131933-80-7; **2b**, 131933-84-1; **2c**·2NH_3, 131933-88-5; **3**·3NH_3, 131933-76-1; **3a**, 123994-72-9; **3b**, 131933-85-2; **3c**·NH_3, 131933-89-6; **4**·3NH_3, 131933-77-2; **4b**, 77976-95-5; **4c**·NH_3, 131933-90-9; **5**·3NH_3, 131933-78-3; **5a**, 131933-81-8; **5b**, 131933-86-3; **5c**·NH_3, 131933-91-0; **6**·3NH_3, 131933-79-4; dGTP·3NH_3, 131933-93-2; ddGTP·NH_3, 132072-09-4; DHPGTP·3NH_3, 131933-94-3; ACVTP·3NH_3, 131933-95-4; ITP·3NH_3, 131933-96-5; 2-BrITP·3NH_3, 131933-97-6; 7-deazaGTP·3NH_3, 131933-98-7; 7-MeGTP, 26554-26-7; 2-bromohypoxanthine, 87781-93-9; 2-bromoinosine, 131933-82-9.

Synthesis and Biochemical Evaluation of Baclofen Analogues Locked in the Baclofen Solid-State Conformation

André Mann,*,† Thierry Boulanger,‡ Barbara Brandau,† François Durant,‡ Guy Evrard,‡ Michel Heaulme,§ Eric Desaulles,∥ and Camille-Georges Wermuth†

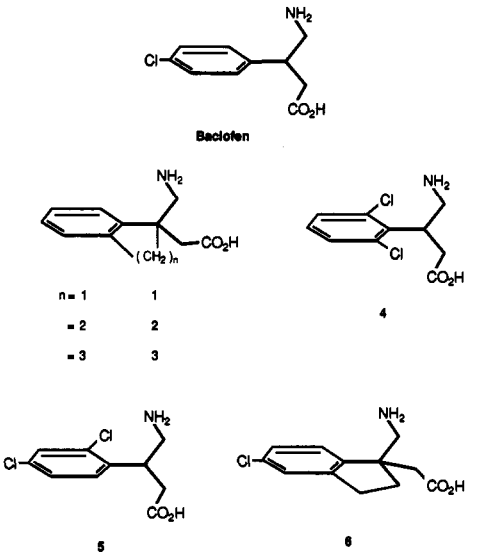
Centre de Neurochimie du CNRS, Département de Pharmacochimie Moléculaire, 5, rue Blaise Pascal, 67084 Strasbourg Cedex, France, Laboratoire de Chimie Moléculaire Structurale, Université Notre-Dame de la Paix, 61, rue de Bruxelles, B-5000 Namur, Belgium, Sanofi Recherche, Rue du Professeur J. Blayac, 34082 Montpellier Cedex, France, and Institut de Physiologie, CNRS URA 309, Université Louis Pasteur, 67084 Strasbourg Cedex, France. *Received June 29, 1990*

The synthesis of six close analogues of baclofen [3-(4-chlorophenyl)-4-aminobutyric acid] (BAC), a potent $GABA_B$ agonist, are reported. The compounds were designed starting from the structural informations contained in the solid state of BAC, regarded as a possible bioactive conformation, in which the *p*-chlorophenyl ring is perpendicular to the GABA backbone. A similar conformational situation was created by rigidifying the BAC structure by means of methylene (**1**), ethylene (**2** and **6**), or propylene (**3**) units, or by introducing chlorine atoms (**4** and **5**) into the ortho positions ("ortho effect"). Only compound **5** showed affinity for the $GABA_B$ receptor. Compound **6** [1-(aminomethyl)-5-chloro-2,3-dihydro-1*H*-indene-1-acetic acid], which was initially considered as representing the optimal mimic of the solid-state conformation of BAC, was surprisingly found inactive. An extensive conformational analysis was performed on compounds **1–6** in order to evaluate their flexibility and the overlap of their conformational population with respect to BAC. For this purpose a distance map was generated from three possible pharmacophoric groups: the amino and the carboxylic functions, and the phenyl ring. Finally, several explanations are proposed to account for the poor affinities of the prepared compounds such as steric hindrance or flexibility demand of the receptor.

Introduction

For the inhibitory neurotransmitter γ-aminobutyric acid (GABA) two major receptor subtypes ($GABA_A$ and $GABA_B$) have been identified on the basis of electrophysiological[1,2] and binding studies.[3] There are evidences that these two receptors play an important part in the central and peripheral nervous system through ion-channel regulation.[4] The overall physiological effects are transmission inhibitions mediated pre- and post-synaptically by the $GABA_A$ sites and presynaptically by the $GABA_B$ sites.[5] A number of specific agonists or antagonists at the $GABA_A$ receptor site have been developed during the last decade.[6] In contrast, 3-(4-chlorophenyl)-4-aminobutyric acid (baclofen, BAC) is the only potent and selective $GABA_B$ agonist described until now. Among the reported $GABA_B$ antagonists such as 5-aminovaleric acid[7] or 3-aminopropanesulfonic acid[8] none has the required potency or selectivity. Phaclofen [3-amino-2-(4-chlorophenyl)-propylphosphonic acid][9] and 2-hydroxysaclofen [3-amino-2-(4-chlorophenyl)-2-hydroxypropanesulfonic acid][10] have been recently presented as peripheral and central BAC antagonists. Finally, the phosphonous analogue of GABA (3-aminopropylphosphinic acid) is reported as a potent ligand for the $GABA_B$ receptors.[11] But the parsimony of the literature data in the $GABA_B$ field does not allow a rational design of new $GABA_B$ ligands.[5,12]

According to recent work, a coupling between $GABA_B$ receptors and GTP binding[13,14] which mediates adenylate cyclase inhibition has been reported; moreover $GABA_B$ receptors modulate the liberation of neurotransmitters via calcium conductance.[15,16] Therefore it seemed worth our searching for new $GABA_B$ agonists or antagonists in order to gain additional knowledge of the $GABA_B$ receptors.



† Centre de Neurochimie du CNRS.
‡ Université Notre-Dame de la Paix.
§ Sanofi Recherche.
∥ Université Louis Pasteur.

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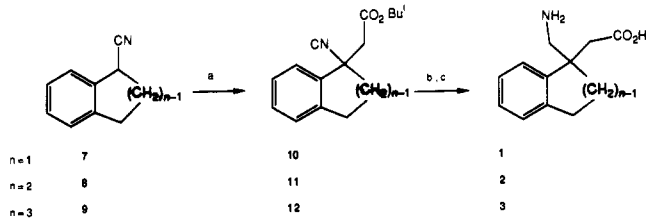
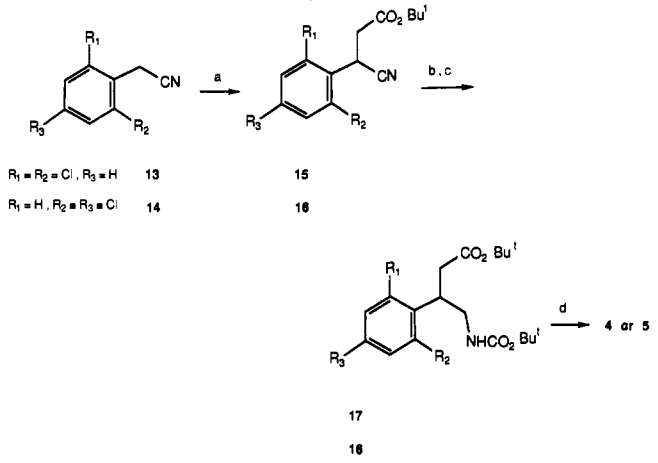
Scheme I[a]



[a] Reagents: (a) LDA, THF, −78 °C, $BrCH_2CO_2$-*t*-Bu; (b) PtO_2, AcOH, H_2; (c) AcOH, HCl.

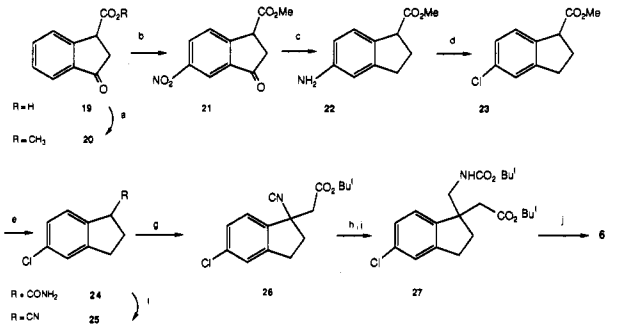
Scheme II[a]



[a] Reagents: (a) LDA, THF, −78 °C, $BrCH_2CO_2$-*t*-Bu; (b) PtO_2, AcOH, H_2; (c) pH 9, $(t\text{-BuO})_2CO$, THF; (d) AcOH, HCl.

Pursuing our efforts in the design and synthesis of GABA-mimetic compounds,[17,18] we present in this paper the synthesis and the biochemical evaluation of six baclofen analogues locked in the baclofen solid-state conformation. Synthesis of rigid analogues of flexible compounds is a classical approach in medicinal chemistry and it proved to be particularly successful in the $GABA_A$ area.[19] As BAC has the same potency as GABA for $GABA_B$ sites, but is totally devoid of $GABA_A$ affinity, it obviously seems to be the ideal starting compound. We turned our attention to the solid-state conformation of (*R*)-BAC (the active enantiomer).[20] Solid-state conformations reduce van der Waals interactions and are potential data sources for the design of rigid analogues. An examination of the X-ray data of (*R*)-BAC revealed that the phenyl ring is almost perpendicular to the GABA backbone, lowering the steric interaction between the phenyl ring and the two ortho hydrogens ("ortho effect").

Scheme III[a]



[a] Reagents: (a) MeOH, H_2SO_4; (b) HNO_3, H_2SO_4, −10 °C; (c) Pd/C, H_2; (d) $NaNO_2$, CuCl; (e) MeOH, NH_3; (f) Cl_3COCl, TEA; (g) LDA, $BrCH_2CO_2$-*t*-Bu; (h) PtO_2, AcOH, H_2; (i) pH 9, $(t\text{-BuO})_2CO$, THF; (j) AcOH, HCl.

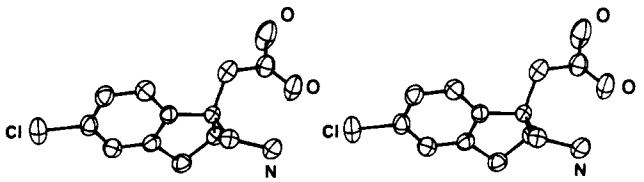


Figure 1. ORTEP stereodrawing of 6·HCl; the hydrogen atoms are not shown.

Hoping to trap the BAC active conformation in this way, we decided to prepare compounds **1–6** in which the ortho effect is grandly enhanced either by the presence of chlorine atoms or by insertion of the carbons in the benzylic and the ortho positions of the phenyl ring, in a four-, five-, or six-membered ring.

Chemistry

Scheme I shows the chemical transformations of 1-cyanobenzocyclobutene, 1-cyanoindan, and 1-cyanotetralin[21,22] to reach the amino acids **1–3**. The presence of only one acidic hydrogen in **7–9** made alkylation with *tert*-butyl bromoacetate after lithiation (LDA in THF) straightforward to yield the gem-substituted nitriles **10–12**. Subsequent catalytic reduction of the nitrile and hydrolytic cleavage of the ester functions afforded compounds **1–3** as hydrochlorides in good yields.

Scheme II outlines the preparation of amino acids **4** and **5**. The synthesis began by lithiation (lithium diisopropylamide, LDA) of the commercially available nitriles **13** and **14** followed by monoalkylation with *tert*-butyl bromoacetate to afford cyano esters **15** and **16** in reasonable yields. To lower the unavoidable dialkylation reaction, inverse addition and HMPA as cosolvent were used. After some experimentations we found the right catalytic conditions to transform the nitriles into the desired amines without hydrogenolysis of the chlorine atoms. In our hands the best conditions were to run the catalytic reduction in acetic acid in presence of PtO_2 at 50 psi and to stop the reaction after a short period of time (30 min), even before the completion of the reduction. The obtained amines and the unreacted nitriles could be easily separated via an

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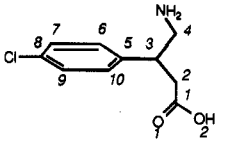
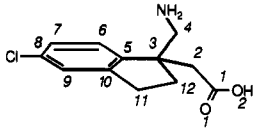
Table I. Crystal and Data Collection Parameters for Compound 6·HCl

crystal parameters at 25 °C		data measurement parameters	
a = 15.169 (2) Å	space group: $P2_1/c$	radiation: graphite-monochromated Mo Kα (λ = 0.710 73 Å)	$-18 \le h \le 18$
b = 12.345 (1) Å	fw = 276.13	diffractometer: Enraf-Nonius CAD-4	$0 \le k \le 15$
c = 7.511 (2) Å	Z = 4	absorption coeff = 4.07 cm^{-1}	$0 \le l \le 9$
V = 1404.3 $Å^3$	d_{calc} = 1.31 g cm^{-3}	$F(000)$ = 616	unique data points with $I \ge 2.5\sigma(I)$: 1813
cryst size: 0.31 × 0.26 × 0.39 mm		θ range = 2–26°	final R factor = 0.040
		unique data points: 2747	max and min heights in final Δ-Fourier map [$e/Å^3$] = 0.49 and −0.36

Table II. Comparison of the X-ray Data of 6·HCl and (*R*)-BAC·HCl[a]





	Å			deg			deg	
distances	BAC·HCl	6·HCl	valence angles	BAC·HCl	6·HCl	torsional angles	BAC·HCl	6·HCl
C(1)–C(2)	1.500 (5)	1.509 (4)	O(1)–C(1)–O(2)	123.8 (3)	122.8 (3)	C(5)–C(3)–C(4)–N	174	170
C(2)–C(3)	1.530 (5)	1.546 (4)	O(1)–C(1)–C(2)	111.2 (3)	111.9 (3)	C(2)–C(3)–C(4)–N	−64	−69
C(3)–C(4)	1.527 (4)	1.529 (4)	O(2)–C(2)–C(3)	114.1 (3)	116.7 (2)	C(1)–C(2)–C(3)–C(4)	181	82
C(1)–O(1)	1.332 (4)	1.298 (4)	C(2)–C(3)–C(4)	111.0 (3)	114.1 (2)	C(1)–C(2)–C(3)–C(5)	−59	−159
C(3)–C(5)	1.523 (4)	1.512 (4)	C(3)–C(4)–N	113.2 (3)	115.3 (2)	C(4)–C(3)–C(5)–C(6)	61	82
C(4)–N	1.499 (4)	1.495 (4)	C(4)–C(3)–C(5)	106.8 (3)	105.4 (2)	C(2)–C(3)–C(5)–C(6)	−59	−41

Table III. Analytical Data and Binding Results for the Prepared Compounds

					IC_{50},[d] μmol	
compds	formula	mp, °C	% yield[b]	method[c]	[^{3}H]GABA binding	[^{3}H]baclofen binding
1	$C_{11}H_{13}NO_2$·HCl	229 dec	66	I	>100	>100
2	$C_{12}H_{15}NO_2$·HCl	193	56	I	>100	>100
3	$C_{13}H_{17}NO_2$·HCl	200–202	47	I	>100	>100
4	$C_{10}H_{11}Cl_2NO_2$·HCl	192–194	83	II	>100	>100
5	$C_{10}H_{11}Cl_2NO_2$·HCl	184–186	75	II	>100	11
6	$C_{12}H_{14}ClNO_2$·HCl	194–196	80	III	>100	>100
GABA					0.03	–
baclofen					–	0.42

[a] The starting nitriles were either commercially available or prepared by literature methods (see Experimental Section). [b] Overall yields from alkylated nitriles. [c] Methods I, II, or III refer to Schemes I, II, or III. [d] The IC_{50} values are means of two experiments done with five different concentrations, and SEM's were less than 10%.

acid–base workup. The resulting amines were transformed without isolation as their *N-tert*-butoxycarbonyl derivatives **17** and **18**, which were fully characterized (see the Experimental Section). Hydrolytic cleavage of the two protecting groups provided amino acids **4** and **5** as hydrochlorides, for which the ^{1}H NMR (200 MHz) data are in agreement with the expected structures.

Scheme III shows the multistep sequence used for the preparation of compound **6**. Esterification with acidic methanol of the known 3-carboxy-1-oxoindan (**19**) yielded methyl ester **20**. A careful nitration afforded nitroindanone **21** in 73% yield after recrystallization. This compound was suitable for a simultaneous catalytic reduction of the nitro function and the benzylic ketone to obtain amino derivative **22**. Thus, compound **23** was readily accessible through a Sandmeyer reaction in the presence of CuCl. Aminolysis of the ester in methanolic ammonia gave rise to amide **24**. Subsequent dehydration with trichloroacetyl chloride in presence of triethylamine[23] yielded nitrile **25**. Proton abstraction (LDA, THF) and alkylation with *tert*-butyl bromoacetate furnished compound **26**. Finally amino acid **6** was obtained following the previously described sequences via carbamate **27**. The analytical data for compound **6** (^{1}H NMR and X-ray) clearly confirmed the presence of the chlorine atom at the 5-position of the aromatic ring (see the Experimental Section and Table III).

Description of X-ray Structure

An X-ray structure determination of **6**·HCl was performed in order to ascertain its structure (Tables I and II and Figure 1). The atomic numbering refers to that used for baclofen.[20] The ethylene bridge in **6**·HCl is indicated by C(11) and C(12). The bond lengths and bond angles are given in Table II together with the values reported for (*R*)-BAC.[20] As shown in this table, the rigidification of baclofen by adding an ethylene bridge between C(10) and C(3) has led to a compound having similar bond lengths and valence angles. Furthermore, BAC and **6**·HCl show similar crystal conformations. The rigidification is particularly efficient for the ammonium-bearing group. Thus, the C(4)–C(3)–C(5)–C(6) torsion angle, indicating the relative orientation toward the chlorophenyl moiety, is 82° for **6**·HCl compared to 61° for BAC. The spatial position of the nitrogen atom is then defined by the C(2)–C(3)–C(4)–N(1) torsion angles, which are equal to −69° and −64° for **6**·HCl and BAC, respectively. The carbon C(2), in the α-position toward the carboxylic function, also presents a similar orientation in both cases, with C(2)–C(3)–C(5)–C(6) torsion angles equal to −41° and −59°. However, the carboxylic groups are situated in different spatial locations with C(1)–C(2)–C(3)–C(4) and C(1)–C(2)–C(3)–C-

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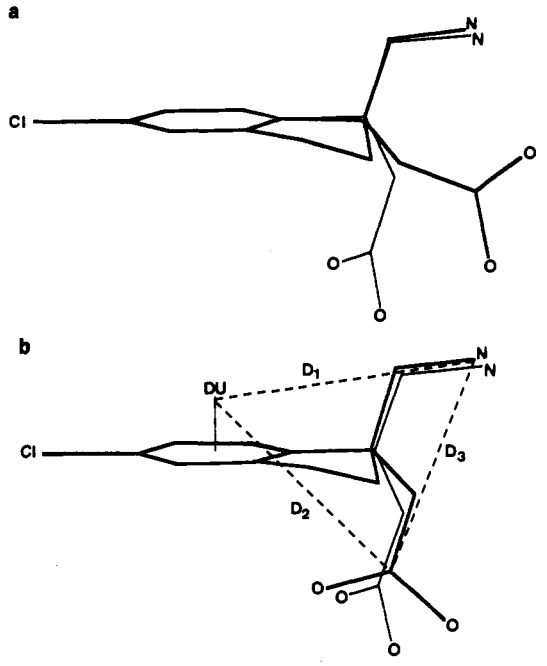

Figure 2. (a) Superposition of the solid-state conformations of BAC and 6·HCl. The molecules are compared by fitting the atoms of the aromatic nuclei (root mean square = 0.08) with use of the FIT option in SYBYL 5.3. The measured distance between the nitrogens (N) is N–N = 1.2 Å and between the carbons (C) from the carboxylic group is C–C = 2.2 Å. Hydrogen atoms are omitted. (b) Computer generated overlay of the solid-state conformation of BAC and one stable conformation of 6 obtained successively with the TWIST and MAXIMIN options in SYBYL 5.3. The measured distances between the nitrogens (N) and carbons (C), from the carboxylic group are N–N = 0.3 Å and C–C = 0.6 Å.

(5) torsional angles equal to −82° and −159° for 6·HCl, and to 181° and −59° for BAC (Figure 2a). In compound 6·HCl a network of multiple intermolecular hydrogen bonds assumes the crystal cohesiveness involving the ammonium and carboxylic moieties, the chloride ion, and a cocrystallized water molecule. An intramolecular hydrogen bond between the ammonium group and the carboxylic function leads to a pseudo seven-membered ring with a O(2)–N distance of 2.76 Å between the carbonyl oxygen and the nitrogen. The C(4)–N carbon–nitrogen distance of 1.495 Å indicates that the nitrogen atom is protonated. The corresponding value observed for BAC is 1.499 Å.

Biological Results

The prepared compounds 1–6 were evaluated in binding experiments for $GABA_A$ and $GABA_B$ affinities according to Snyder[24] and Bowery.[3] At $GABA_A$ sites, the IC_{50} values for all compounds were greater than 100 μM. As expected, the introduction of an aromatic region at the β-position on the GABA frame seems prohibitive for GABA binding. For the $GABA_B$ sites only compound 5 shows a moderate affinity ($IC_{50} = 11 \times 10^{-6}$ M), but for compounds 1–4 and 6 the observed values are largely above 10^{-4} M (Table III).

Discussion

The aim of this work was an attempt to identify the bioactive conformation of BAC at the $GABA_B$ receptor. We used the structural informations contained in the solid-state conformation of BAC, where the amino and carboxylic functions respectively occupy each of the half-space delimited by the phenyl ring. As a working hypothesis, we assumed that the bioactive conformation of BAC is mimicked by the solid-state one of BAC or at least included in the conformational subset generated around the above mentioned spatial relationship. In this conformational population the steric interactions between the phenyl ring and the two hydrogens are reduced. Accordingly compounds 1–4 were designed to amplify the ortho effect observed in the crystal structure by the introduction of methylene, ethylene, or propylene bridges or a chlorine atom. These additional units prevent or restrict the free rotation of the aromatic ring and give rise to compounds which freeze the solid-state conformation of BAC. But, unexpectedly, none of these compounds showed a significant affinity for the $GABA_B$ receptor. Initially we imputed the negative results observed with compounds 1–4 either to the absence of a chlorine atom in the para position of the aromatic ring (this argument is supported by the moderate affinity reported for benzofuranyl BAC analogues also devoid of chlorine[25]) and/or to the bulkiness introduced in the vicinity of the ortho position. To appraise these two elements we prepared compound 5, in which steric hindrance as well as the *p*-chloro substituent are present together. Biological activity was found for compound 5, suggesting some steric tolerance in the ortho position and the necessity of chlorine in the para position. We then undertook the synthesis of compound 6: the indan ring was selected on account of comparable values for the length of the benzylic bond in 6 and BAC, 1.51 and 1.52 Å,[26] respectively, rather than the benzocyclobutene ring with a larger bond length (1.56 Å)[26] or the tetrahydronaphthalene ring with a similar length (1.52 Å)[26] but with a too large extravolume with respect to that of BAC. The presence in compound 6 of the *p*-chloro substituent let us anticipate a good affinity for the $GABA_B$ recognition sites. But as it appears (Table III), compound 6 is devoid of $GABA_B$ binding at 10^{-4} M. This result is disappointing in the sense that except the blockade of one rotatable bond, compound 6 contains all the functional and structural features of BAC. At this stage we decided to get the crystalline structure of 6 to ensure its chemical formula and to see whether some unanticipated features would explain the lack of biological activity. In fact the chemical structure was clearly confirmed by the X-ray results.

To find an explanation for the poor affinities observed for compounds 1–6, we investigated their flexibility with respect to BAC using conformational analysis. In order to characterize properly the accessible conformers for each compound, a distance map was created with the SYBYL SEARCH routine. One of the major advantage of distance maps is to provide an adequate way to visualize and handle the conformational data of compounds where multiple rotatable bonds have to be considered. If we assume that the aromatic ring and the amino and the carboxylic functions are substructures involved in receptor recognition, a three-point pharmacophore for the $GABA_B$ receptor may be hypothesized involving the nitrogen atom, the carbon atom from the carboxyl, and a dummy atom (DU) 1 Å above the aromatic plane. Therefore for each conformer three distances (d_1, d_2, and d_3; for definition see Figure 3a) can be measured and each conformer is now represented as a point in a three-dimensional space (distance map), where the coordinate axis are the intergroup distances. After identification of the main rotatable bonds, steps of 5° were assigned to the dihedral angles, and an energy cutoff of 5 kcal/mol was set to disclose the stable

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(26) The data were taken from the fragment library of SYBYL.

Table IV. Conformational Data for BAC and Compounds 1–6

	BAC	1	2 and 6[a]	3	4	5
distance ranges,[b] Å						
d_1	2.80–5.55	3.00–4.95	2.70–4.85	2.70–4.90	2.85–5.50	2.85–5.50
d_2	3.20–5.60	4.40–5.50	4.35–5.55	4.30–5.60	3.15–5.60	3.15–5.60
d_3	2.90–5.10	2.95–5.15	2.85–5.05	2.90–5.05	2.90–5.05	2.95–5.05
number of conformations	7797	1124	443	251	1982	6916
points in the distance map[c]	936	495	212	129	415	829
points in the distance map after constraint		108	82	20	300	576
ratio of % overlap[d]		12	9	2	32	61

[a] Owing their structural resemblance, compounds 2 and 6 are indistinguishable in the SEARCH procedure. [b] The distance maps are available as supplementary material. [c] Each point may represent more than one conformation. [d] Percentage of overlap with respect to the distance map of BAC.

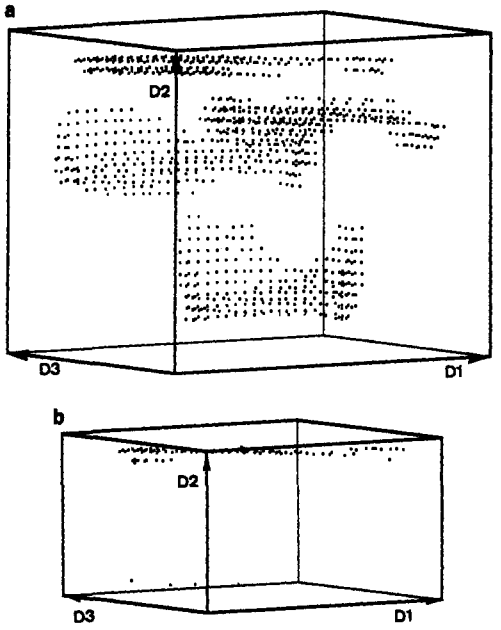

Figure 3. (a) Distance map for BAC. The coordinate axis are the distances defined as d_1, distance from N to DU; d_2, distance from C (carboxylic carbon) to DU; d_3, distance from N to C (carboxylic carbon). The DU atom defines a point 1 Å above the aromatic plane (see the text). The distance ranges are $2.7 < d_1 < 5.5$ Å; $3.1 < d_2 < 5.5$; $2.8 < d_3 < 5.1$ Å. (b) Distance map after constraint for compound 6. The grid size was set at 0.1 Å. The coordinate axis are related to part a. The distance ranges are $2.7 < d_1 < 4.8$ Å; $4.3 < d_2 < 5.5$ Å; $3.2 < d_3 < 5.1$ Å.

conformers. The results of the computational calculations are reported on Table IV.

Accordingly for each compound, the evaluation of the number of conformers and/or points in the distance map provide a direct measurement of flexibility. The rank in decreasing flexibility is BAC > 5 > 4 > 1 > 2, 6 > 3 and is directly related to the steric hindrance present at the ortho positions of the phenyl ring. On the other hand, the data obtained for the distances d_1, d_2, and d_3 are roughly comparable in ranges for compounds 1–6 with respect to those for BAC, except for the d_2 ranges of compounds 1–3 and 6, which are significantly smaller. This difference originates mainly from the rigidification around the benzylic bond and intimates that the carboxylic acid is more affected in its mobility than the primary amine in compounds 1–3 and 6. To quantify more precisely the conformational overlap with BAC, we carried out a SEARCH procedure on compounds 1–6 with the distance map of BAC as constraint (Figure 3a). Interestingly, compound 5, which retains some affinity, shows 61% overlap whereas compound 4 reaches only 32% and the other compounds 10% or less (see Table IV and Figure 3b for the distance map with constraint of compound 6 as one example). With these results in hand, it is tempting to assume that conformational flexibility is a requisite to secure $GABA_B$ affinity. Finally, to disclose for compounds 1–6 conformers matching the solid state of BAC, we made use of the TWIST option in SYBYL. Several stable conformers were found after energy minimization and Figure 2b displays one example for compound 6. Finally, the conformational analysis demonstrates that compounds 1–6 are good candidates not only to explore the solid state of BAC but also to mimic other stable conformers of BAC.

Conclusion

Taking all our data together, the following concluding remarks can be drawn: (i) if the bioactive conformation of BAC at the $GABA_B$ receptor is close to its solid-state conformation, compounds 1–6 are less active or inactive for steric reasons; for example in 6 the ethylene bridge may occupy an area of bulk intolerance at the receptor and the intramolecular hydrogen bond may prevent receptor recognition; (ii) if the solid-state conformation of BAC·HCl does not correspond to the active conformation of BAC at the $GABA_B$ receptor, compounds 1–6 delineate an unproductive subset in the set of the conformations accessible to BAC; for example conformers of compound 6 are overlapping some stable conformers of BAC; (iii) if a certain degree of conformational flexibility is required for binding to and activation of $GABA_B$ receptors, then going from compound 5 to 6, the decrease in binding is consistent with the reduced flexibility of their structures. Finally, even though we did not obtain conclusive arguments, our work may be of use for further investigations about the bioactive conformation of BAC at the $GABA_B$ receptors, especially when a conformational study has to be undertaken.

Experimental Section

Melting points were obtained on a calibrated Kofler hot-stage apparatus and are uncorrected. IR spectra were measured with Pye-Unicam SP3-300S spectrophotometer and ^1H NMR spectra recorded on a WP-80 (80 MHz) or WP 200 (200 MHz) Bruker spectrometer using Me_4Si as external or internal reference. Analytical results indicated by elemental symbols were within 0.4% of the theoretical values. Unless otherwise stated, all reactions were carried out under an argon atmosphere with dry solvents. THF was distilled from sodium–benzophenone and methylene chloride from CaH_2 prior to use. The organic solvents were removed by evaporation under reduced pressure with a rotary evaporator. The column chromatographies were performed by using a flash chromatography technique. Compounds 7–9 were prepared according to literature procedures.[21,22]

***tert*-Butyl 7-Cyanobicyclo[4.2.0]octa-1,3,5-triene-7-acetate (10).** To a solution of lithium diisopropylamide prepared from *n*-butyllithium (16 mL of a 1.5 M solution in hexane) and diisopropylamine (25 mL) in THF (60 mL) was added at −78 °C nitrile 7 (3.1 g, 2.4 mmol) dissolved in THF (5 mL). After 30 min a solution of *tert*-butyl bromoacetate (5 g, 25 mmol) in THF (10 mL) was added. After one night of stirring, saturated NH_4Cl was added, followed by ethyl ether. The organic layer was washed with brine, dried, and concentrated under reduced pressure to

an oil which was distilled under high vacuum to yield **10** as a syrupy liquid (4.4 g, 75%): bp 195 °C (bath) (0.1 mm); IR ($CHCl_3$) 2210 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 1.45 (9 H, s), 2.80 (2 H, s), 3.50 (2 H, AB syst, J_{AB} = 14 Hz), 7.35–7.45 (4 H, m).

The above procedure was used for the preparation of **11** and **12**.

7-(Aminomethyl)bicyclo[4.2.0]octa-1,3,5-triene-7-acetic Acid (1). In a Parr bottle a solution of **10** (1.5 g, 6.2 mmol) in MeOH (40 mL) containing 2 mL of concentrated HCl and PtO_2 (100 mg) was shook under hydrogen (60 psi) overnight. The catalyst was removed by filtration through Celite. After evaporation, the remaining syrup (no starting material in TLC) was taken up in a mixture of AcOH (10 mL) and concentrated HCl (5 mL) and refluxed for 3 h. The solvent was removed and the residue crystallized in 2-propanol to yield **1** (0.92 g, 66%), mp 229 °C (from 2-propanol–ether). Anal. ($C_{11}H_{14}ClNO_2$) C, H, N.

The above procedure was used for the preparation of **2** and **3**. For analytical data see Table III.

***tert*-Butyl 3-(2,6-Dichlorophenyl)-3-cyanopropanoate (15).** To a solution of lithium diisopropylamide (8 mmol) prepared from *n*-butyllithium (1.5 M; 5.3 mL, 8 mmol) and diisopropylamine (1.2 mL, 8 mmol) dissolved in THF (30 mL) was added, at −78 °C, 2,6-dichlorophenylacetonitrile (**13**; 1.35 g, 7.2 mmol) in THF (5 mL). After 30 min at this temperature, dry HMPA (0.98 mL, 8 mmol) was added and the mixture was introduced via a cannula under nitrogen over 15 min to a solution of *tert*-butyl bromoacetate (3 g, 16 mmol) in THF (10 mL) at −78 °C. The reaction was quenched after 3 h at room temperature with saturated ammonium chloride and extracted with ether. The organic extracts were combined, washed with water, and dried over sodium sulfate. Concentration under reduced pressure afforded an oily residue which was chromatographed on silical gel eluting with hexane–ether (4:1), followed by a bulb-to-bulb distillation to yield **15** (2 g; 80%): bp 150 °C (0.1 mm); IR ($CHCl_3$) 2210 cm^{-1}; ^1H NMR (CD_3OD, 200 MHz) δ 1.44 (9 H, s, $C(CH_3)_3$), 2.90 (1 H, A from ABX, J = 16 and 6 Hz, CH_AH_B), 3.20 (1 H, B from ABX, J = 16 and 9 Hz, CH_AH_B), 5.27 (1 H, X from ABX, $J_{AX} + J_{BX}$ = 15 Hz, −C*H*−), 7.45 (3 H, m, ArH).

Compound **16** was prepared according to the above procedure.

***tert*-Butyl 4-[*N*-(*tert*-Butoxycarbonyl)amino]-3-(2,6-dichlorophenyl)butyrate (17).** A solution of **15** (1.5 g, 5 mmol) in acetic acid (80 mL) was hydrogenated at 60 psi for 1 h in presence of PtO_2 (150 mg) as catalyst. After filtration and evaporation at reduced pressure, the residue was taken up in hexane (30 mL) and water (60 mL). The aqueous layer was rendered alkaline (NaOH, 1 N, pH 9) and di-*tert*-butyl dicarbonate (2.5 g, 12 mmol) dissolved in THF (20 mL) was added. After overnight stirring, the residue obtained after evaporation was partitioned in dichloromethane (60 mL) and water (40 mL). The organic layer was washed with water and saturated brine, dried (Na_2SO_4), and evaporated under reduced pressure to yield an oily residue, which was purified by chromatography on silica gel, eluting with hexane–ether (7:3). Compound **17** was isolated as an oil which solidified slowly (0.95 g, 54%) at room temperature: ^1H NMR (CD_3OD 200 MHz) δ 1.27 (9 H, s, 3 × CH_3), 1.39 (9 H, s, 3 × CH_3), 2.71 (1 H, A from ABX, J = 14 and 6 Hz, $CH_AH_BCO_2H$), 3.15 (1, B from ABX, J = 14 and 8 Hz, $CH_AH_BCO_2H$), 3.52 (1 H, A from ABX, J = 14 and 8 Hz CH_AH_BNH), 3.52 (1 H, B from ABX, J = 14 and 8 Hz, CH_AH_BNH), 4.21–4.46 (1 H, m, −C*H*−), 7.2–7.35 (3 H, m, Ar*H*).

(2,6-Dichlorophenyl)-4-amino-3-butyric Acid (4). A solution of ester **17** (0.75 g, 2 mmol) in a mixture of acetic acid (25 mL) and concentrated hydrochloric acid (5 mL) was refluxed for 2 h. The reaction mixture was evaporated under reduced pressure to yield **4**, as a white solid (0.480 g, 83%): mp 192–194 °C (from methanol-ether); ^1H NMR (CD_3OD, 200 MHz) δ 2.95 (1 H, A from ABX, J = 16 and 6 Hz, $CH_AH_BCO_2H$), 3.17 (1 H, B from ABX, J = 16 and 8 Hz, $CH_AH_BCO_2H$), 3.49 (1 H, A from ABX, J = 14 and 6 Hz, $CH_AH_BNH_2$), 3.52 (1 H, B from ABX, J = 14 and 4 Hz, $CH_AH_BNH_2$), 4.40–4.55 (1 H, m, −CH−), 7.27–7.52 (3 H, m, ArH). Anal. ($C_{10}H_{12}Cl_3NO_2$) C, H, N.

The above experimental procedure was used for the preparation of **5**. For analytical data see Table III.

Methyl 3-Oxo-2,3-dihydro-1*H*-indene-1-carboxylate (20). To a solution of **19** (12 g, 68 mmol) in MeOH (250 mL) was added concentrated H_2SO_4 (2 mL) and the mixture was refluxed for 10 h. After evaporation, the residue was partitioned between ether (300 mL) and water (100 mL), and the organic layer was washed with K_2CO_3 (5% solution) and brine and dried (Na_2SO_4). After evaporation under reduced pressure the residue was distilled under vacuum to yield **20** (26 g, 80%): bp 130 °C (bath) (0.1 mm); ^1H NMR (80 MHz, $CDCl_3$) δ 3.05 (2 H, AB from ABX, J = 20, 8 and 4 Hz), 4.25 (1 H, X from ABX, $J_{AX} + J_{BX}$ = 12 Hz), 7.15–7.75 (4 H, m, Ar*H*).

Methyl 3-Oxo-5-nitro-2,3-dihydro-1*H*-indene-1-carboxylate (21). To a well-stirred mixture of H_2SO_4 (63 mL of a 98% solution) and nitric acid (11.7 mL of a 65% solution) was added at −10 °C a solution of **20** (6.5 g, 34 mmol) in nitromethane (5 mL). The addition rate was carefully adjusted to raise the temperature from −10 to −5 °C during 30 min. The reaction mixture was stirred further for 15 min at this temperature (attention has to be paid to temperature and reaction time!). After ice–water hydrolysis (250 mL), the yellow precipitate was collected and extracted with dichloromethane. The organic phase was washed with a potassium bicarbonate solution (5%) and with saturated brine and dried (Na_2SO_4). After solvent evaporation, the crystalline residue was taken up in hot diisopropyl ether to yield nitro adduct **21** (5.15 g, 73%). Recrystallization gave yellow needles: mp 128–130 °C (from 2-propanol); ^1H NMR ($CDCl_3$, 80 MHz) δ 2.95 (1 H, A from ABX, J = 19 and 8 Hz, CH_AH_B), 3.30 (1 H, B from ABX, J = 19 and 4 Hz, CH_AH_B), 4.32 (1 H, X from ABX, $J_{AX} + J_{BX}$ = 12 Hz, $CHCO_2Me$), 7.7–8.1 (1 H, m, ArH), 8.2–8.5 (2 H, m, Ar*H*). Anal. ($C_{11}H_9NO_5$) C, H, N.

Methyl 5-Amino-2,3-dihydro-1*H*-indene-1-carboxylate Hydrochloride (22). A solution of **21** (10 g, 43 mmol) in acetic acid (120 mL) containing perchloric acid (6 mL) and catalyst (Pd/C, 10 %, 500 mg) was shaken in a Parr bottle under hydrogen (60 psi) during 6 h. After filtration through Celite, the organic phase was evaporated under reduced pressure. The residue was made alkaline with aqueous ammonia at 0 °C. The obtained oil was extracted with ether, washed with brine, dried (Na_2SO_4), and concentrated. The residual syrup was taken up in a mixture of 2-propanol/diisopropyl ether and treated with an etheral solution saturated with anhydrous HCl. The resulting crystals were recovered and identified as **22**·HCl (8 g, 83%): mp 220–221 °C (from 2-propanol–ether); ^1H NMR ($CDCl_3$, 80 MHz) δ 2.05 (2 H, s, NH_2), 2.10–2.45 (2 H, m, CH_2CH_2Ar), 2.65–2.85 (2 H, m, CH_2CH_2Ar), 3.80 (3 H, s, OCH_3), 4.05 (1 H, t, J = 6 Hz, −CH−), 6.40–6.50 (2 H, m, Ar*H*), 6.90–7.10 (1 H, m, Ar*H*). Anal. ($C_{11}H_{14}ClNO_2$) C, H, N.

Methyl 5-Chloro-2,3-dihydro-1*H*-indene-1-carboxylate (23). To a mixture of hydrochloride **22** (7.5 g, 33 mmol) in aqueous solution (37.2 mL of H_2O and 15 mL of concentrated HCl) was added at −5 °C a solution of sodium nitrite (2.65 g, 38 mmol) in water (10 mL). After the addition, the reaction medium was homogeneous with a yellow color and stirring was continued for 1 h at 0 °C. In the meantime a cuprous chloride (4.5 g, 45 mmol) solution in concentrated HCl (35 mL) was prepared. While the two flasks are maintained at 0 °C, the diazonium solution was introduced by means of a cannula to the cuprous salt. After 3 h at room temperature, the organic products were extracted from the black mixture, with dichloromethane (3 × 100 mL), washed with sodium hydroxide (10%), water, and brine; the organic layer was dried (Na_2SO_4) and evaporated under reduced pressure; and the oily residue was distilled to yield **23** (5.7 g, 80%): bp 150 °C (0.2 mm); ^1H NMR ($CDCl_3$, 80 MHz) δ 2.20–2.60 (2 H, m, CH_2CH_2Ar), 2.90–3.10 (2 H, m, CH_2CH_2Ar), 3.80 (3 H, s, OCH_3), 4.15 (1 H, t, J = 7 Hz, −CH−), 7.25–7.50 (3 H, m, Ar*H*).

5-Chloro-2,3-dihydro-1*H*-indene-1-carboxamide (24). To methanol (40 mL) saturated with ammonia was added ester **23** (1.3 g, 6.2 mmol). After 1 week at room temperature no starting material was apparent via TLC. The solvent was evaporated and the crystalline residue was taken up in ether and filtered to yield amide **24** (1.2 g, 92%), mp 160–161 °C (from ethyl acetate). Anal. ($C_{10}H_{10}NOCl$) C, H, N.

1-Cyano-5-chloro-2,3-dihydro-1*H*-indene (25). To a stirred mixture of carboxamide **24** (1.6 g, 8.2 mmol) and triethylamine (1.3 mL, 9.1 mmol) in dry dichloromethane (60 mL) was added dropwise a solution of trichloracetyl chloride (1 mL, 9 mmol) in dry dichloromethane (15 mL) at 0 °C. After the end of the addition, the mixture was treated, respectively, with ice water (25 mL), sodium hydroxyde solution (30 mL, 2 N), sulfuric acid

(30 mL, 2 N), and water. The organic phase was dried (Na_2SO_4) and concentrated to yield nitrile **25** as an yellow oil after chromatography on silica gel eluting with hexane–ether 9:1 (1.3 g, 90%): IR ($CHCl_3$) 2210 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 2.15–2.45 (2 H, m, CH_2CH_2Ar), 2.75–2.90 (2 H, m, CH_2CH_2Ar), 4.05 (1 H, t, J = 7 Hz, CH), 7.15–7.40 (3 H, m, ArH).

***tert*-Butyl 1-Cyano-5-chloro-2,3-dihydro-1*H*-indene-1-acetate (26).** To a solution of lithium diisopropylamine prepared from *n*-butyllithium (5.3 mL of a 1.35 M solution in hexane) and diisopropylamine (1 mL, 7.1 mmol) in THF (20 mL) was added at −78 °C nitrile **25** (1.3 g, 6.7 mmol) dissolved in THF (5 mL). After a period of 30 min at −78 °C, a solution of *tert*-butyl bromoacetate (2 g, 10 mmol) was rapidly added and the reaction was allowed to reach room temperature overnight. A saturated ammonium chloride solution (10 mL) was added, followed by ethyl ether extraction. The usual workup, brine, drying, and evaporation under reduced pressure, yielded an oil which was purified by chromatography on silica gel eluting with hexane ether (8:2). Nitrile **26** was isolated as an pale yellow oil (1.75 g, 81%): IR ($CHCl_3$) 2215 cm^{-1}; ^1H NMR ($CDCl_3$, 80 MHz) δ 1.50 (9 H, s), 2.5–3.35 (6 H, m), 7.25–7.35 (3 H, m).

***tert*-Butyl 1-[[*N*-(*tert*-Butoxycarbonyl)amino]-methyl]-2,3-dihydro-1*H*-indene-1-acetate (27).** A solution of nitrile **26** (1.2 g, 6 mmol) in acetic acid (80 mL) was hydrogenated at 60 psi for 1 h in the presence of PtO_2 (150 mg) as catalyst. The reaction mixture was filtered through Celite and evaporated to dryness. The residue was partitioned between water (50 mL) and hexane (50 mL). Some starting material can be recovered at this stage. The aqueous layer was rendered alkaline (pH 9) with NaOH (1 N) and a solution of di-*tert*-butyl dicarbonate (1.74 g, 8 mmol) in THF (30 mL) was added. The mixture was left at room temperature overnight with vigorous stirring. After evaporation, the residue was taken up in dichloromethane (60 mL). The organic layer was washed with water and saturated brine, dried (Na_2SO_4), and evaporated under reduced pressure to yield an oily residue which was purified by chromatography on silica gel, eluting with hexane–ether (7:3). Compound **27** was isolated as an oil (0.680 g; 35%): ^1H NMR ($CDCl_3$, 80 MHz) δ 1.25 (2 H, s, 3 × CH_3), 1.35 (9 H, s, 3 × CH_3), 2.05–2.40 (4 H, m), 2.80–3.05 (2 H, m), 3.40–3.50 (2 H, m), 7.10–7.30 (3 H, m).

1-(Aminomethyl)-5-chloro-2,3-dihydro-1*H*-indene-1-acetic Acid Hydrochloride (6). A solution of **27** (0.480 g, 1.6 mmol) was refluxed for 2 h in a mixture of acetic acid (25 mL) and concentrated hydrochloric acid (5 mL). After evaporation under reduced pressure the crystalline powder was filtered with ether to yield **6** (0.350 g, 80%): mp 194–196 °C (from diisopropyl ether–2-propanol); ^1H NMR (CD_3OD, 200 MHz) δ 2.20–2.29 (2 H, m, CH_2CH_2Ar), 2.77 (2 H, AB system J_{AB} = 16 Hz, CH_2CO_2H), 2.98–3.05 (2 H, m, CH_2Ar), 3.37 (2 H, AB system J_{AB} = 16 Hz, CH_2CO_2H), 2.98–3.05 (2 H, m, CH_2Ar), 3.37 (2 H, AB system J_{AB} = 13 Hz, CH_2NH_2), 7.26 (2 H, m, Ar*H*), 7.31–7.35 (1 H, m, Ar*H*).

Single-Crystal X-ray Analysis of 6·HCl. Crystals were obtained by slow evaporation of an ethyl acetate–ethanol solution. A colorless prismatic crystal having dimensions of 0.31 × 0.26 × 0.39 mm was used for all X-ray measurements. D_m was not measured. Following the ω–θ scan method, intensities were collected on a Enraf-Nonius CAD-4 diffracometer equipped with a graphite monochromator and MoKα radiation (λ = 0.71073 Å). Lattice parameters were obtained from least-squares refinement of 25 medium angle reflections. A molecule of water has co-crystallized. The linear absorption coefficient was 4.07 cm^{-1} and no correction was made. Data were corrected for Lorentz and polarization effects. The structure was solved by direct methods using the SHELX 86[27] program. All heavy atoms were directly found. A water molecule was found in a first difference Fourier map full-matrix; least-squares method was used to refine positional and anisotropic thermal parameters. All hydrogen atoms were located on a difference Fourier map and were fixed with isotropic temperature factors. The final R value was 0.040 and R_w was 0.052. All refinement calculations were performed with the SHELX 76 program,[28] structural analysis with X-RAY 76,[29] and perspective view were drawn with ORTEP.[30]

Biochemical Assays. [^{3}H]GABA Binding Assay. Experiments were performed using classical Triton-treated membranes.[24] Binding assays were carried out at 4 °C for 5 min. The reaction mixture in a final volume of 2 mL contained 0.2 mL of synaptic membrane suspension (about 1 mg of protein), 0.2 mL of [^{3}H]-GABA, 25–40 Ci/mmol (New England Nuclear, Boston, MA), in a final concentration of 2.9 nM, 0.2 mL of unlabeled drug, and 1.4 mL of 50 mmol Tris/citrate buffer, pH 7.1. At the end of the incubation the mixture was quickly filtered through Whatman GF/C filters. The filters were washed with 10 mL of ice-cold 50 mmol Tris/citrate buffer, pH 7.1, and bound radioactivity was evaluated by scintillation counting.

[^{3}H]Baclofen Binding Assay. Affinity of compounds for the $GABA_B$ site was estimated by the [^{3}H]baclofen binding assay, 30–50 Ci/mmol (New England Nuclear, Boston, MA). Experiments were performed according to Hill and Bowery[3] with minor modifications (final concentration of [^{3}H]baclofen was 26.7 nM instead of 20 nM and incubation time was 30 min instead of 10 min).

Molecular Modeling. The study was performed using the SYBYL 5.3 software package[31] on a Vax Station 2000 and a Evans-Sutherland PS390 graphic terminal.

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Registry No. 1·HCl, 132205-59-5; 2·HCl, 132233-06-8; 3·HCl, 132205-60-8; 4·HCl, 60611-50-9; 5·HCl, 132205-61-9; **6**, 132205-79-9; 6·HCl, 132205-62-0; **7**, 93854-80-9; **8**, 132205-63-1; **9**, 132205-64-2; **10**, 132205-65-3; **11**, 132205-66-4; **12**, 132205-67-5; **13**, 3215-64-3; **14**, 6306-60-1; **15**, 132205-68-6; **16**, 132205-69-7; **17**, 132205-70-0; **18**, 132205-71-1; **19**, 40452-27-5; **20**, 132295-74-0; **21**, 132205-72-2; **22**, 132205-73-3; **23**, 132205-74-4; **24**, 132205-75-5; **25**, 132205-76-6; **26**, 132205-77-7; **27**, 132205-78-8; *tert*-butyl bromoacetate, 5292-43-3.

Supplementary Material Available: Tables of X-ray parameters for 6·HCl, distance maps for compounds 1–6 with and without constraint (9 pages); structure factors for 6·HCl (16 pages). Ordering information is given on any current masthead page.

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