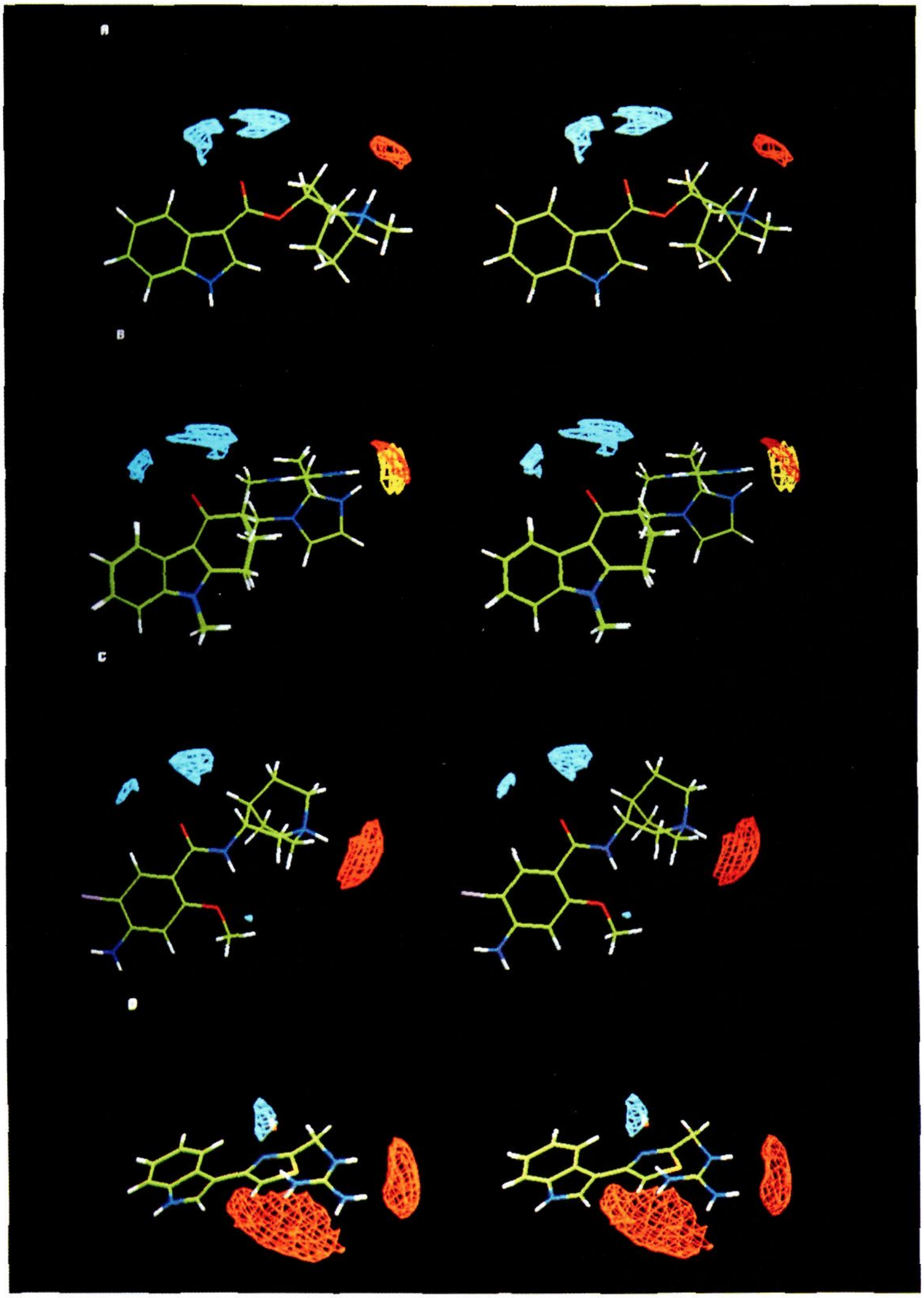


Figure 1. (Stereo view) Contours represent areas of favorable interaction between 5-HT$_3$ receptor ligands and a carboxylate oxygen probe (red, yellow) or a serine-like hydroxyl probe (blue) generated with the GRIN & GRID program. Part A shows **1** (ICS-205-930): red = −8.0 kcal/mol, blue = −4.4 kcal/mol. Part B shows **2** (ondansetron): red/yellow = −7.5 kcal/mol, blue = −4.4 kcal/mol. Part C shows **3** (zacopride): red = −8.5 kcal/mol, blue = −4.8 kcal/mol. Part D shows **4**: red = −11.5 kcal/mol, blue = −5.4 kcal/mol.

the serine probe, and the red (or yellow) contour is for the carboxyl probe.

Compound **1** (1A) has areas of interaction in which the protonated amine function and the carbonyl function could interact with a carboxylate oxygen (red contour) and a serine-like hydroxyl (blue contours), respectively. The serine probe interaction with the carbonyl of the ester is divided into two areas, one for each set of lone-pair electrons associated with the carbonyl oxygen. The hydrogen on the indole nitrogen is also capable of interacting with a carboxylate oxygen, although it is not shown in this figure due to the energy level chosen for contouring. The interaction energy between a carboxylate oxygen and a protonated nitrogen is greater than that of a neutral nitrogen. In order to visualize the interactions with the indole NH, a lower energy value must be used for con-

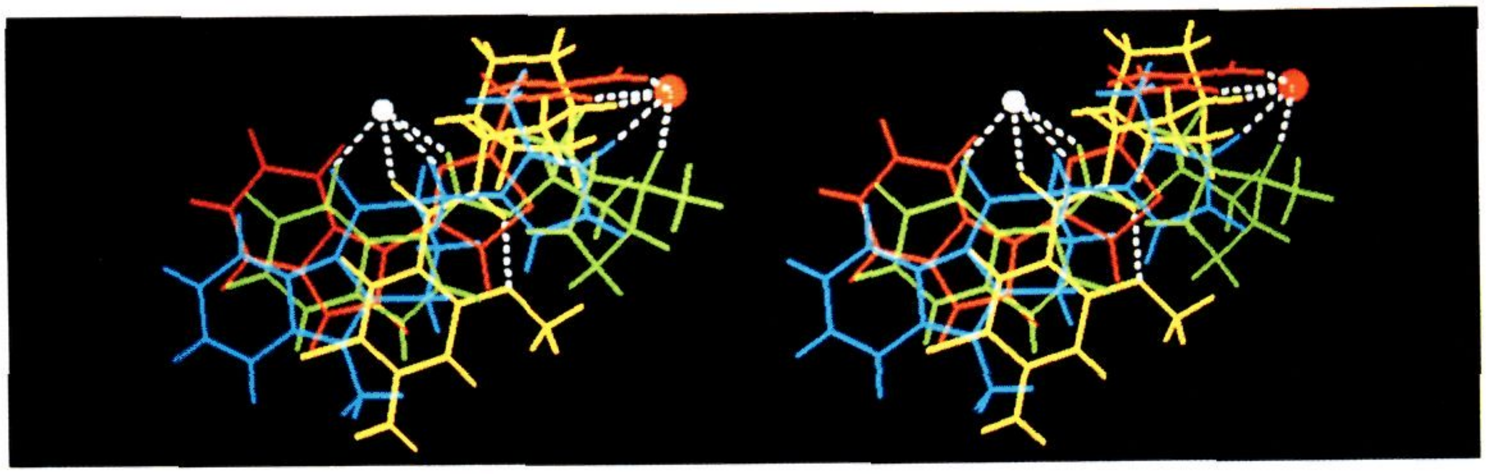
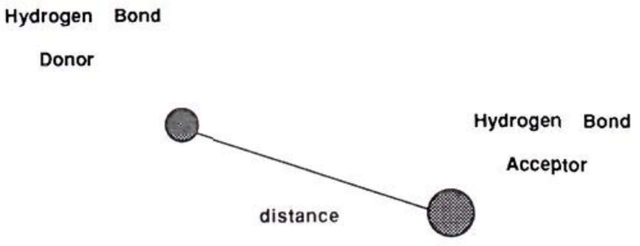


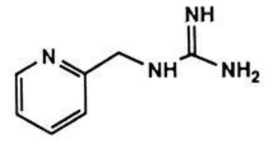
Figure 2. (Stereo view) Overlay of specific 5-HT$_3$ receptor ligands **1** (green), **2** (blue), **3** (yellow), and **4** (red) based on the three-component pharmacophore. The red sphere represents the preferred position of the carbonyl oxygen probe and the white sphere represents the preferred position of the hydrogen in the serine-like hydroxyl probe.

Table II. Distance between the Two Electrostatic Components of the Pharmacophore

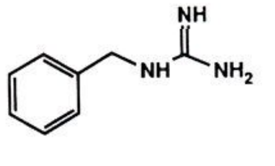


compound	distance, Å		compound	distance, Å		
1	4.65	**7.70**	**3**	**7.71**	10.65	
2			**4**			
axial	**7.66**	10.90	minimized	0.50	5.22	6.88
equatorial	8.88	11.20	overlap	1.20	6.15	**7.64**

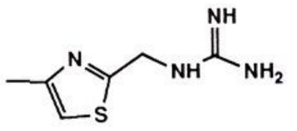
Chart II. Minimum Structure Containing Necessary Pharmacophore for 5-HT$_3$ Binding



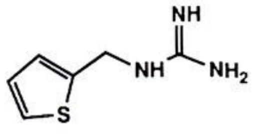
5 (K$_i$ = 82 ± 31 nM)



7 (K$_i$ > 1.0 μM)

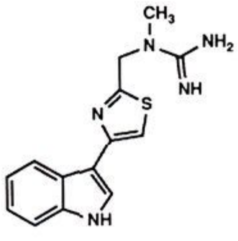


6 (K$_i$ = 116 ± 15 nM)

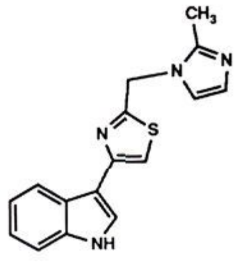


8 (K$_i$ > 1.0 μM)

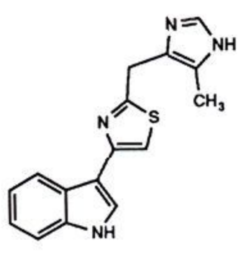
Chart III. Structure–Activity Relationships within the Thiazole Series



9 (K$_i$ > 1.0 μM)



10 (K$_i$ > 1.0 μM)



11 (K$_i$ = 10 ± 3 nM)

touring. This interaction area was assumed not to be critical, since methylation at this position, as in **2**, does not result in a lack of 5-HT$_3$ receptor binding affinity. Therefore, this contour is not displayed.

Figure 1B shows the pseudoaxial conformation for compound **2** overlayed onto the pseudoequatorial conformer. The carbonyl moiety of **2** is coincidental in both conformations and results in the same blue contour for interaction with a serine-like hydroxyl. The red and yellow contours represent the interaction of the pseudoaxial and pseudoequatorial conformers with the carboxylate probe, respectively. Compound **4** (1D) displays the three expected red contours for the carboxylate probe, resulting from the distribution of the positive charge among all five hydrogens of the protonated guanidine residue. Thus, any one of the three red contours could be involved in a hydrogen bond with a receptor.

Similarities among the four compounds were identified and two regions, one for hydrogen-bond accepting and one for hydrogen-bond donating, were common to all molecules. The point of strongest interaction within each contour was established, and the distance between the hydrogen-bond-donating area and hydrogen-bond-accepting area was measured. Table II shows these distances *for each compound. Compounds* **1–3** *have two distances*, one for each of the two blue contours. Compound **4** has three distances, one for each of the three red contours. Identified in Table II is the common distance between regions of interest (approximately 7.7 Å) that **1**, the pseudoaxial conformation of **2**, and **3** all share. the pseudoequatorial conformation of **2** does not share this common distance, with 8.88 Å being the smallest distance. Compound **4**, in its minimum energy conformation, does not share the common distance of 7.7 Å between electrostatic regions. It was necessary to rotate the bond between the thiazole ring and the side chain carbon (bond "a") 20° in order to get a distance that was similar to that of the other compounds. This conformational change results in the loss of an intramolecular electrostatic interaction between the nitrogen in the thiazole ring and one of the hydrogens of the charged guanidine moiety. This rather small change results in an increase in conformational energy of 7.3 kcal/mol when calculated in a vacuum. In order to get a more meaningful value, the effect of aqueous solvent was taken into account. A dynamics simulation was performed on each conformation at 300 K in a 15-Å cube of water with periodic boundary conditions.[16] Each conformation was held rigid while the water underwent 10 ps of simulation with the verlet algorithm in CHARMM. After the 10-ps simulation, the temperature was reduced to 0 K, and the final coordinates were minimized with the conjugate gradient algorithm in CHARMM to a tolerance of 0.01 kcal/mol. Thus, the 20° rotation results in a 2.3 kcal/mol increase in conformational energy relative to the global minima when solvent effects are incorporated into the calculation. This energy difference is not considered large enough to preclude this from being the binding conformation.

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(16) Brooks, C. L., III; Karplus, M.; Pettitt, B. M. in *Proteins: A Theoretical Perspective of Dynamics, Structure, and Thermodynamics*; Prigogine, I., Rice, S. A., Eds.; John Wiley & Sons: New York, 1988, pp 36–38.