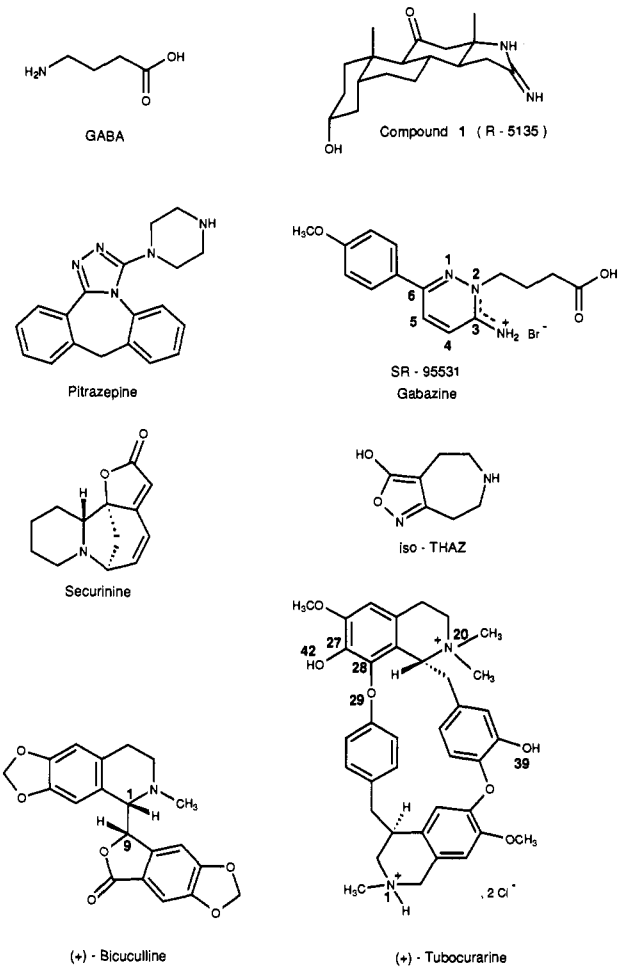
Introduction

We have recently shown that pyridazinyl-GABA derivatives with a butyric acid chain linked to the N_2 nitrogen of a 3-aminopyridazine are selective and competitive $GABA_A$ antagonists.[1-4] Structure–activity relationships studies led to the compound SR 95531 (gabazine; ChartI). Its biochemical, pharmacological, and electrophysiological study has already been extensively described elsewhere.[5-7] A synthesis of radiolabeled gabazine has also been reported.[8] [^{3}H]Gabazine is commercially available[9] and it was shown to be a highly specific ligand of the $GABA_A$ receptor.[10,11]

However, few studies were undertaken on the stereoelectronic requisites associated with a $GABA_A$ antagonist activity.[12-15] Taking advantage of the selective and potent antagonist activity of gabazine, the aim of our study was to compare this compound with other $GABA_A$ antagonists. The structures of (+)-bicuculline, (−)-securinine, (+)-tubocurarine, iso-THAZ, R-5135 (compound 1), and pitrazepine were selected for this purpose (Chart I and Table I). Using ab initio molecular orbital calculations performed on crystalline atomic coordinates, attempts were made to identify in each structure the functional groups that are involved in receptor recognition and binding. In complement to these theoretical data, a more topological study of all antagonists using computer graphics facilities was performed in order to determine the salient stereoelectronic requirements for a potent and selective $GABA_A$ antagonism.

On the basis of previous successful studies,[35-37] the active analogue approach[38] was used to map the $GABA_A$ receptor. Starting from a template, which is generally a potent, selective, and conformationally constrained molecule, the active conformations of more flexible analogues can be deduced via systematic conformational search using geometrical parameters of the template as constraints. This approach is not immediately applicable to the whole set of antagonists with respect to their conformational freedom. Therefore, the seven antagonists described in this paper were divided into two classes, depending on their affinity and selectivity for the $GABA_A$ receptors (Table I). A template structure from each class was then selected. The first class includes potent antagonists (1, pitrazepine,

Chart I. Structure of GABA and Seven $GABA_A$ Receptor Antagonists



and gabazine) which have IC_{50} values ([^{3}H]GABA binding) lower than 10 μM, but which also exhibit high affinities

† Centre de Neurochimie et Université Louis Pasteur.
‡ Present address: Departement Pharmazie, ETH-Zentrum, Clausiusstrasse 21, CH-8092 Zürich, Switzerland.
§ Facultés Universitaires Notre-Dame de la Paix.

(1) Wermuth, C. G.; Bizière, K. Pyridazinyl-GABA derivatives: A New class of synthetic $GABA_A$ antagonists. *Trends Pharm. Sci.* **1986**, *7*, 421–424.
(2) Chambon, J. P.; Feltz, P.; Heaulme, M.; Restle, S.; Schlichter, R.; Bizière, K.; Wermuth, C. G. An arylaminopyridazine derivative of gamma-aminobutyric acid (GABA) is a selective and competitive antagonist at the $GABA_A$ receptor site. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 1832–1836.

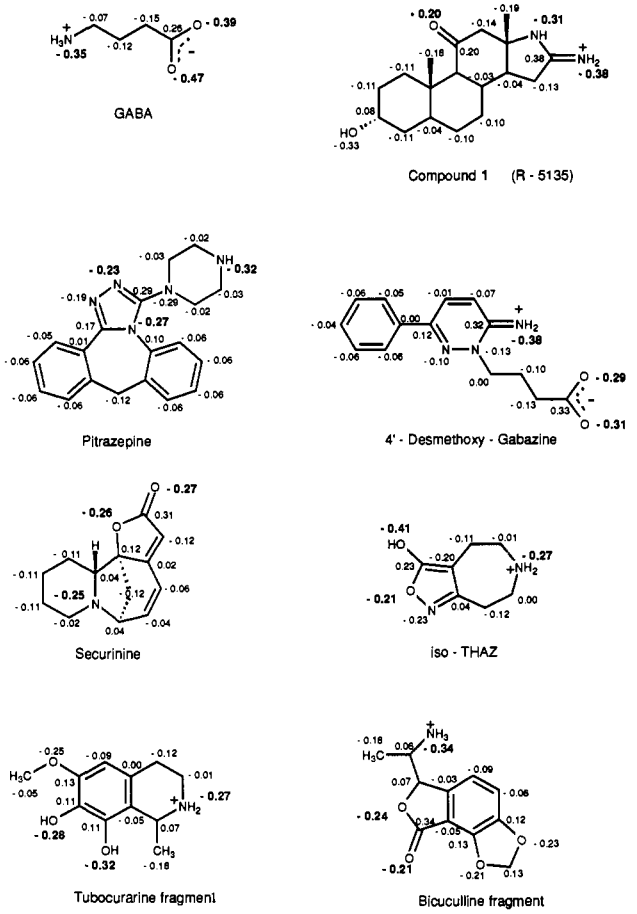
0022-2623/92/1835-1969$03.00/0

Table I. Affinity and Selectivity Profiles of $GABA_A$ Antagonists

compound	[^{3}H]GABA binding, μM	other activities	ref	class
gabazine	0.14[a]	none	1–11	I
bicuculline	15[b]	acetylcholinesterase inhibitor	16–19	II
iso-THAZ	16[b]	glycine antagonist	20–22	II
tubocurarine	58[b]	nicotinic receptor blocker	23–26	II
pitrazepine	0.24[b,c]	glycine antagonist, BZD ligand[d]	27–30	I
compound 1	0.020[b]	glycine antagonist, BZD ligand[d]	31–33	I
securinine	50[b]	none	34	II

[a] K_i values. [b] IC_{50} values. [c] [^{3}H]Muscimol binding. [d] BZD = benzodiazepine.

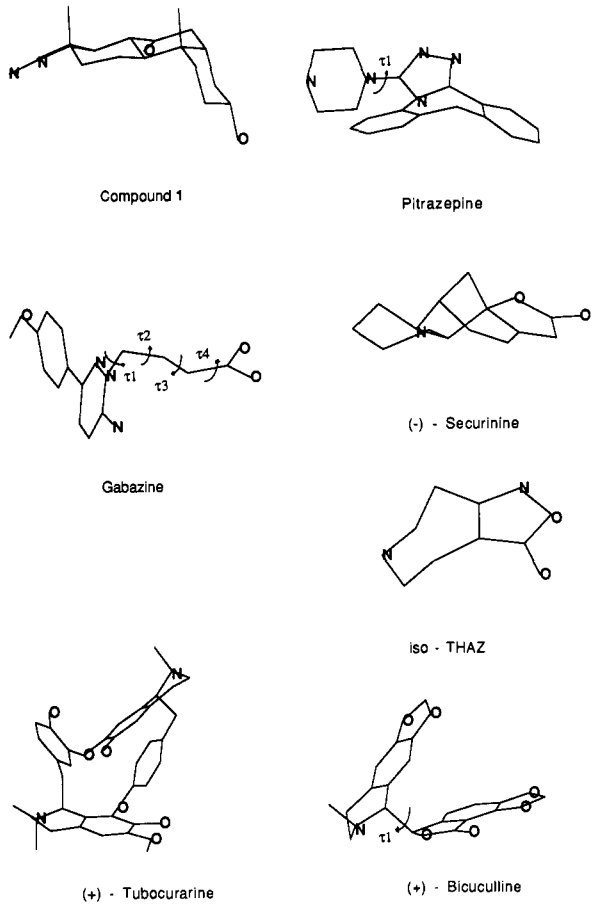
Chart II.[a] Atomic Charges of GABA and $GABA_A$ Antagonists Computed by Ab Initio Calculations from X-ray Conformations[a]



[a] The charges representing anionic and cationic sites are displayed in boldface.

for the strychnine-sensitive glycine or the benzodiazepine receptor sites (except for gabazine, which is selective for

Chart III. Putative Active Conformations of the $GABA_A$ Antagonists at Their Receptor Site



the $GABA_A$ receptor). In the second class, we selected molecules which are weaker antagonists, with IC_{50} values

(3) Bourguignon, J. J.; Schlewer, G.; Melikian, A.; Chantreux, D.; Molimard, J. C.; Heaulme, M.; Chambon, J. P.; Bizière, K.; Wermuth, C. G. Aminopyridazines: Derivatives of SR 95103, structure-activity relationships. *Pharmacologist* **1985**, *27*, 518.

(4) Wermuth, C. G.; Bourguignon, J. J.; Schlewer, G., Gies, J. P.; Schoenfelder, A.; Melikian, A.; Bouchet, M. J.; Chantreux, D.; Molimard, J. C.; Heaulme, M.; Chambon, J. P.; Bizière, K. Synthesis and Structure–Activity Relationships of a Series of Aminopyridazine Derivatives of gamma-Aminobutyric Acid Acting as Selective GABA-A Antagonists. *J. Med. Chem.* **1987**, *30*, 239–249.

(5) Heaulme, M.; Chambon, J. P.; Leyris, R.; Molimard, J. C.; Wermuth, C. G.; Bizière, K. Biochemical Characterization of the Interaction of Three Pyridazinyl-GABA Derivatives with the $GABA_A$ Receptor Site. *Brain Res.* **1986**, *384*, 224–231.

(6) Michaud, J. C.; Mienville, J. M.; Chambon, J. P.; Bizière, K. Interactions between three pyridazinyl-GABA derivatives and central GABA and glycine receptors in the rat. An *in vivo* microiontophoretic study. *Neuropharmacology* **1986**, *25*, 1197–1203.

(7) Santucci, V.; Fournier, M.; Chambon, J. P.; Bizière, K. Electroencephalographic study of SR 95103, a $GABA_A$ antagonist: Interaction with inhibitory amino acids and muscimol. *Eur. J. Pharmacol.* **1985**, *114*, 219–222.

(8) Melikian, A.; Schlewer, G.; Hurt, S.; Chantreux, D.; Wermuth, C. G. Synthesis of tritiium-labelled 2-(3′-carboxypropyl)-3-imino-6 para-methoxy phenyl-2,3-dihydropyridazine ([^{3}H] SR 95531). *J. Labeled Compd. Radiopharm.* **1987**, *24*, 267–274.

(9) Du Pont, NEN Products, Boston, MA 02118.

(10) Heaulme, M.; Chambon, J. P.; Leyris, R.; Wermuth, C. G.; Bizière, K. Specific binding of a phenyl-pyridazinium derivative endowed with $GABA_A$ receptor antagonist activity to rat brain. *Neuropharmacology* **1986**, *25*, 1279–1283.

(11) Heaulme, M.; Chambon, J. P.; Leyris, R.; Wermuth, C. G.; Bizière, K. Characterization of the Binding of [^{3}H]SR 95531, a $GABA_A$ Antagonist to Rat Brain Membranes. *J. Neurochem.* **1987**, *48*, 1667–1686.

(12) Steward, E. G.; Borthwick, P. W.; Clarke, G. R.; Warner, D. Agonism and antagonism of gamma-aminobutyric acid. *Nature (London)* **1975**, *256*, 600–602.

(13) Wermuth, C. G.; Rognan, D. Modeling of Antagonists of the GABA-A Receptor. *Actual Chim. Thér.* **1987**, *14*, 215–233.

(14) Boulanger, T.; Vercauteren, D. P.; Durant, F.; Andrē, J. M. 3- and 5-Isoxazolol Zwitterions: *An ab initio* Molecular Orbital Study relating to GABA Agonism and Antagonism, *J. Theor. Biol.* **1987**, *127*, 479–489.

(15) Pooler, C. W.; Steward, E. G. Structural factors governing agonist and antagonist activity in the $GABA_A$ system. *Biochem. Pharmacol.* **1988**, *37*, 943–945.

Table II. Pharmacophore Elements of $GABA_A$ Antagonists

compound	anionic site	cationic site	intercharge distance,[a] Å	dihedral angles,[b] deg τ_1	τ_2	τ_3	τ_4	ΔE,[c] kcal/mol
compound 1	ketone	amidine	5.14					
pitrazepine	triazole	secondary amine	4.71	67				1.69
gabazine	carboxylic acid	primary amine	4.80	240	33	180	0	2.30
securinine	lactone	tertiary amine	4.62					
iso-THAZ	isoxazolol	secondary amine	4.40					
tubocurarine	O_{29}–C_{28}–C_{27}–O_{42} centroid	quaternary ammonium	4.63					
bicuculline	lactone	quaternary ammonium	4.65	120				2.40

[a] Distance between anionic and cationic sites. For delocalized systems, the centroid of the corresponding atoms were selected. [b] Dihedral angle values of flexible antagonists found after conformational analysis using the intercharge distance of compound 3 as constraint. [c] Energy deviation from the minimum found by molecular mechanics.

higher than 10 μM (bicuculline, securinine, iso-THAZ, and tubocurarine).

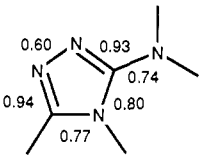


Figure 1. π-Electron overlap population calculated for the triazolic fragment of pitrazepine. Delocalization occurs mainly over the triazole ring with a minor participation of the exocyclic nitrogen atom.

As starting data, available crystal atomic coordiantes were chosen for all compounds. The structural study of the antagonists could then be performed in two steps: (i) geometry optimization and atomic charges calculations using ab initio molecular orbital methods and (ii) molecular modeling with respect to the previous electronic analysis, using SYBYL software.[39]

Results

Starting from X-ray diffraction data on GABA[40] we performed an ab initio geometry optimization and recorded net atomic charges (expressed in electrons) (Chart II). Particular interesting values are the atomic charges −0.47e, 0.26e, and −0.39e computed for the three carboxylate atoms and −0.35e for the nitrogen atom. This charge is negative although the nitrogen atom is protonated. In fact, the positive charge is shared by the hydrogen atoms of the amino group and the neighboring carbon (not shown on Chart II; see ref 14 for a more detailed study). However,

(16) Curtis, D. R.; Duggan, A. W.; Felix, D.; Johnston, G. A. R. GABA, Bicuculline and Central Inhibition. *Nature (London)* **1970**, *226*, 1222–1224.
(17) Johnston, G. A. R.; Beart, P. M.; Curtis, D. R.; Game, C. G. A.; McCulloch, R. M.; McLachlan, R. M. Bicuculline methochloride as a GABA antagonist. *Nature (London)* **1972**, *240*, 219–220.
(18) Svenneby, G.; Roberts, E. Bicuculline and N-methylbicuculline-competitive inhibitors of brain acetylcholinesterase. *J. Neurochem.* **1973**, *21*, 1025–1026.
(19) Svenneby, G.; Roberts, E. Elevated acetylcholine content in mouse brain after treatment with bicuculline and picrotoxin. *J. Neurochem.* **1974**, *23*, 275–277.
(20) Arnt, J.; Krogsgaard-Larsen, P. GABA agonists and potential antagonists related to muscimol. *Brain Res.* **1979**, *177*, 395–399.
(21) Krogsgaard-Larsen, P.; Hjeds, H.; Curtis, D. R.; Leah, J. D.; Peet, M. J. Glycine Antagonists Structurally Related to Muscimol, THIP, or Isoguvacine. *J. Neurochem.* **1982**, *39*, 1319–1324.
(22) Brehm, L.; Krogsgaard-Larsen, P.; Schaumburg, K.; Johansen, J. S.; Falch, E.; Curtis, D. R. Glycine Antagonists. Synthesis, Structure, and Biological Effects of Some Bicyclic 5-Isoxazolol Zwitterions. *J. Med. Chem.* **1986**, *29*, 224–229.
(23) Triggle, D. J.; Triggle, C. R. *Chemical Pharmacology of the Synapse*; Academic Press: London, 1976; p 370.
(24) Maksay, G.; Ticku, M. K. Diazotization and Thiocyanate Differentiate Agonists from Antagonists for the High- and Low-Affinity Receptors of gamma-Aminobutyric Acid. *J. Neurochem.* **1984**, *43*, 261–268.
(25) Hill, R. G.; Simmonds, M. A.; Straughan, D. W. Convulsive properties of alpha-tubocurarine and cortical inhibition. *Nature (London)* **1972**, *240*, 51–52.
(26) Simmonds, M. A. Classification of some GABA antagonists with regard to site of action and potency in slices of rat cuneate nucleus. *Eur. J. Pharmacol.* **1982**, *80*, 347–358.
(27) Gähwiller, B. H.; Maurer, R.; Wüthrich, H. J. Pitrazepin, a novel $GABA_A$ antagonist. *Neurosci. Lett.* **1984**, *45*, 311–316.
(28) Kemp, J. A.; Marshall, G. R.; Wong, E. H. F.; Woodruff, G. N. Pharmacological studies on pitrazepin, a $GABA_A$ receptor antagonist. *Br. J. Pharmacol.* **1985**, *85*, 237P.
(29) Braestrup, C.; Nielsen, M. Interaction of pitrazepin with the GABA/benzodiazepine receptor complex and with glycine receptors. *Eur. J. Pharmacol.* **1985**, *118*, 115–121.
(30) Curtis, D. R.; Gynther, B. D. Pitrazepin: A Central glycine and GABA antagonist. *Eur. J. Pharmacol.* **1986**, *131*, 311–313.
(31) Hunt, P.; Clements-Jewery, S. A. steroid derivative, R 5135, antagonizes the GABA/benzodiazepine receptor interaction. *Neuropharmacol.* **1981**, *20*, 357–361.
(32) Simmonds, M. A.; Turner, J. P. Antagonism of inhibitory amino acids by the steroid derivative RU5135. *Br. J. Pharmacol.* **1985**, *84*, 631–635.
(33) Curtis, D. R.; Malik, R. Glycine antagonism by RU5135. *Eur. J. Pharmacol.* **1985**, *110*, 383–384.
(34) Beutler, J. A.; Karbon, E. W.; Brubaker, A. N.; Malik, R.; Curtis, D. R.; Enna, S. J. Securinine Alkaloids: A New Class of GABA Receptor Antagonist. *Brain Res.* **1985**, *330*, 135–140.
(35) Hibert, M. F.; Gittos, M.W.; Middlemiss, D. N.; Mir, A. K.; Fozard, J. R. Graphics Computer-Aided Receptor Mapping as a Predictive Tool for Drug Design; Development of Potent, Selective, and Stereospecific Ligands for the $5\text{-}HT_{1A}$ Receptor. *J. Med. Chem.* **1986**, *31*, 1087–1093.
(36) Mayer, D.; Naylor, C. B.; Motoc, I.; Marshall, G. R. A unique geometry of the active site of angiotensin-converting enzyme consistent with structure–activity studies. *J. Comput.-Aided Mol. Des.* **1987**, *1*, 3–16.
(37) Kaminski, J. J.; Puchalski, C.; Solomon, D. M.; Rizvi, R. K.; Conn, D. J.; Elliott, A.; Lovey, R. G.; Guzik, H.; Chiu, P. J. S.; Long, J. F.; McPhail, A. T. Antiulcer Agents. 4. Conformational Considerations and the Antiulcer Activity of Substituted Imidazo[1,2-a]pyridines and Related Analogues. *J. Med. Chem.* **1989**, *32*, 1686–1700.
(38) Marshall, G. R.; Barry, C. D.; Bosshard, H. E.; Dammkoehler, R. A.; Dunn, D. A. The Conformational Parameter in Drug Design: The Active Analog Approach. In *Computer-Assisted Drug Design*, ACS Symposium Series 112; Olson, E. C., Christoffersen, R. E., Eds; American Chemical Society: Washington, DC, 1979; pp 205–226.
(39) TRIPOS Associates Inc., St-Louis, MO 63144 (5.2. release).
(40) Steward, E. G.; Player, R. B.; Warner, D. The Crystal and Molecular Structure of gamma-Aminobutyric Acid Determined at Low Temperature. *Acta Crystallogr. Sect. B*, **1973**, *B29*, 2038–2040.

we assume that binding of GABA to the $GABA_A$ receptor is mainly represented by two ionic interactions involving a delocalized carboxylate function (anionic site) and a protonated nitrogen (cationic site).

The next objective was to recognize the main electronic and topological features associated with the high affinity and strong antagonist properties of the first group of antagonists: compound 1 (R-5135), pitrazepine, and gabazine. The rigid steroid R-5135 was chosen as template structure for this first class. Its hydrobromide salt is shown in the crystal conformation on Chart III. The structure of this amidine was optimized by the semiempirical MNDO method; it has also been determined by X-ray diffraction.[41] Ab initio calculations, using either theoretical or experimental geometries, yielded identical charge distributions. The oxygen atom in position 11 presents an atomic charge of −0.20e (Chart II) so that the carbonyl function may act as a possible interaction site. The charges of both nitrogen atoms are −0.31 and −0.38e for the endo and exo atoms, respectively. Each of these nitrogens can separately act as an equivalent of the protonated nitrogen of GABA (−0.35e). In fact, the amidinic system within R-5135 is highly delocalized, so that the positive charge is spread over three atoms; thus the interaction with the receptor may imply the whole amidinic system. As a consequence we can define for compound 1 as well as for GABA itself, a cationic site of interaction (amidinic group protonated under physiological conditions) and an anionic site (the carbonyl function in position 11). The distance between the centroid of the amidinic moiety and the midpoint of the C=O bond, 5.14 Å, was thus considered as a starting reference of the "intercharge" distance in $GABA_A$ antagonists.

The structure of the free base form of pitrazepine has been solved by X-ray diffraction.[42] The analysis of the crystal packing, which includes cocrystallized water molecules, shows the ability of pitrazepine to develop hydrogen bonds by its secondary amine. The two benzo groups are located far from the piperazinotriazole heterocyclic system and thus produce only a slight steric hindrance in its vicinity. Starting from the experimental geometry, ab initio calculations were then performed. A charge of −0.32e was found for the secondary amine (free base) of the piperazine ring. The guanidinic nitrogen atoms of the triazole ring carry relatively symmetrically distributed net charges of −0.27e and −0.23e (Chart II). Compared to the values found for the carboxylic group of GABA (−0.39e and −0.47e), these values are significantly lower. However, they are of the same order of magnitude than those found for lactones (bicuculline and securinine, see below). The π-electron overlap population calculations show also that the delocalization of the triazole ring does not overlap with the exocyclic piperazine nitrogen (Chart II). The non conjugated nature of this nitrogen is clearly evidenced by the values reported in Figure 1, which shows that a single bond links the piperazine and the triazole rings. Furthermore, the comparison of the triazole ring with a carboxylic group is strongly in favor of an isosteric and isoelectronic mimicry between the triazolic N–C–N atoms and the carboxylic O–C–O atoms of GABA. The triazole ring of pitrazepine can thus be identified to an anionic site of interaction. The cationic site must then be the secondary amino group of the piperazinyl ring (atomic charge −0.32e as compared to −0.35e for GABA).

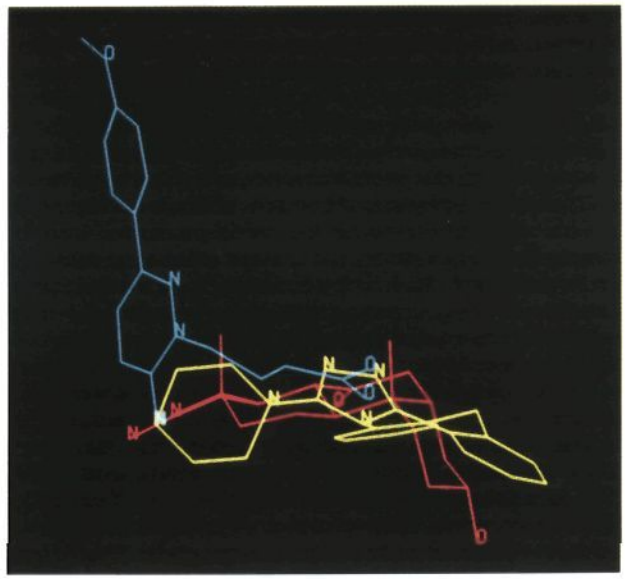
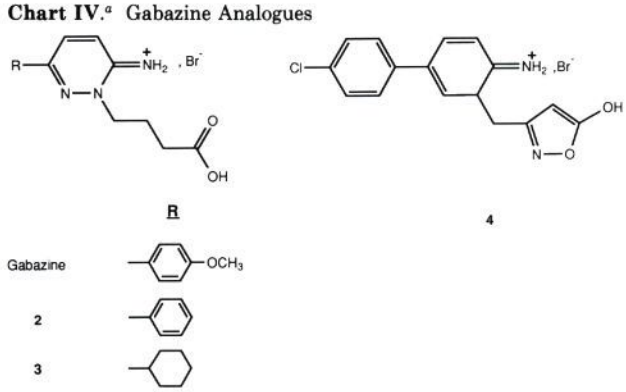


Figure 2. Superposition of compound 1 (red) on pitrazepine (yellow) and gabazine (cyan) in their active conformation at the $GABA_A$ receptor sites. Hydrogen atoms have been omitted for clarity.

Chart IV.[a] Gabazine Analogues



[a] Crystal structures of compounds 2 and 3 were used to obtain atomic coordinates of gabazine. The conformationally restrained analogue 4 allows fixing of two dihedral angles of the gabazine carboxypropyl side chain, thus simplifying its conformational analysis.

As it was not possible to fit both ionic interaction sites of compound 1 and pitrazepine considering only the solid-state conformations, we performed a conformational analysis of pitrazepine taking into account only the rotatable bond (see Experimental Section). The intercharge distance found for the rigid steroid 1 (5.14 Å) was used as constraint to disclose the active conformation of pitrazepine. After a systematic conformational search and relaxation by molecular mechanics (MAXIMIN 2[43]), we selected the conformer shown in Chart III. This conformation with a τ_1 dihedral angle of 67° corresponds to a low-energy conformation close to the global minimum (Table II). The intercharge distance between the centroid of the N–C–N

(41) Boulanger, T.; Vercauteren, D. P.; Evrard, G.; Durant, F. Comparison between Optimized and Crystal Structures of R5135, a Potent Steroidic GABA-A Antagonist: *Ab Initio* Molecular Orbital Charge Population Analysis. . *J. Mol. Struct.* **1989**, *212*, 315–322.

(42) Boulanger, T.; Vercauteren, D. P.; Evrard, G.; Durant, F. Crystal Structure and Quantum Electronic Analyses of Pitrazepin, a Gamma-Aminobutyric Acid (GABA) Receptor Antagonist. *J. Chem. Soc. Perkin Trans 2* **1987**, 217–221.

(43) Clark, M.; Cramer, R. D., III; Van Opdenbosch, N. Validation of the general purpose TRIPOS 5.2 force field. *J. Comput. Chem.* **1989**, *10*, 982–1012.

triazole and the secondary piperazinyl amine is shorter (4.71 Å) than that observed for the steroid 1 (5.14 Å). However, both molecules have their main interaction sites located in a same three-dimensional region (Figure 2), making possible a similar interaction with the $GABA_A$ receptor.

To close the study of this first group of antagonists, we analyzed the pyridazinyl-GABA derivative gabazine. Although its three-dimensional structure has not yet been established by X-ray diffraction, the crystal structures of very close analogues SR 95128 (**2**) and SR 95305 (**3**) (6-phenyl and 6-cyclohexyl analogues of gabazine, respectively) are available (Chart IV).[44,45] Both structures show a similar electronic delocalization of the aminopyridazine ring system. Starting from the crystal atomic coordinates of compound **2**, the atomic charges computed for the carboxylic oxygens are –0.31e and 0.29e (Chart II). These values are in agreement with an undissociated carboxylic group and thus are lower than those observed for the anionic form of the GABA zwitterion (–0.47e and –0.39e). The atomic charges for the nitrogen atoms are –0.10e, –0.13e, and –0.36e for the N_1 and N_2 nitrogens of the pyridazine ring and for the exocyclic nitrogen, respectively. In contrast to the amidinic moiety of steroid **1**, for which both nitrogen atoms were electronically identical (0.31e and –0.38e), the N_1 and N_2 atomic charges of compound **2** present only a minor contribution, comparable to that of carbon atoms. With an atomic charge of –0.36e, we propose that only the protonated exocyclic nitrogen should be involved in receptor interaction as a cationic site. The structure of gabazine was easily derived from that of compound **2** by adding a *p*-methoxy group using SYBYL standard fragments. Here again, a conformational analysis of the flexible carboxypropyl side chain was necessary to identify the active conformation. However, this analysis could be limited to the τ_1 and τ_2 dihedral angles (see Chart III) since conformational blockade of the τ_3 and τ_4 dihedral angles such as that observed in the muscimol derivative **4** is still compatible with potent and selective $GABA_A$ antagonist properties (Chart IV).[46] Torsional angle values of 180° and 0° for τ_3 and τ_4 were thus assigned during the conformational search procedure. The analysis was restricted to the τ_1 and τ_2 angles using the 5.14 Å value as constraint for the intercharge distance. The energy-minimized selected conformer shown on Chart III was compared with compound **1** and pitrazepine (Figure 2). When protonated nitrogens are fitted, the carboxylic acid function of gabazine can be well matached either to the carbonyl function of compound **1** or to the isoelectronic N–C–N triazolic system of pitrazepine. This fit was achieved with a low-energy conformation of gabazine (Table II). For this conformer, the intercharge distance (4.80 Å) between the exocyclic amino group and the centroid of the carboxylic function lays in the same range than that observed for the two previous antagonists.

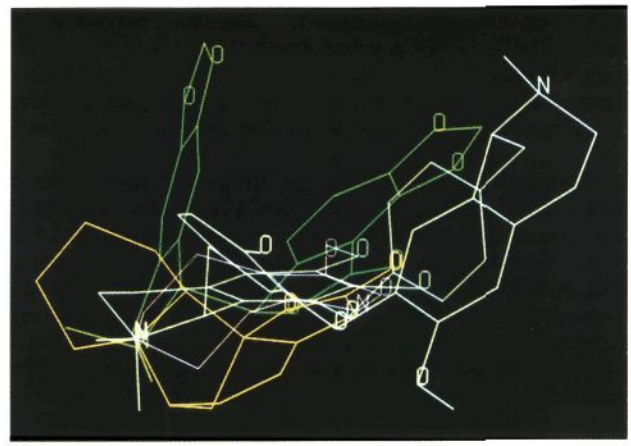


Figure 3. Superposition of (–)securinine (red orange) on iso-THAZ (magenta), tubocurarine (white), and bicuculline (green) in their active conformation at the $GABA_A$ receptor sites. Hydrogen atoms have been omitted for clarity.

For the second class of $GABA_A$ antagonists, which includes securinine, iso-THAZ, tubocurarine, and bicuculline (Chart I), (–)-securinine was chosen as template. The crystal structure of its hydrobromide has been solved by X-ray diffraction.[47] Ab initio calculations were performed using this experimentally observed geometry. The atomic charges computed for the exo- and endo-cyclic lactonic oxygen atoms are –0.27e and –0.26e, respectively. These values remain very close to that observed for the triazolic N–C–N atoms of pitrazepine for example (Chart II). The protonated tertiary amine has a charge of –0.29e and may represent the cationic site of interaction, the lactone being here a pseudoanionic interaction site. The intercharge distance computed from the tertiary amine to the lactone centroid (4.62 Å) is slightly shorter than that found for the other less potent antagonists (Table II).

The three-dimensional structure of iso-THAZ has not been solved by X-ray diffraction, but the known experimental structures of closely related analogues (THAZ[48] and isomuscimol[49]) gave us easy access to the atomic coordinates of the zwitterionic form. The computed atomic charges of both oxygen atoms are –0.41e and –0.21e, as compared with –0.47e and –0.39e for GABA itself (Chart II).[14] The slight decrease of the charge on the exocyclic oxygen is due to a delocalization of the negative charge over the whole isoxazole heterocycle, rather than toward the only endocyclic oxygen atom, which is thus less negative. However, the observed charges appear to be strong enough to insure receptor recognition. The nitrogen atom charge is –0.27e for iso-THAZ versus –0.35e for GABA. The observed decrease is due to the insertion of the nitrogen atom into a secondary amine function for which the positive charge is spread over the two neighboring carbon atoms. This value can again be compared with the charge of the secondary amine of pitrazepine (–0.32e as free base). This result confirms that protonation of a secondary amine leads to a decrease of the nitrogen atomic charge of approximately 0.05e. According to the previous study, the centroid

(44) Van der Brempt, C.; Evrard, G.; Durant, F. Structure of an Aminopyridazine Derivative. *Acta Crystallogr.* **1986**, *C42*, 1206–1208.

(45) Boulanger, T.; Vercauteren, D. P.; Evrard, G.; Durant, F. Structural and Electronic Comparative Analysis of Aminopyridazines showing Anti-GABA Activity. Crystal Structure of 2-(Carboxy-3′-Propyl)-3-Amino-6-Cyclohexylpyridazinium Bromide. *Ab initio* Mulliken Population Analysis of the 6-Phenyl-substituted Analog Compared to GABA. *J. Crystallogr. Spectrosc. Res.* **1987**, *17*, 561–573.

(46) Melikian, A.; Schlewer, G.; Chambon, J. P.; Heaulme, M.; Wermuth, C. G. Muscimol Can Replace GABA in the "Pyridazinyl-GABA-Type" Antagonists. *Actual. Chim. Thér.* **1988**, *15*, 337–341.

(47) Imado, S.; Shiro, M.; Horii, Z. Structure of Securinine Hydrobromide Dihydrate and the Molecular Structure of Securinine. *Chem. Pharm. Bull.* **1965**, *13*, 643–651.

(48) Brehm, L.; Larsen, N. A. L. 5,6,7,8-Tetrahydro-4H-isoxazolo-[4,5]azepin-3-ol Monohydrate, a Structural Analogue of GABA, *Acta Crystallogr.* **1976**, *B32*, 3336–3339.

(49) Honoré, T.; Brehm, L. 3-Aminomethyl-5-isoxazolol Monohydrate (Isomuscimol). *Acta Crystallogr.* **1978**, *B34*, 3417–3419.

Table III. Possible Interaction Modes of (+)-Tubocurarine to the $GABA_A$ Receptor

interaction mode	anionic site	cationic site	intercharge distance, Å
A	N_1	O_{39}	6.48
B	N_1	O_{42}	8.70
C	N_{20}	O_{39}	7.30
D	N_{20}	O_{42}	6.05

of the delocalized O_{exo}–C–O_{endo}–N atoms of the isoxazole ring was superposed on the lactone centroid of securinine (Figure 3). In the overlay, both nitrogen atoms are well matched. These two molecules, which bind to the $GABA_A$ receptor with micromolar affinities, share a similar spatial pattern, even if the intercharge distance is shorter for iso-THAZ (4.40 Å) than for securinine (4.62 Å).

The structure of (+)-tubocurarine (Chart I) has been solved twice by X-ray diffraction in its dichloride pentahydrate[50] and dibromide methanol solvate salts.[51] To avoid large computer-disk space needs, we extracted a simplified structural fragment (Chart II). The results for this reduced structure show a charge of –0.25e on the methoxy oxygen versus –0.28e and –0.32e for the ones of the phenolic functions. These values are lower than those observed for the lactonic oxygen atoms of securinine (–0.26e and –0.27e). In addition, we observe that the electronic environment of the median phenolic group is symmetric. Like in iso-THAZ, the atomic charge found for the nitrogen atom is –0.27e. Tubocurarine presents two benzyltetrahydroisoquinoline rings bearing two phenolic functions (atoms 39 and 42) and two nitrogens, a tertiary (atom 1) and a quaternary one (atom 20; see Chart I for atomic numeration). If we consider two hydroxyl groups as negative-charged areas and two nitrogen atoms as putative cationic sites, (+)-tubocurarine may be recognized by the $GABA_A$ receptor through four possible intercharge orientations (Table III). The interaction modes B and C would probably not be effective due to their too long intercharge distances between the selected oxygen and the nitrogen atoms (8.70 and 7.30 Å, respectively) as compared with the previous antagonists. Between the two other possibilities (6.48 Å for mode A and 6.05 Å for mode D), the last one was chosen since the corresponding intercharge distance is the closest to the previously reported values. Furthermore, the selected oxygen atom (O_{42}) belongs to the most acidic phenolic function.

If we consider as an electron-rich area the tetraatomic O_{29}–C_{28}–C_{27}–O_{42} system (Chart I), its centroid is 4.63 Å from the ammonium nitrogen. This centroid can be overlaid on the securinine lactone and makes possible the adjustment of both nitrogen atoms (Figure 3). In such an interaction mode, the largest part of tubocurarine (top of Figure 3) is far enough from the two interaction areas (bottom of Figure 3) and cannot hinder the interaction with the receptor site.

Finally, (+)-bicuculline was examined. Starting from available X-ray data,[52] an ab initio study was performed on a simplified partial structural element (Chart II). In this fragment, the tertiary amino group of (+)-bicuculline was replaced by a primary amine. As expected for this function, the nitrogen atom has a charge value of –0.34e, which is almost identical to the value found for the GABA ammonium group (–0.35e). The atomic charges of the lactonic oxygen atoms (–0.24e and –0.21e) in the phthalide moiety are lower than those GABA (–4.47e and –0.39e). However, the corresponding carrier carbon atom has a higher charge on the phthalide (+0.34e) than on the GABA carboxylate (+0.26e) (see Chart II). As a consequence, the relative charge difference between the oxygen and the carbon atoms are on the same order of magnitude in both molecules (ca. 0.6e). On the other hand, negative charges for the lactonic oxygen atoms of bicuculline are lower than those found for the corresponding atoms in securinine (Chart II). This may be due to the α,β–γ,δ-insaturation of securinine.

If the crystalline conformation of (+)-bicuculline is considered, the intercharge distance between the centroid of the lactone and the nitrogen is rather short (4.40 Å). After conformational analysis, we found that it was possible to increase this distance (from 4.40 to 4.65 Å) by modifying the dihedral angle τ_1 (H–C_1–C_9–H from 90° to 120°; see Chart I for atomic numeration). This can be achieved without having to overcome a significant energy barrier (Table III). The resulting conformer, shown in Chart III, was superposed with the previous antagonists of class II (Figure 3). By fitting all nitrogen atoms, the lactonic O–C–O atoms of bicuculline can be overlaid perfectly with the "anionic sites" of the other molecules.

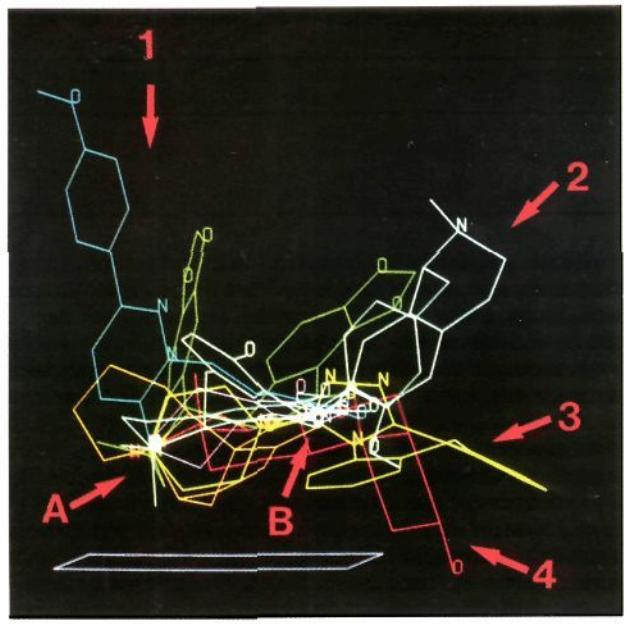


Figure 4. Overlay of the seven antagonists: 1 (red), pitrazepine (yellow), gabazine (cyan), securinine (red orange), iso-THAZ (magenta), tubocurarine (white), and bicuculline (green). The plane of the interaction core with the $GABA_A$ receptor is displayed in magenta. Arrows indicate different sites of interactions with the receptor: A (cationic site), B (anionic site), 1–4 (additional binding sites).

Discussion

The seven antagonists have been fitted together in Figure 4. All structures possess a "cationic site" of interaction (quaternary ammonium, tertiary or secondary amine) distant by about 5 Å from an "anionic site" of interaction (carbonyl, carboxyl, lactone, triazole). The delocalized anionic site does not need to be a carboxyl

(50) Codding, P. W.; James, M. N. G. The Crystal and Molecular Structure of a Potent Neuromuscular Blocking Agent: *d*-Tubocurarine Dichloride Pentahydrate. *Acta Crystallogr.* **1973**, *B29*, 935–942.
(51) Reynolds, C. D.; Palmer, R. A. The Crystal Structure, Absolute Configuration and Stereochemistry of (+)-tubocurarine Dibromide Methanol Solvate: A Potent Neuromuscular Blocking Agent. *Acta Crystallogr.* **1976**, *B32*, 1431–1439.
(52) Gorinsky, C.; Moss, D. S. Crystal and Molecular Structure of Bicuculline. *J. Cryst. Mol. Struct.* **1973**, *3*, 299–307.

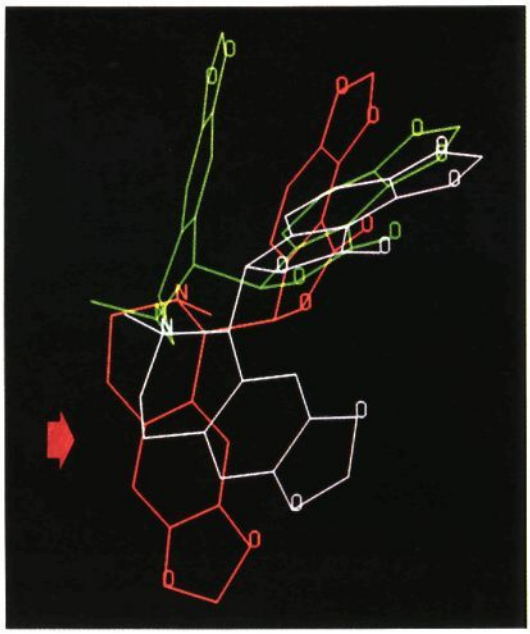


Figure 5. Superposition of (+)-bicuculline (green) on its inactive isomers: (−)-bicuculline (magenta) and adlumidine (red). Occupation of the area indicated by an arrow prevents binding to the $GABA_A$ receptor.

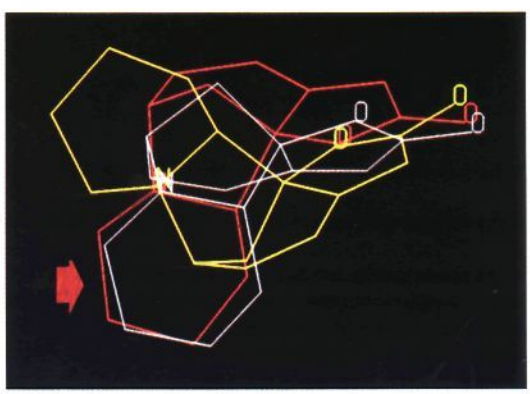


Figure 6. Superposition of (−)-securinine (red orange) on its inactive isomers: allosecurinine (magenta) and virosecurinine (red). Occupation of the area indicated by an arrow prevents binding to the $GABA_A$ receptor.

group as for GABA itself. These features explain the possibility for all described compounds to bind to the $GABA_A$ receptor. In fact, the first event in the receptor–ligand interaction is the approach and recognition of the receptor. If we consider that recognition occurs through two main ionic interactions (labeled A and B, Figure 4), the bulky parts of all structures are located above the interaction plane colored in magenta in totally different directions (labeled from 1 to 4, Figure 4). An interaction core with the receptor can thus be delimited. It accounts for binding of only the two ionic sites and allows few steric bulk tolerance. This could explain the inactivity of enantiomers or regioisomers of some antagonists: (−)-bicuculline and adlumidine (isomers of (+)-bicuculline) (Figure 5), allosecurinine and virosecurinine (isomers of securinine) (Figure 6), and SR 95132 (4-phenyl analogue of gabazine) (Figure 7).

All these inactive analogues possess cationic and anionic sites with correct intercharge distances, but are too sterically hindered in the vicinity of the interaction core region. Occupation of these forbidden regions (indicated by arrows, Figures 5–7) prevents recognition of binding to the $GABA_A$ receptor.

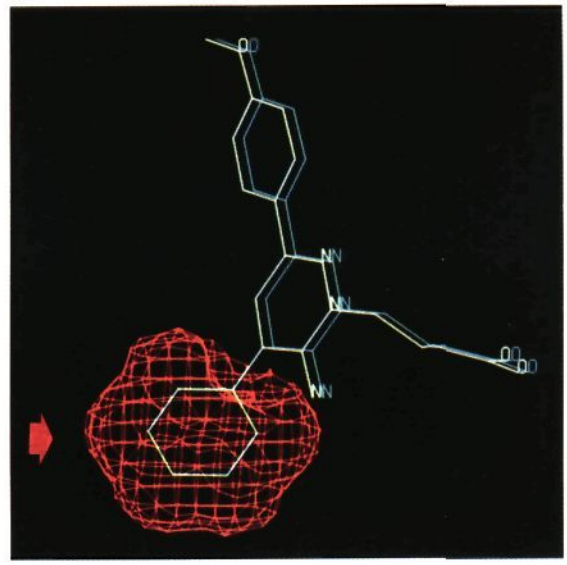


Figure 7. Superposition of gabazine (cyan) on its inactive 4-phenyl analogue SR-95132 (white). Occupation of the area indicated by an arrow prevents binding to the $GABA_A$ receptor.

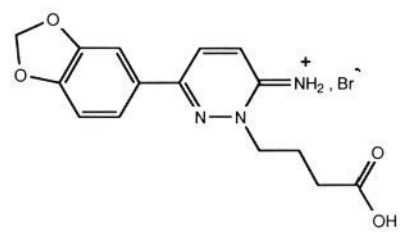


Figure 8. Structure of the designed compound **5** (SR-96073).

The antagonist effect of all these ligands can be explained according to the Ariëns theory[53] by the presence of additional binding sites, for example, substituted aromatic or aliphatic rings, which are not essential for a specific interaction with the receptor but reinforce binding of the interaction core. Such additional links may prevent activation and three-dimensional structure modification of the receptor protein. When few additional sites are available (securinine and iso-THAZ), a weak antagonist effect is reported (see Table II). When bulky additional binding sites are present (gabazine, pitrazepine, and compound 1), a stronger antagonist effect is described. The spatial orientation of the additional binding sites is also determinant. Thus, in spite of its bulky additional binding site, tubocurarine remains a weak $GABA_A$ antagonist. On the other hand the orientation of extra binding sites could influence the specificity of the $GABA_A$ antagonists for their receptor. When additional links are located in the elongation of the anionic site as for steroid 1 or pitrazepine (sites 3 and 4, Figure 4), recognition of the $GABA_A$ receptor is associated with binding to benzodiazepine receptor sites.[29,31] On the other hand, the presence of additional binding sites in the elongation of the cationic site of interaction as for bicuculline and gabazine (site 1, Figure 4) confers a better selectivity for the $GABA_A$ receptor.[2,34]

(53) Ariëns, E. J.; Beld, A. J.; Rodrigues de Miranda, J. F.; Simonis, A. M. The Pharmacon-Receptor-Effector Concept: A Basis for Understanding the Transmission of Information in Biological Systems. In *The Receptors*, O'Brien, R. D., Ed.: Plenum: New York, 1979; Vol. 1, pp 33–91.

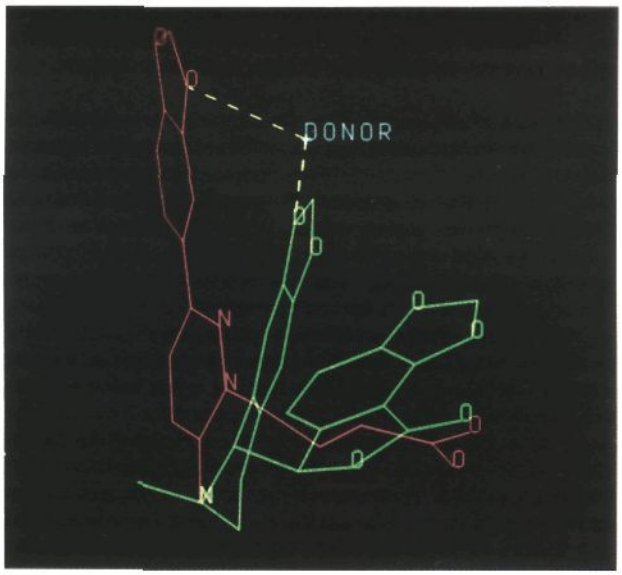


Figure 9. Hypothetical hydrogen bond (yellow broken lines) common to bicuculline (green) and compound **5** (red). The dummy atom (DONOR) corresponds to a hydrogen-bond donor, located in the $GABA_A$ receptor site at a distance of 2.8 Å from two hydrogen-bond acceptors (oxygen atoms of both antagonists).

An interesting point is the presence of similar moieties (*p*-methoxyphenyl for gabazine, (methylenedioxy)phenyl for bicuculline) in the same three-dimensional region. They could perhaps interact with the same part of the receptor, as suggested by very similar structure–activity relationships in both families.[54,55] In particular, a common hydrogen bonding to the receptor may be speculated. To test this hypothesis, the 3,4-(methylenedioxy) analogue of gabazine was designed (compound **5**, Figure 8). It is perfectly able to accept, as does bicuculline, a hydrogen bond from the $GABA_A$ receptor (Figure 9). Its meta-substituted oxygen atom is better located than the para-substituted one (present in gabazine) with respect to the hypothetical hydrogen-bond donor (Figure 9) so that an enhanced affinity for the $GABA_A$ receptor could be expected. Compound **5** was synthesized (as SR-96073) and was, as predicted, three times more potent than gabazine as $GABA_A$ antagonist (K_i = 0.05 μM, [^{3}H]GABA binding). Furthermore, it was shown to share the same selectivity for $GABA_A$ receptors than gabazine itself.[56]

These experimental data fully support our model and the existence of a unique hydrogen-bonding site (previously defined in Figure 4 as interactions 3 and 4) in the elongation of the cationic site of interaction. This feature, associated with the lack of additional sites 1 and 2 (Figure 4), could be responsible for the selectivity of gabazine congeners toward the $GABA_A$ receptor sites. In addition, the presence of the intact GABA structure in such compounds defines the same binding core as for the endogenous ligand. The absence of this common GABA moiety could explain the less specific $GABA_A$ antagonist activity of bicuculline (see Table II) even if the same hydrogen-bonding possibilities as for gabazine derivatives are present.

If binding of antagonists in terms of selectivity can be rationalized, the properties determining the potency of the antagonist effect are more complex. We can examine first the relation between intercharge distances and $GABA_A$ antagonist activity. It is interesting to notice that compounds possessing a distance around 5 Å (steroid **1**, pitrazepine, and gabazine) are very potent antagonists. Thus, if we choose for pitrazepine the centroid of the five-membered triazole ring (see Chart II) as anionic site rather than the N=C—N amidine, the intercharge distance rises from 4.71 to 5.26 Å, which is a value close to that found for the rigid compound **1** (5.14 Å). Similar considerations can be made for gabazine. We mentioned earlier a 4.8-Å distance for the conformer selected after conformational analysis. The carboxypropyl side chain can obviously adopt more extended conformations so that intercharge distances above 5 Å are also possible for some low-energy conformers. At the opposite, weaker antagonists such as securinine, iso-THAZ, or bicuculline have shorter intercharge distances (around 4.5 Å, see Table II). Since they have either totally rigid structures (securinine, iso-THAZ) or already full extended conformations (bicuculline), there is no possibility to increase the intercharge distances up to a 5-Å value, which may be critical for optimal binding to the $GABA_A$ receptor.

Furthermore, the two main interaction sites do not need to be ionized; this is particularly exemplified by steroid **1**, which interacts by a simple carbonyl function. To compensate a weaker "anionic" interaction, the tetracyclic steroid skeleton may provide other additional links and reinforce binding to the receptor. Similarly, the carboxyl group of gabazine derivatives can be replaced by a neutral cyano group, without changing $GABA_A$ antagonist properties,[57] the cyano analogues having the unique property to present a binding potentiated by the chaotropic thiocyanate anion.[57] These discrepancies cannot be explained to date by receptor mapping techniques. Only access to the three-dimensional structure of the $GABA_A$ binding site will hopefully bring in the near future new insights in the ligand receptor interaction and help to delineate some of the unexplained observations.

Conclusion

A set of seven $GABA_A$ antagonists has been studied by ab initio charge calculations and molecular modeling studies. A common pharmacophore could be defined and it revealed the following features: (i) all structures share a cationic and an anionic site of interaction, distant by about 5 Å, that delimit a specific interaction core with the $GABA_A$ receptor; (ii) the binding core allows only limited steric bulk tolerance; and (iii) the nature and orientation of additional binding sites determine the selectivity and affinity for the $GABA_A$ receptor. Their presence in the elongation of the anionic site confers affinity for benzodiazepine receptor sites. In contrast, their location in the elongation of the cationic site confers a higher selectivity for the $GABA_A$ receptor. The development of a putative hydrogen bond to the receptor was validated by the design of a new compound which was shown to be a potent and

(54) Kardos, J.; Blasko, G.; Kerekes, P.; Kovacs, I.; Simonyi, M. Inhibition of [^{3}H]GABA binding to rat brain syntaptic membranes by bicuculline and related alkaloids. *Biochem. Pharmacol.* **1984**, *33*, 3537–3545.

(55) Melikian-Badalians, A. Syntheses and Structure–Activity Relationship Studies of a New Series of GABA Antagonists at the GABA-A Receptor. Ph.D Thesis, Université Louis Pasteur, Strasbourg, France, 1987.

(56) Melikian, A.; Schlewer, G.; Chambon, J. P.; Wermuth, C. G. GABA-A antagonists derived from muscimol and thiomuscimol. *J. Med. Chem.* Submitted for publication.

(57) Wermuth, C. G.; Chambon, J. P.; Heaulme, M.; Melikian, A.; Schlewer, G.; Leyris, R.; Biziére, K. The sensitivity of gamma-aminobutyric acid antagonists to thiocyanate is related to the absence of a function anionic group in their structure. *Eur. J. Pharmacol.* **1987**, *144*, 375–378.

selective $GABA_A$ antagonist.

Experimental Section

Ab Initio Calculations. Computations were considered at the restricted Hartree–Fock (RHF) MO-LCAO-SCF level of the electronic theory. Within this framework, they have been performed at the STO-3G degree of sophistication. All calculations were done with the GAUS<<SIAN 82 program[58] adapted to an IBM 4341-2 and FPS 16 computer system (under VM/CMS). Net atomic charges and overlap populations were calculated by the Mulliken analysis.[59]

Molecular Modeling. The molecular modeling study was performed using the SYBYL software package[39] on a micro VAX II and using an E&S PS 390 graphics station. All molecules were built from existing crystallographic coordinates (GABA,[40] steroid 1,[41] pitrazepine,[42] securinine,[47] tubocurarine,[51] and bicuculline[52]) or derived from solid-state conformations of analogues (compounds 2 and 3[44,45] for gabazine and THAZ[48] and isomuscimol[49] for iso-THAZ). Hydrogen atoms were placed at standard bond distances and angles. The potential energy of each structure was then refined by a molecular mechanics procedure (MAXIMIN 2[43]) until the root mean square energy gradient was less than 0.05 kcal/mol Å. Bonded and 1,4-electrostatic terms were used with previously calculated ab initio atomic charges and a distance-dependent dielectric function. This iterative process using a combination of first-derivative and nonderivative methods reveals only local minima, depending on the starting geometries. The local minimum having the lowest potential energy is called in the text the global minimum.

After energy minimization, the three flexible molecules (pitrazepine, gabazine, and bicuculline) were submitted to a systematic conformational search (SEARCH[60]) based on the screening of van der Waals contact distances. A set of rotatable bonds was identified for each structure (see Chart III and Table II), and all possible rotamers were evaluated. To limit the conformational hyperspace, the following constraints were added: (i) rotation of all rotatable bonds with a stepwise increment of 1°, (ii) definition of a van der Waals radius factor of 0.90 (multiplicative factor of the atomic radius), (iii) elimination of all conformations having an energy 15 kcal/mol higher than the global minimum, and (iv) use of the intercharge distance of compound 1 (5.15 Å) as constraint for those of examined molecules, within a ±0.5-Å range.

The selected conformers (Chart III) were then further relaxed by energy minimization and fitted to their reference antagonist (compound 1 for pitrazepine and gabazine, securinine for bicuculline) in order to match cationic and anionic sites of all antagonists.

Acknowledgment. We thank Drs. Michel Heaulme and Jean-Pierre Chambon, for determining the IC_{50} value of compound 5, and Marlyse Wernert, for the excellent secretarial assistance. We acknowledge the National Foundation for Scientific Research (FNRS), IBM-Belgium, and the Facultés Universitaires Notre-Dame de la Paix (FNDP) for the use of the Namur Scientific Computing Facility.

Registry No. SR 95531, 104104-50-9; R-5135, 78774-26-2; Iso-THAZ, 58893-45-1; (+)-bicuculline, 485-49-4; (−)-securinine, 5610-40-2; (+)-tubocurarine, 57-95-4; pitrazepine, 90685-01-1.

Supplementary Material Available: Cartesian coordinates of all compounds fitted in their active conformations (14 pages). Ordering information is given on any current masthead page.

(58) Binkley, J. S.; Frisch, M. J.; De Frees, D. J.; Raghavachari, K.; Whiteside, R. A.; Shlegel, H. B.; Fluder, E. M.; Pople, J. A. GAUSSIAN 82; Carnegie-Mellon University, Pittsburgh, PA, 1983.

(59) Mulliken, R. S. Electronic Population Analysis on LCAO-MO Molecular Wave Functions. *J. Chem. Phys.* **1955**, *23*, 1833–1840.

(60) Dammkoehler, R. A.; Karasek, S. F.; Berkley Shands, E. F.; Marshall, G. R. Constrained search of conformational hyperspace. *J. Comput.-Aided Mol. Des.* **1989**, *3*, 3–21.