

# Superimposition-based protocol as a tool for determining bioactive conformations. I. Application to ligands of the glycine receptor (GlyR)

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The natural templates (NT) approach, which is a superimposition-based protocol that has been successfully employed in several studies, is here applied to ligands of the glycine ligand-gated ion channel receptor. Bioactive conformations for glycine and its analogs were obtained using strychnine (a natural and specific competitive antagonist) as template. Experimental evidence was used to guide the superimposition protocol. Three essential regions have been defined in strychnine's structure that serve as a pharmacophore for agonist and antagonist activities. Reasonable alignments of known ligands were found in the majority of the cases. Molecular mechanics (i.e., conformational searches for the relatively flexible ligands) and molecular dynamics (for relatively rigid ligands such as strychnine and 5,6,7,8-tetrahydro-4H-isoxazolo[3,4-d]azepin-3-ol) were used to assess the energetic accessibility of the proposed bioactive conformations. © 2001 by Elsevier Science Inc.

**Keywords:** natural templates, glycine receptor, neurotransmitters, molecular modelling, superposition

## INTRODUCTION

Glycine (Figure 1) is one of the two major inhibitory neurotransmitters in the central nervous system,<sup>1</sup> GABA being the

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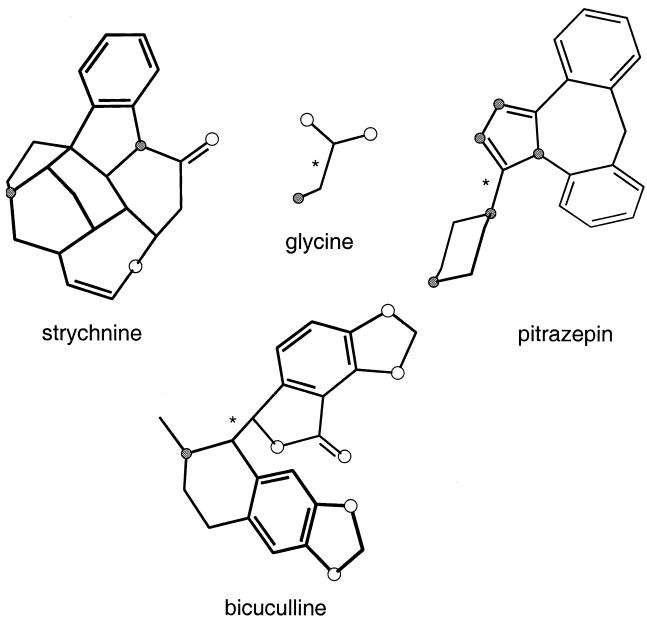
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other. These neurotransmitters interact with their receptors (ligand-gated ion channel receptors, LGICR), which control the opening of an associated channel. These channels are selective for the transit of chloride ions, which are in greater concentration in the extracellular medium. The inrush of chloride anions into the postsynaptic neuron causes hyperpolarization and thus inhibits the likelihood of the neuron firing.

From the structural point of view, neurotransmitters are small, highly flexible molecules. This characteristic makes it difficult to find the conformation in which they bind to their respective receptors: the bioactive conformation. When this conformation is known, it can be relatively easy to explain activity trends among agonist and antagonist ligands. Furthermore, binding modes within the receptor cavity can be envisaged, and, in a last step, new drug designs can be proposed. A three-dimensional X-ray structure of a receptor is a valuable tool for discriminating between a set of designs; for example, when docking ligand designs and assessing fit and binding. In the absence of such structural data on the receptor (as is the present case), use of natural and specific competitive antagonists with rigid structures is the best alternative for developing pharmacophoric models.

The natural templates (NT) technique uses relatively rigid alkaloids to which small, flexible molecules are fit. The technique has been useful for generating pharmacophoric models pertinent to several important LGICRs (5-HT<sub>3</sub>, nACh, GABA<sub>A</sub>, and Gly).<sup>2,3</sup> With the NT-derived pharmacophoric models in hand, other computational chemistry strategies have been employed. For instance, the three-dimensional quantitative structure-activity (3D-QSAR) method, comparative molecular field analysis (CoMFA), was applied in the case of 5-HT<sub>3</sub>.<sup>4</sup> In another example, molecular dynamics simulations were applied to a model of nAChR.<sup>5</sup> As with any scientific



**Figure 1.** Glycine (the neurotransmitter), strychnine (the template, competitive antagonist), and bicuculline and pitrazepin (competitive antagonists). Shaded and open circles correspond to nitrogen and oxygen atoms, respectively. All other atoms are carbons, and hydrogen atoms have been deleted from the drawings to make the structures less cluttered. Asterisks refer to rotatable bonds used in the conformational searches.

model, some revisions of the NT-derived pharmacophoric models can be, and have been, incorporated to account for new experimental evidence when it has become available.<sup>5,6</sup>

In this paper we describe molecular modelling using the NT protocol for ligands of the glycinergic receptor. We use strychnine (Figure 1) as the 3-D template to locate essential features for recognition of ligands that bind to the same receptor. Different classes of ligands are superimposed onto the template, and activity trends are justified. All the molecules were subjected to conformational search analysis to explore the energetic accessibility of proposed bioactive conformations. Experimental results are used throughout the paper as a guide for superimpositions.

A brief description of the protocol employed is as follows: (1) a common fragment of the natural template is obtained based on specific competitive antagonists, and the bioactive conformation for glycine is proposed; (2)  $\alpha$ -amino acid ligands (either agonists or antagonists) are fitted to the fragment, and glycine's bioactive conformation is confirmed; and (3) finally,  $\beta$ -amino acids are used to test the proposed model, giving an independent confirmation of the proposed pharmacophores.

## METHODOLOGY

Biological activity data was used from published studies, and the relevant values for the molecules studied are shown in Table 1.<sup>7,8,9</sup> All the molecular modelling was done using QUANTA/CHARMM software<sup>10</sup> running on a Silicon Graphics ([www.sgi.com](http://www.sgi.com)) workstation. When available, molecular structures were obtained from published crystallographic stud-

**Table 1. Biological activity data for the studied compounds**

Molecule	K <sub>i</sub> (nM) <sup>a</sup>	EC <sub>50</sub> (mM) <sup>b</sup>	IC <sub>50</sub> (mM) <sup>c</sup>
Strychnine	7.0	—	—
Pitrazepin	580	—	—
Bicuculline	6900	—	—
Glycine	2200	0.2	—
L- $\alpha$ -Alanine	—	3.1	—
D- $\alpha$ -Alanine	—	9.0	—
D-Serine	—	—	8.5
L-Serine	—	5.1	—
L-Proline	—	—	414
D- $\alpha$ -ABA	—	—	3.6
L- $\alpha$ -ABA	—	—	6
ACHC	—	—	15
$\alpha$ -AIBA	—	—	2.9
ISONIPE	—	—	0.23
$\beta$ -Alanine	13600	—	11.6
Taurine	29000	1.7	0.7
ISOTHAZ	1700	—	—

<sup>a</sup> Taken from reference 7.

<sup>b</sup> Effective concentrations taken from reference 8.

<sup>c</sup> All inhibitory concentrations were taken from reference 8, except the value for  $\beta$ -alanine, which was taken from reference 9.

ies: strychnine,<sup>11</sup> bicuculline,<sup>12</sup> pitrazepin,<sup>13</sup> and ISOTHAZ (5,6,7,8-tetrahydro-4H-isoxazolo[3,4-d]azepin-3-ol).<sup>14</sup> Structures for glycine, L- $\alpha$ -alanine, D- $\alpha$ -alanine, L-serine, D-serine, L-proline, D- $\alpha$ -ABA (D- $\alpha$ -aminobutyric acid), L- $\alpha$ -ABA, ACHC (1-amino-cyclohexane-1-carboxylic-acid),  $\alpha$ -AIBA ( $\alpha$ -amino-*iso*-butyric acid), ISONIPE (*iso*-nipecotinic acid),  $\beta$ -alanine, and taurine were assembled within QUANTA using standard bond lengths and bond angles. All molecules (agonists and antagonists) were built as protonated species as they exist at physiological pH. Molecules existing predominantly as zwitterions were built in this form.

Atomic charges were assigned using CHARMM Param 23.0 parameter set, and molecular mechanics energy minimizations and molecular dynamics (MD) were done using the CHARMM force field.<sup>15</sup> For all calculations described herein, the dielectric of the medium was set to unity, and the cut-off distances for both the electrostatic and Lennard-Jones nonbonded interactions were set to 14 Å.

All the molecules were energy minimized with the Adopted-Basis Newton-Raphson algorithm. Structures were considered fully optimized when the energy changes between successive iterations were less than 0.01 kcal/mol. Energy minimization with a tighter convergence criterion ( $10^{-5}$  kcal/mol) was also performed on some of the compounds (glycine, L- $\alpha$ -alanine, D- $\alpha$ -alanine, L-serine, D-serine, L-proline, D- $\alpha$ -ABA, L- $\alpha$ -ABA, ACHC,  $\alpha$ -AIBA, and ISONIPE) to test the effect of running a larger number of minimization cycles on these flexible molecules. The two minimized structures (from the  $10^{-2}$  and  $10^{-5}$  criteria, respectively) were then superimposed using all the atoms except the hydrogens, and the RMS distance measured. In all cases, the RMS values were <0.3 Å. The energy difference between the two minimized structures was always below 1 kcal/mol, except for D- $\alpha$ -ABA (1.8 kcal/mol)

and ACHC (3.2 kcal/mol). Although in these two cases the energy difference could be significant, the low RMS values indicate that both conformations could fit the template equally well.

The ligand molecules were flexibly fit to the template strychnine. This technique recognizes that molecules are inherently flexible and can change their shape by rotation around single bonds. Unless explicitly stated, all atoms in the ligand (except hydrogens) were used for fitting. The superimposed structures were energy minimized with negligible alteration of the conformation, thereby indicating that these conformations are located at a local energy minimum. Conformational searches were performed on the more relevant free rotatable bonds to locate the global energy minimum conformations. The systematic search method (grid scan), where each specified torsional angle is varied over a grid of equally spaced values, was used. Except where explicitly stated, the global minima are the same as the local minima mentioned above, and this is the conformation used for the analysis.

Tables 2, 3, and 4 collect the number of torsional angles (NTA), step size for the torsional angle increment (in degrees), and the number of conformations (NC) calculated for each molecule. One criterion for judging the superimposition of the ligands and template is the root-mean square (RMS) distance between selected atoms in the ligands and the equivalent atoms in the template. Another criterion is the distance between the protonated amine nitrogen in the ligand and template; this heteroatom plays an important role with respect to the relative activity of the ligands. The tables contain the RMS values and the N···N separations (in Å). These distances were computed after flexibly fitting the molecules.

## RESULTS AND DISCUSSION

### Strychnine as Natural Template

The use of strychnine as the NT for aligning GlyR ligands is justified by three experimental considerations.

1. Strychnine has been experimentally characterized as a potent GlyR competitive antagonist ( $K_i = 7.0$  nM) with relatively high specificity.<sup>16</sup>
2. Binding studies have demonstrated that strychnine and glycine have the same binding site, but their interactions within residues in the receptor cavity are somewhat different.<sup>8,17–19</sup>
3. It is known experimentally that strychnine interacts with the mouth of the channel (probably with Arg 271) and thereby produces blockage.<sup>20</sup>

Hence, from an experimental point of view, it seems appropriate to use strychnine as the NT for GlyR. But what makes strychnine especially attractive for computational studies like this is the molecule's conformational rigidity. A relatively rigid template is valuable because there are fewer possibilities of how it can fit the receptor and how small molecules can be aligned to the template.

To demonstrate conformational rigidity of strychnine, a short molecular dynamics simulation was performed using Quanta/CHARMM. The MD calculations used the same force field, parameter set, dielectric constant, and cut-off as in the molecular mechanics calculations. A velocity Verlet algorithm was used to integrate Newton's equation of motion using 1 fs time steps. The SHAKE algorithm was used to constrain bond

**Table 2. Selected results from conformational analyses of the competitive antagonists, bicuculline and pitrazepin, and the neurotransmitter, glycine (in three different alignments), and the superimposition of these molecules with respect to strychnine<sup>a</sup>**

Molecule	NTA	Step size	NC	RMS (Å)	N···N (Å)
Bicuculline	1	5°	72	0.14	0.32
Pitrazepin	1	5°	72	0.25	0.37
Glycine	1	15°	24	0.28	1.84
Glycine <sup>b</sup>	—	—	—	0.32	2.38
Glycine <sup>b</sup>	—	—	—	0.39	3.13

<sup>a</sup> NTA = number of torsional angles. NC = number of conformations.

<sup>b</sup> Alternate alignments to strychnine (see text).

**Table 3. Selected results from conformational analysis and superimposition protocol for  $\alpha$ -amino acids**

Molecule	NTA	Step size	NC	RMS (Å)	N···N (Å)
L- $\alpha$ -Alanine	1	30°	12	0.22	1.86
D- $\alpha$ -Alanine	1	30°	12	0.25	1.83
L-Serine	3	45°	512	0.27	1.74
D-Serine	3	45°	512	0.45	2.46
L-Proline	1	5°	72	0.27	1.74
D- $\alpha$ -ABA	2	5°	5184	0.41	2.75
D- $\alpha$ -ABA <sup>a</sup>	—	—	—	0.29	3.01
D- $\alpha$ -AGA <sup>a</sup>	—	—	—	1.06	2.61
D- $\alpha$ -ABA	2	5°	5184	0.35	3.13
L- $\alpha$ -ABA <sup>a</sup>	—	—	—	0.67	2.16
L- $\alpha$ -ABA <sup>a</sup>	—	—	—	0.65	2.17
ACHC	1	5°	72	0.45	2.70
ACHC <sup>a</sup>	—	—	—	0.50	3.15
$\alpha$ -AIBA	3	45°	8	0.83	2.29
$\alpha$ -AIBA <sup>a</sup>	—	—	—	0.31	1.79
$\alpha$ -AIBA <sup>a</sup>	—	—	—	0.34	3.21

<sup>a</sup> Alternate alignments to strychnine (see text).

**Table 4. Selected results from conformational analysis and superimposition protocol for  $\beta$ -amino acids**

Molecule	NTA	Step size	NC	RMS (Å)	N···N (Å)
ISONIPE	1	5°	72	0.30	0.97
$\beta$ -Alanine <sup>a</sup>	2	5°	5184	0.21	0.22
$\beta$ -Alanine <sup>b</sup>	—	—	—	0.19	2.41
Taurine <sup>a</sup>	2	5°	5184	0.13	0.11
Taurine <sup>b</sup>	—	—	—	0.10	2.51

<sup>a</sup> Antagonist conformation.

<sup>b</sup> Agonist conformation.

lengths and bond angles to their equilibrium values with a relative tolerance of  $10^{-9}$ . The simulation protocol involved adding kinetic energy to the system for 6 ps, thereby bringing the temperature of the system to 600 K. The system's kinetic and potential energies were allowed to fully equilibrate for 25

ps. Following this, full simulation was carried out for 100 ps. During this period, molecular structures were sampled at 20-ps intervals, yielding 5 structures for post-simulation processing.

Seven structures (X-ray, the energy minimized model, and five from the MD simulation) were analyzed and compared in terms of energy and structural parameters. The minimized structure is -42.7 kcal/mol lower in energy than the X-ray structure and exhibits an RMS distance of 0.0963 Å with respect to the X-ray structure. The six modeled structures are almost the same in energy (within 0.001 kcal/mol) and three-dimensional shape (RMS distance within 0.01 Å of each other). This similarity shows that strychnine is quite rigid and an excellent template.

For purposes of deriving a pharmacophore, three main interaction sites can be identified in strychnine's structure (Figure 2): (A) a cationic region, i.e., the protonated or quaternary nitrogen; (B) a  $\pi$  region, i.e., planar and rich in delocalized electron density; and (C) an electronegative region, i.e., the oxygen of the carbonyl group. Some ligands do not occupy region C, prompting us to use pharmacophoric regions A and B as the main superimposition points. In light of our own findings, we postulate that the so-called C region of strychnine is the one that interacts with the mouth of the channel.

The same MD protocol was employed on the relatively rigid ligand ISOTHAZ. The average energy difference between the model and the 5 structures obtained from the simulations is about 3.5 kcal/mol. Although, in principle, this energy difference could be significant, the average value for the RMS is below 0.1, which indicates that the structures are very similar three-dimensionally. This means that any of the structures could be successfully overlapped with the template to find the bioactive conformation.

### Bicuculline and Pitrazepin Competitive Antagonists: Determination of the Glycine Bioactive Conformation

Bicuculline<sup>21</sup> ( $K_i = 6900$  nM) has only one acceptable way of being superimposed on the strychnine structure. The six aromatic carbon atoms and the protonated amine nitrogen were used for fitting purposes. As seen in Table 2, the RMS value is excellent (0.14 Å), and the N···N distance is small (0.32 Å). This superimposition also allows bicuculline to display one part of its structure toward pharmacophoric region C, which is

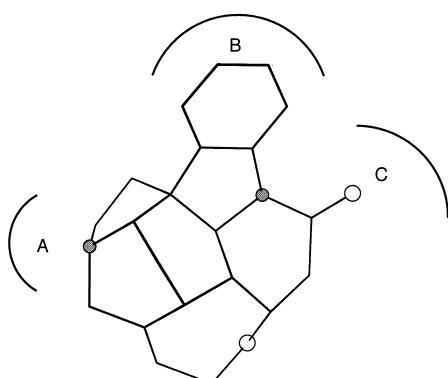


Figure 2. Three pharmacophoric areas defining the glycine bioactive conformation.

thought to be related to blockage of the channel and to antagonistic activity. The superimposition shown in Color Plate 1(a) shows the three pharmacophoric sites are satisfied.

Pitrazepin<sup>22</sup> ( $K_i = 580$  nM) is also a relatively rigid molecule with only one rotatable bond (Figure 1). Three atoms of the triazole ring (i.e., the carbon attached to the piperazine ring and its two adjacent nitrogens) and the protonated amine nitrogen are used for fitting, yielding an RMS value of 0.25 Å. Pitrazepin's protonated amine nitrogen is close to that of strychnine (N···N separation of 0.37 Å) as seen Table 2 and Color Plate 1(b). The triazole ring acts as an equivalent to pharmacophoric region B. The benzene ring in region C can cause blockage of the channel. As in the case of bicuculline, the three pharmacophoric sites are thus fulfilled.

Based on these observations, Color Plate 2(a) shows the proposed alignment and bioactive conformation for the neurotransmitter glycine. The RMS value and N···N distance were 0.28 and 1.84 Å, respectively (Table 2). As can be inferred from Color Plate 2(a), two alternative superimpositions (with the same conformation for glycine) are possible. These alternatives position glycine's protonated amine nitrogen in opposite directions with respect to the equivalent nitrogen in strychnine. The RMS value and N···N distances are, respectively, 0.32 and 2.38 Å for one of them (Color Plate 2(b)), and 0.39 and 3.13 Å for the other (Color Plate 2(c)). Neither alternative fits the pharmacophore as well as the proposed bioactive superimposition.

### Amino Acid-type Ligands Acting at GlyR

Glycine-related ligands constitute a set of compounds able to act as either agonists or antagonists. For these compounds, agonistic activity is given by their effective concentration EC<sub>50</sub> values and their antagonist activity by the inhibitory concentration IC<sub>50</sub> values (Table 1), as reported by Schmieden and Betz using voltage-clamp experiments.<sup>8</sup>

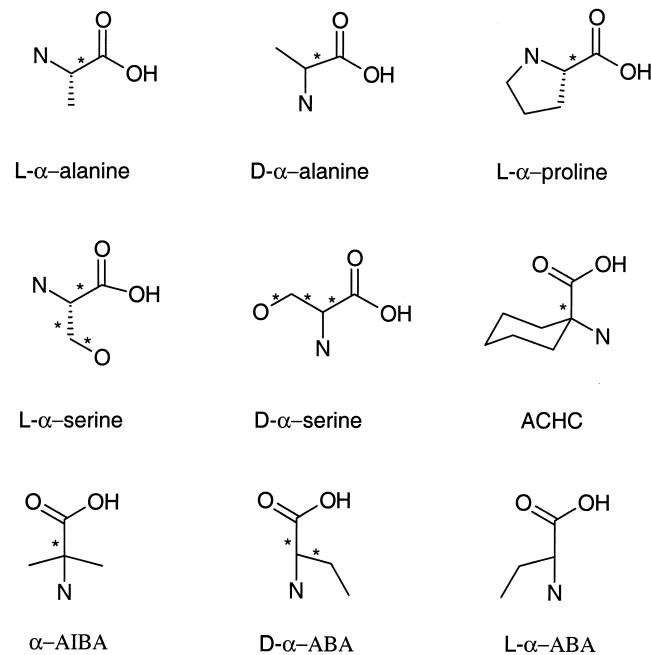


Figure 3.  $\alpha$ -Amino acids studied. Asterisks indicate rotatable bonds for the conformational searches.

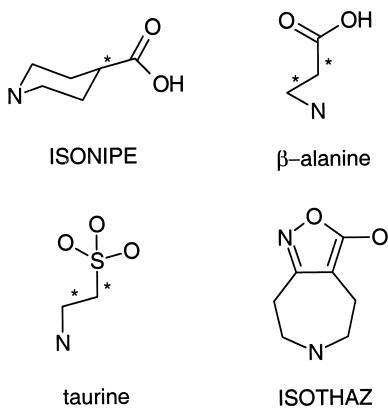


Figure 4.  $\beta$ -Amino acids studied. Asterisks indicate rotatable bonds used in the conformational searches.

#### $\alpha$ -Amino acids: Confirmation of the proposed bioactive conformation for glycine

The chemical structures of the  $\alpha$ -amino acids studied here are depicted in Figure 3. L- $\alpha$ -Alanine ( $EC_{50} = 3.1$  mM) and D- $\alpha$ -alanine ( $EC_{50} = 9.0$  mM) have been characterized as relative potent agonists compared to glycine ( $EC_{50} = 0.2$  mM). L- $\alpha$ -Alanine superimposed with strychnine is shown in Color Plate 3(a). The RMS value and N···N distance are 0.22 and 1.86 Å, respectively (Table 3). As in the case of glycine, other alternatives are possible, and for the same reasons as before they are ruled out. The same situation occurs with D- $\alpha$ -alanine, whose superimposition is shown in Color Plate 3(b). The RMS value and N···N distance are 0.25 and 1.83 Å, respectively (Table 3).

A hydroxyl group at  $C_\beta$  atom of D- and L-alanine generates D- and L-serine. Whereas L-serine is an agonist ( $EC_{50} = 5.1$  mM), D-serine is an antagonist ( $IC_{50} = 8.5$  mM). Both enantiomers are stabilized by an intramolecular hydrogen bond creating a five-member pseudo-ring that fits perfectly with its equivalent in strychnine (Color Plates 4(a) and 4(b)). L-Serine, as agonist, displays its protonated amine nitrogen at the same spatial location as does glycine. For L-serine the RMS value and N···N distance from fitting are 0.27 and 1.74 Å, respectively; for D-serine the fits are 0.45 and 2.46 Å, respectively (Table 3). Introduction of a pyrrolidine ring in the glycine structure produces L- and D-proline. L-Proline behaves as an agonist.<sup>23</sup> There are no relevant bioactivity data available for D-proline, and it was not included in our study. The superimposition of L-proline is shown on Color Plate 4(c). As seen in Table 3, the RMS value and N···N distance are 0.27 and 1.74 Å, respectively, the same as for L-serine. The good spatial agreement between pyrrolidine's five-member ring and its equivalent in strychnine structure is apparent.

D- $\alpha$ -ABA and L- $\alpha$ -ABA are antagonists with  $IC_{50}$  values of 3.6 and 6.0 mM, respectively. As in the case of glycine and L- $\alpha$ - and D- $\alpha$ -alanine, two other alternatives are possible besides the one we advocate (Color Plate 5(a)). In the preferred superimposition for D- $\alpha$ -ABA, the RMS value and N···N distance are 0.41 and 2.75 Å, respectively (Table 3). One of the alternatives (Color Plate 5(b)) has a better RMS value (0.29 Å) but a longer N···N distance (3.01 Å). The other alternative (Color Plate 5(c)) has a shorter N···N distance (2.61 Å) but a very poor RMS value (1.06 Å), and the carboxylate group is

almost perpendicular compared with the pharmacophoric area B in strychnine.

In the case of L- $\alpha$ -ABA, the proposed alignment has an RMS value of 0.35 and an N···N distance of 3.13 Å (Color Plate 6(a)). Both alternatives have higher and similar RMS values (0.67 and 0.65 Å) and similar N···N distances (2.16 and 2.17 Å) (Color Plates 6(b) and 6(c)). The second alternative displays the carboxylate group perpendicular to pharmacophoric area B in strychnine, as does D- $\alpha$ -ABA, and therefore it is not a favorable choice.

ACHC, which is a weak antagonist ( $IC_{50} = 15$  mM), can be superimposed on strychnine in two reasonable ways. The first one (Color Plate 7(a)), which has the amino group in an equatorial position, causes the carboxylic group to deviate from the plane of the aromatic ring of strychnine, whereas the protonated amine nitrogen is in a position equivalent to D-serine studied before (Color Plate 4(a)). The six-membered ring is located at the same spatial location as its equivalent in strychnine. The RMS value and N···N distance are 0.45 and 2.70 Å, respectively. Another possibility (Color Plate 7(b)), which has the amino group in an axial position, should be considered. For it, the protonated amine nitrogen is in a direction opposite from the previous one, and the six-membered ring location is consistent with the same ring in pitrazepin (Color Plate 1(b)). The conformation is stabilized by an intramolecular hydrogen bond, which in turn creates a five-membered pseudo-ring located at the same spatial area as its equivalent ring in strychnine. The RMS value and N···N distance are 0.50 and 3.15 Å, respectively. From Color Plate 7, we cannot rule out either of these two possible alignments; both fit the template about equally. Hence, both situations must be considered.

$\alpha$ -AIBA has been characterized as an antagonist with an  $IC_{50}$  value of 2.9 mM. Three superimpositions are possible (Color Plate 1(b)). One of them (Color Plate 8(a)) is out of consideration because of an orthogonal disposition of the carboxylate group in relation with the  $\pi$  area in strychnine. The RMS value and N···N distance for this alignment are 0.83 and 2.29 Å, respectively. The other two remaining alignments (Color Plate 8(b) and 8(c)) have similar RMS values, 0.31 and 0.34 Å; the main difference between them is the N···N distance: 1.79 Å vs. 3.21 Å. Taking into account the criteria we have been following, the alignment shown in Color Plate 8(c) seems to be the conformation that better fits the model.

#### $\beta$ -Amino acids: Validation of strychnine as template

These ligands (Figure 4) have the characteristic of an extra rotatable bond in the side chain. Experimental results show that  $\beta$ -amino acids usually display both agonistic and antagonistic activities.<sup>8</sup>

ISONIPE, a  $\gamma$ -amino acid, has been characterized as a competitive antagonist<sup>8</sup> and will serve as a template for the antagonist conformation for  $\beta$ -amino acids. Superimposition using all atoms except the hydrogens and the protonated amine nitrogen is shown in Color Plate 9. The carboxylate group is well situated in the  $\pi$  region (B), and the piperidine nitrogen is close to the corresponding nitrogen in strychnine. The RMS value and N···N distance are 0.30 and 0.97 Å, respectively (Table 4).

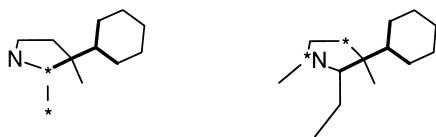
$\beta$ -Alanine and taurine are two prototypes for  $\beta$ -amino acids. Minimum energy conformations obtained from the conformational search show that both molecules can exhibit an intramolecular hydrogen bond, but in this conformation neither of the molecules could be superimposed on the template. To put

$\beta$ -alanine in an antagonist conformation (Color Plate 10(a)) costs approximately 20 kcal/mol above the global energy minimum. The antagonist conformation has an RMS value of 0.21 Å and an N $\cdots$ N distance of 0.22 Å. The same excellent fit is found for taurine antagonist conformation, which is approximately 27 kcal/mol above its global minimum; the RMS value and N $\cdots$ N distance are 0.13 and 0.11 Å, respectively (Color Plate 10(b)).

Similar results are found for the agonist conformation. In the case of  $\beta$ -alanine (Color Plate 11(a)), the difference with respect to the global minimum is almost the same as before (approximately 20 kcal/mol) with an RMS value of 0.19 Å and N $\cdots$ N distance of 2.41 Å. For taurine's agonist conformation (Color Plate 11(b)), which is approximately 20 kcal/mol above the global minimum, the RMS value and N $\cdots$ N distance are 0.10 and 2.51 Å, respectively. The separation of the nitrogens is much greater than in the case of the antagonist conformation.

In these last two examples ( $\beta$ -alanine and taurine), there is a relatively large difference in energy (20–30 kcal/mol) between the global minimum energy conformations and either the antagonist or agonist conformations proposed here. However, the significant point is that the agonist or antagonist conformations differ from each other by only about 1 kcal/mol for  $\beta$ -alanine and about 7 kcal/mol for taurine. With such low barriers of interconversion between the agonist and antagonist conformations, it is easy to understand their dual behavior on the receptor. It is well known that the bioactive conformation frequently is not the minimum energy conformation. The former can, in principle, be as much as 30 kcal/mol above the latter.<sup>24</sup> This assumption was used by Hibert et al., for instance, in their study of 5-HT<sub>3</sub> receptor antagonists.<sup>25</sup>

As a final example, one muscimol derivative can be considered. ISOTHAZ, which behaves as a competitive antagonist,<sup>7</sup> seems to interact with a different binding site than does strychnine,<sup>26</sup> a binding site that more resembles that of  $\beta$ -alanine and glycine. Superimposition of ISOTHAZ and strychnine is shown in Color Plate 12. The RMS value and N $\cdots$ N distance are 0.12 and 1.95 Å, respectively. Due to ISOTHAZ's relative rigidity, the proposed bioactive conformation may adopt an agonist-like fit instead of an antagonist-like one. Such an idea is in agreement with the experimental evidence<sup>26</sup> and also confirms the agonist conformation in  $\beta$ -amino acids. Thus, even though ISOTHAZ is a competitive antagonist, it appears to share a binding site like that of agonists, and this justifies superimposing it like an agonist.



Agonist pharmacophore      Antagonist pharmacophore

Figure 5. Depiction of the pharmacophores obtained by the NT protocol using strychnine as template. The bonds in bold represent the glycine structure. Asterisks denote possible locations for the protonated amine nitrogen. The glycine molecule is superimposed on arbitrary substructures taken from strychnine.

## CONCLUSIONS

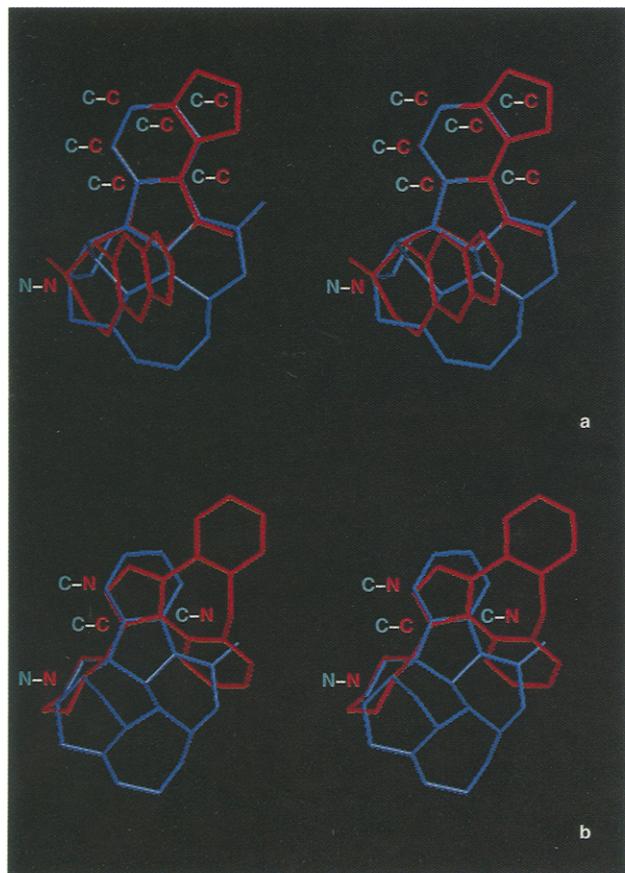
By using a relatively rigid natural template that binds to the same receptor as a set of small, flexible ligands, it is possible to deduce from molecular modelling the bioactive conformations of the latter molecules. Strychnine, which is a natural and specific competitive antagonist, served as the template for ligands of the glycinergic ligand-gated ion channel receptors. Pharmacophores for agonist and antagonist activity were obtained. These are summarized in Figure 5. The main difference between the agonist and antagonist pharmacophores is the location of the protonated amine nitrogen. These models (Figure 5) are proposed for further elucidation of the bioactive conformations of ligands mediating the glycinergic ion channels.

## REFERENCES

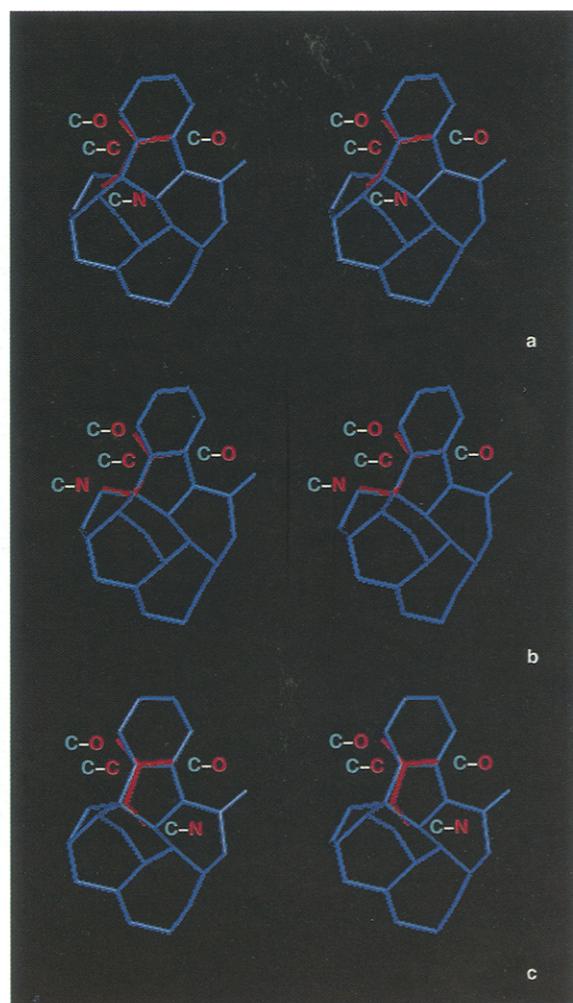
- Aprison, M.H., and Werman, R. The distribution of glycine in cat spinal cord and roots. *Life Sci.* 1965, **4**, 2075–2083
- Aprison, M.H., Gálvez-Ruano, E., and Lipkowitz, K.B. Identification of a second glycine-like fragment on the strychnine molecule. *J. Neurosci. Res.* 1995, **40**, 396–400
- Aprison, M.H., Gálvez-Ruano, E., and Lipkowitz, K.B. Comparison of binding mechanisms at cholinergic, serotonergic, glycinergic, and GABAergic receptors. *J. Neurosci. Res.* 1996, **43**, 127–136
- Morreale, A., Gálvez-Ruano, E., Iriepa-Canalda, I., and Boyd, D.B. Arylpiperazines with serotonin-3 antagonist activity: A comparative molecular field analysis. *J. Med. Chem.* 1998, **41**, 2029–2039
- Gálvez-Ruano, E., Iriepa-Canalda, I., Morreale, A., and Lipkowitz, K.B. A computational model of the acetylcholine binding site. *J. Comput.-Aided Mol. Design*, 1999, **13**, 57–68
- Gálvez-Ruano, E., Iriepa, I., and Morreale, A. Natural templates for N. S. receptor ligands. I. Competitive antagonist alkaloids at the glycinergic, GABAergic, cholinergic, and serotonergic receptors. *Trends Heterocyclic Chem.*, 1997, **5**, 135–144
- Braestrup, C., Nielsen, M., and Krosgaard-Larsen, P. Glycine antagonists structurally related to 4,5,6,7-tetrahydroisoazolo[5,4-c]pyridin-3-ol inhibit binding of [<sup>3</sup>H]strychnine to rat brain membranes. *J. Neurochem.* 1986, **47**, 691–696
- Schmieden, V., and Betz, H. Pharmacology of the inhibitory glycine receptor: Agonism and antagonism of amino acids and piperidine carboxylic acid compounds. *Mol. Pharmacol.* 1995, **48**, 919–927
- Johnson, G., Drummond, J.T., Boxer, P.A., and Bruns, R.F.  $\beta$ -Proline analogues as agonists at the strychnine-sensitive glycine receptor. *J. Med. Chem.* 1992, **35**, 233–241
- Molecular Simulations Inc., San Diego, CA, USA ([www.msi.com](http://www.msi.com))
- Mostad, A. Structural study of the strychnine molecule in crystals of the free base and of the nitric acid complex. *Acta Chem. Scand., Ser. B* 1985, **39**, 705–716
- Lipkowitz, K.B., Gilardi, R.D., and Aprison, M.H. Molecular mechanics and X-ray crystallographic analysis of bicuculline salt conformations. *J. Mol. Struct.* 1988, **178**, 305–313

- 13 Boulanger, T., Vercauteran, D.P., Evrard, G., and Durant, F. Crystal structure and quantum electronic analyses of pitrazepin, a gamma-aminobutyric acid (GABA) receptor antagonist. *J. Chem. Soc., Perkin Trans. II* 1989, 217–221
- 14 Brehm, L., Krogsgaard-Larsen, P., Schaumburg, K., Johansen, J.S., Falch, E., and Curtis, D.R. Glycine antagonists. Synthesis, structure, and biological effects of some bicyclic 5-isoxazolol zwitterions. *J. Med. Chem.* 1986, **29**, 224–229
- 15 Brooks, B.R., Brucolieri, R.E., Olafson, B.D., States, D.J., Swaminathan, S., and Karplus, M. CHARMM: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* 1983, **4**, 187–217
- 16 Curtis, D.R., Hösli, L., and Johnston, G.A.R. A pharmacological study of the depression on spinal neurons by glycine and related amino acids. *Exp. Brain Res.* 1968, **6**, 1–18
- 17 Rajendra, S., Vandenberg, R.J., Pierce, K.D., Barry, P.H., and Schofield, P.R. The unique extracellular disulfide loop of the glycine receptor is a principal ligand binding element. *EMBO J.* 1995, **13**, 2987–2998
- 18 Vandenberg, R.J., French, C.R., Barry, P.H., Shine, J., and Shofield, P.R. Antagonism of ligand-gated ion channel receptors: Two domains of the glycine receptor  $\alpha$  subunit form the strychnine-binding site. *Proc. Natl. Acad. Sci. USA* 1992, **89**, 1765–1769
- 19 Vandenberg, R.J., Handford, C.A., and Schofield, P.R. Distinct agonist- antagonist-binding sites on the glycine receptor. *Neuron*, 1992, **9**, 491–496
- 20 Young, A.B., and Snyder, S.H. The glycine synaptic receptor: Evidence that strychnine binding is associated with the ionic conductance mechanism. *Proc. Natl. Acad. Sci. USA* 1974, **71**, 4002–4005
- 21 Betz, H., and Becker, C.-M. The mammalian glycine receptor: Biology and structure of a neuronal chloride channel protein. *Chem. Int.* 1988, **13**, 137–146
- 22 Curtis, D., and Gynther, B.D. Pitrazepin: a central glycine and GABA antagonist. *Eur. J. Pharmacol.* 1986, **131**, 311–313
- 23 Langosch, D., Betz, H., and Becker, C.-D. Molecular structure and developmental regulation of the inhibitory glycine receptor. In: *Glycine Neurotransmission*, Ottersen, O.P. and Storm-Mathisen, J., Eds., Wiley, New York, 1990, pp. 67–82
- 24 Spark, M.J., Winkler, D.A., and Andrews, P.R. Conformational analysis of folates and folate analogues. *Int. J. Quantum Chem., Quantum Biol. Symp.* 1982, **9**, 321–333
- 25 Hibert, M.F., Hoffmann, R., Miller, R.C., and Carr, A.A. Conformation-activity relationship study of 5-HT<sub>3</sub> receptor antagonists and definition of a model for this receptor site. *J. Med. Chem.* 1990, **33**, 1594–1600
- 26 Marvizón, J.C.G., Vázquez, J., García Calvo, M., Mayor, F. Jr., Gómez, A.R., Valdivieso, F., and Benavides, J. The glycine receptor: Pharmacological studies and mathematical modeling of the allosteric interaction between the glycine- and strychnine-binding sites. *Mol. Pharmacol.* 1986, **30**, 590–597

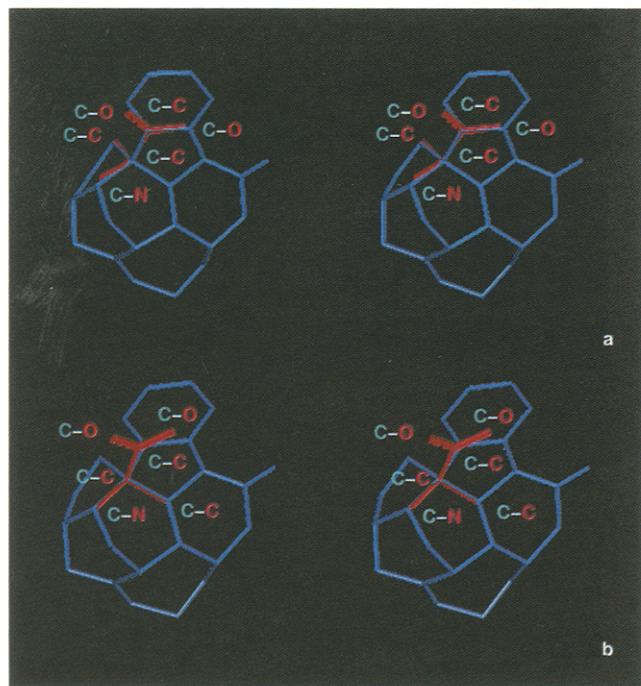
**Superimposition-based protocol as a tool for determining bioactive conformations. I. Application to ligands of the glycinergic receptor (GlyR)**



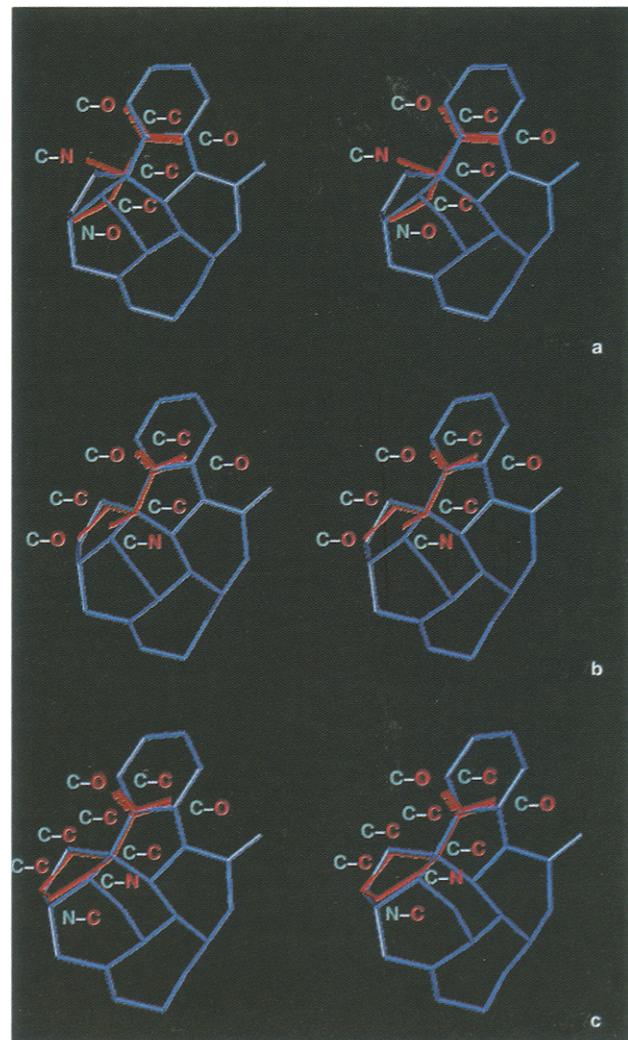
Color Plate 1. Stereo images of (a) bicuculline superimposed with strychnine, and (b) pitrazepin superimposed with strychnine. In this and subsequent figures, the stereo is in the relaxed eye format. Also, in this and subsequent figures, carbon atoms are in black, nitrogen atoms in blue, and oxygen atoms in red; hydrogen atoms are omitted for clarity. Labels indicate which pairs of atoms are being aligned; the labels are colored the same as the respective molecules.



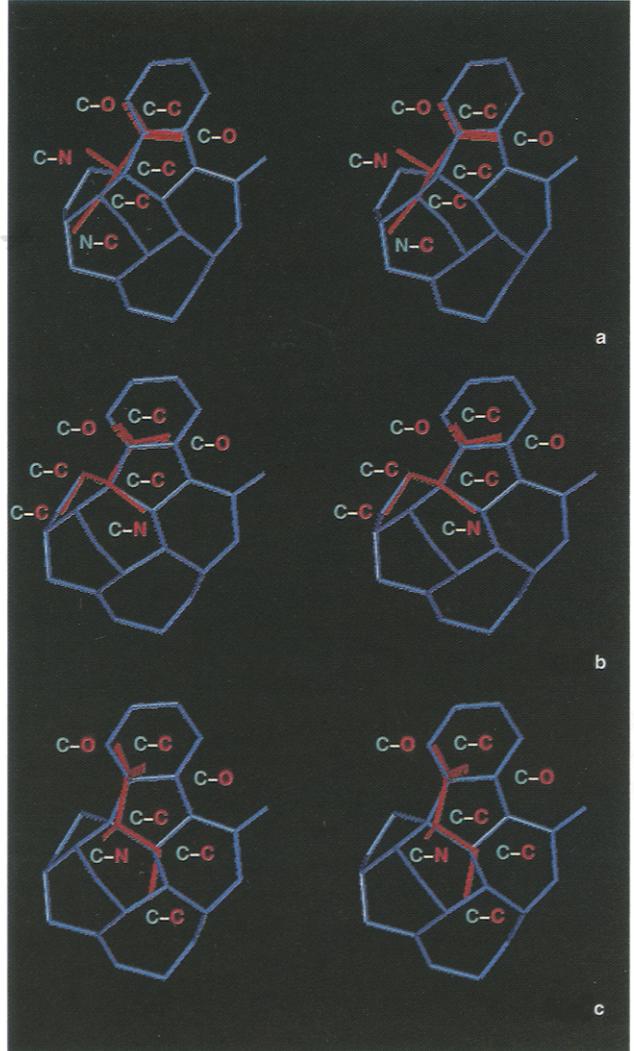
Color Plate 2. Stereo images of three different possibilities of superimposing glycine with strychnine. (a) Proposed bioactive conformation. (b and c) Two other possibilities.



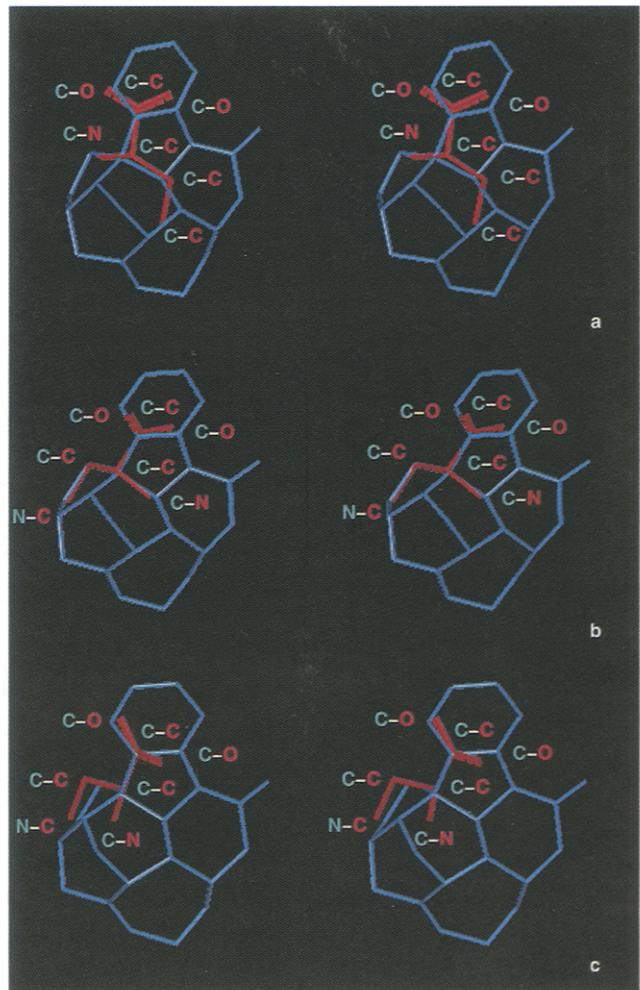
Color Plate 3. Stereo images of (a) L- $\alpha$ -alanine superimposed with strychnine, and (b) D- $\alpha$ -alanine superimposed with strychnine.



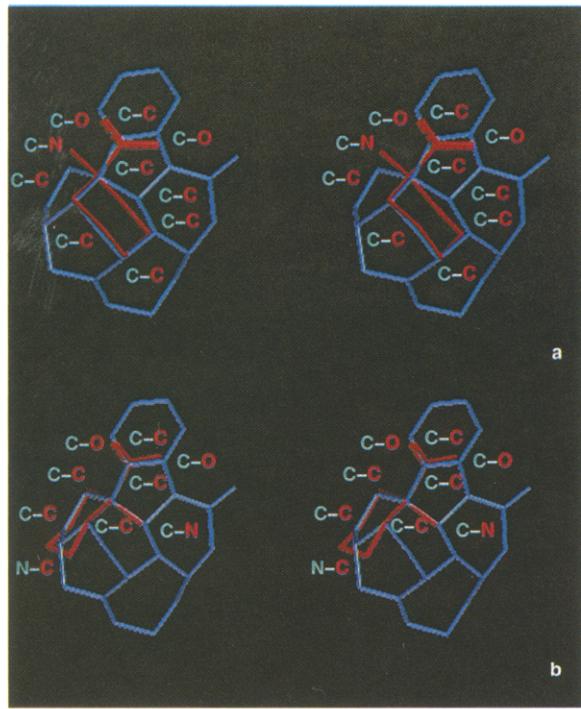
Color Plate 4. Stereo images of (a) L-serine superimposed with strychnine, (b) D-serine superimposed with strychnine, and (c) L-proline superimposed with strychnine.



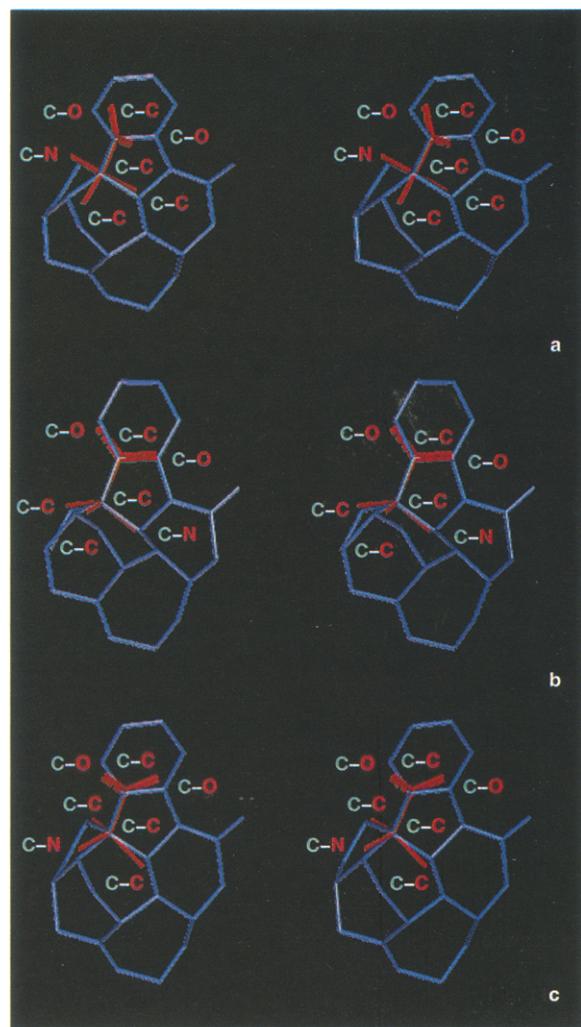
Color Plate 5. Stereo images of D- $\alpha$ -ABA superimposed with strychnine. (a) Proposed bioactive conformation. (b) and c) Two other possibilities.



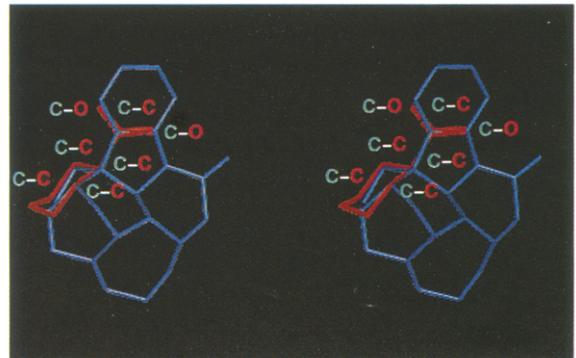
Color Plate 6. Stereo images of L- $\alpha$ -ABA superimposed with strychnine. (a) proposed bioactive conformation. (b) and c) Two other possibilities.



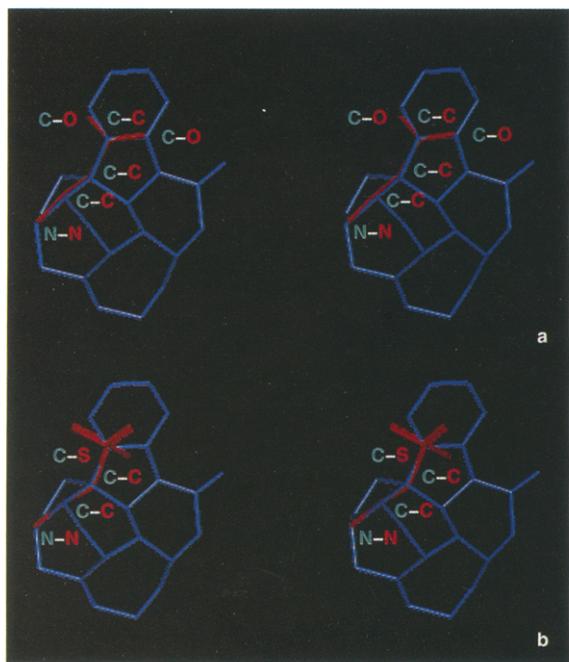
Color Plate 7. Stereo images of ACHC superimposed with strychnine. Two proposed bioactive alternatives.



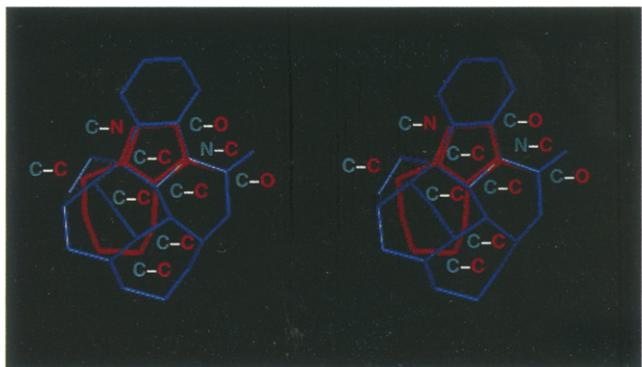
Color Plate 8. Stereo images of  $\alpha$ -AIBA superimposed with strychnine. (a and b) Alternative superimpositions. (c) Proposed bioactive conformation.



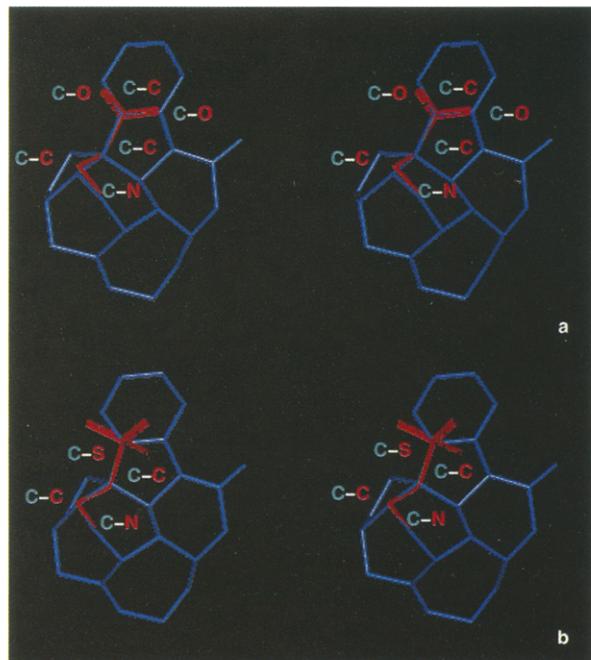
Color Plate 9. Stereo images of ISONIPE superimposed with strychnine.



Color Plate 10. Stereo images of the (a) antagonist conformation for  $\beta$ -alanine superimposed with strychnine, and the (b) antagonist conformation for taurine superimposed with strychnine.



Color Plate 12. Stereo images of ISOTHAZ superimposed with strychnine.



Color Plate 11. Stereo images of the (a) agonist conformation of  $\beta$ -alanine superimposed with strychnine, and the (b) agonist conformation of taurine superimposed with strychnine.