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Molecular Modeling of 5-HT_3 Receptor Ligands

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EVANS, S. M., A. GALDES AND M. GALL. *Molecular modeling of 5-HT_3 receptor ligands.* PHARMACOL BIOCHEM BEHAV **40**(4) 1033–1040, 1991.—Ligands of various chemical classes (e.g., indoles, indazoles, benzamides, carbazoles, and quinolines) have demonstrated high affinity for the 5-HT_3 receptor in radiolabeled ligand-binding studies, and have shown 5-HT_3 receptor antagonistic activity in functional assays which utilize the excitatory effects of 5-HT on enteric neurons and autonomic afferents. Several 5-HT_3 antagonists are currently being evaluated for potential use in the treatment of migraine, schizophrenia, and anxiety, and a few have already demonstrated high efficacy as antiemetics in cancer chemotherapy. The purpose of this presentation is to highlight the significant structure-affinity relationships (SAFIR) and common geometrical features among 5-HT_3 receptor ligands, and to describe the three-dimensional pharmacophore for the 5-HT_3 recognition site derived from computational techniques. The chemical template containing the recognition elements (functional groups) for the 5-HT_3 receptor are: an aromatic or heteroaromatic ring system, a coplanar carbonyl group, and a nitrogen center, interrelated by well-defined distances. Two "binding shapes" or "active shapes" for 5-HT_3 ligands have been identified from detailed conformational analyses.

Molecular modeling 5-HT_3 receptor Serotonin Pharmacophore Structure-activity relationships Three-dimensional Antiemetic

SEVERAL groups working independently are studying 5-HT_3 receptor ligands through state-of-the-art computational techniques. This paper presents the three-dimensional structure-affinity relationships that have evolved from molecular modeling studies of 5-HT_3 receptor antagonists. A discussion of serotonin receptor subtypes and standard serotonergic ligands is included in this symposium (22), while general aspects of the complex pharmacology of 5-HT receptor systems have been reviewed elsewhere (19, 32, 49). Many of the 5-HT_3 antagonists currently available are nonselective, yet their high affinity for the 5-HT_3 receptor provides a rationale for structural comparison and a foundation for constructing hypotheses about the chemical functionality comprising the pharmacophore which binds to the 5-HT_3 receptor.

PHARMACOLOGICAL SIGNIFICANCE OF 5-HT_3 ANTAGONISTS

5-HT_3 antagonists constitute ligands selective for the M-receptor originally characterized by Gaddum and Picarelli (20) in 1957 from an isolated guinea pig ileum preparation (15). These ligands are a novel class of therapeutic agents (32, 38, 66) with high efficacy as antiemetics (11, 27, 59) and show potential for treating secretory and motility disorders of the gastrointestinal tract (14,26). These agents also show promise in the treatment of central nervous system conditions such as anxiety, psychoses, pain and/or migraine (31, 38, 66). In addition, they seem to modulate certain behavioral abnormalities (61). The development of more selective antagonists has given impetus to research in this area and to the further characterization of the 5-HT_3 receptor, one of the main serotonergic receptor subtypes with clinical importance.

5-HT_3 receptors/recognition sites are found in the enteric nervous system (24, 44, 52), the sympathetic and parasympathetic autonomic nervous systems, the sensory nervous system, and the central nervous system (2, 34, 50). Their molecular size has been determined (25,39). In many instances, they are directly coupled to a monovalent cation channel, as shown by electrophysiological studies (8,17). The biological responses of 5-HT_3 antagonists have been characterized by using a number of functional assays that rely on the antagonism of the excitatory effects of serotonin on enteric neurons and autonomic afferents (38), e.g., depolarization and reflex bradycardia of the rat vagus nerve, contraction of the guinea pig ileum, and tachycardia induced in the isolated rabbit heart. The clinical antiemetic response (16,43) has been demonstrated by using cisplatin-induced emesis models (36) in the ferret (58) and dog (13,21).

Recently, radioligand binding studies have characterized the 5-HT_3 recognition site in the central nervous system (23, 27, 45, 50, 51, 68), with the specific brain location varying among species. Areas of localization have included rat entorhinal cortex (4, 33, 35, 65), rat and ferret area postrema (the site of the chemoreceptor trigger zone involved in emesis) (34), and the human amygdala, hippocampus, and area postrema (1, 3, 34, 54, 63). Electrophysiological and receptor binding studies have also utilized in vitro systems, namely membranes from the neuronal cell lines N1E-115 mouse neuroblastoma cells (30,40) and NG108-15 neuroblastoma-glioma cells (29, 47, 57).

These biological observations have provided two significant contributions to the understanding of 5-HT_3 receptor mechanisms and structure-affinity relationships (SAFIR): 1) the existence of a single, saturable, high-affinity binding site; and 2) the parallel correlation between the rank order of the antagonists' affinity for the 5-HT_3 receptor and their potency determined in the various functional assays.

STRUCTURES OF 5-HT_3 ANTAGONISTS

Whereas there exist few 5-HT_3 agonists, most of which are nonselective and therefore of limited use, there are several well-

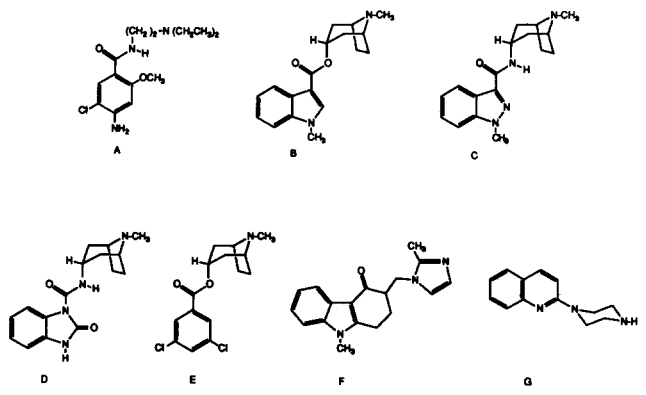

FIG. 1. Representative chemical structures (classes) of 5-HT_3 antagonists: (A), metoclopramide (benzamide); (B), ICS 205-930 (indole); (C), LY 278584 (indazole); (D), DAU 6215 (benzimidazolone); (E), MDL 72222 (benzoate); (F), ondansetron (carbazole); (G), quipazine (quinoline).

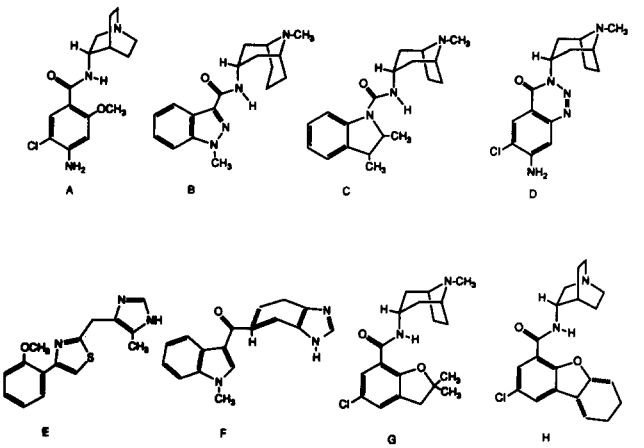

FIG. 2. New 5-HT_3 antagonists: (A), (S)-zacopride; (B), BRL 43694 (granisetron); (C), an indolinecarboxamide; (D), a benzotriazinone; (E), a 2-[4(5)-methyl-5(4)-imidazolylmethyl]thiazole; (F), YM 060; (G), LY 277359 (a benzofuran carboxamide); (H), RG 12915 (a hexahydrobenzofuran).

known, structurally diverse classes of 5-HT_3 antagonists (Fig. 1). Some of these ligands are nonspecific; for example, metoclopramide is primarily a D_2 dopaminergic antagonist (27), and ICS 205-930, a potent 5-HT_3 antagonist described by Richardson in 1985, is also a weak 5-HT_4 antagonist (22).

On the basis of radioligand binding data, Peroutka and Schmidt (60) compiled an extensive list of potent 5-HT_3 receptor ligands (inhibition constants $K_i \leq 10$ nM). From a composite analysis of structure-affinity relationships, they determined the chemical similarities among these diverse structures and proposed a two-dimensional pharmacophore for the 5-HT_3 receptor site: a 6-atom aromatic ring separated from an embedded nitrogen by a maximum of seven atoms. Two important connectivity relationships were noted: 1) the distance from the aromatic ring center to the nitrogen, measured in sterically acceptable conformations, was 6.0 to 7.8 Å; and 2) the first two bonds originating from the aromatic ring were always coplanar with the aromatic portion of the molecule.

The two-dimensional pharmacophore was generated from the superimposition of each ligand in a single arbitrary conformation in which the nitrogen was placed in the same plane as the aromatic ring. Since most of the ligands, however, are not planar, the resulting pharmacophore does not provide insight into the three-dimensional characteristics of molecular volume and shape, both of which are conformation-dependent properties. Nevertheless, the two-dimensional pharmacophore was helpful in developing a detailed set of topological descriptors, chemical "rules" that describe 5-HT_3 antagonists. These rules were used as a qualitative tool to successfully predict the 5-HT_3 receptor binding affinity of previously untested compounds.

THREE-DIMENSIONAL PHARMACOPHORE FOR 5-HT_3 ANTAGONISTS

We have expanded Peroutka's topological model (60) to include three-dimensional concepts, generated by studying conformation-affinity relationships of potent 5-HT_3 receptor antagonists. Peroutka's work relied on arbitrary three-dimensional structures, since the conformational energy of the molecules was not considered. The model constructed from superimposition of structurally diverse ligands therefore gave a wide range for the "aromatic ring to nitrogen" distance and provided no information on overall geometric shape. Since the structure of the 5-HT_3 receptor has not yet been determined, our studies were also restricted to analyses of similarities among 5-HT_3 receptor ligands. However, we performed detailed conformational analyses to identify all low-energy structures and sort them into conformational classes. We then superimposed similar conformational classes to identify common three-dimensional shapes. We have thus identified a pharmacophore for the 5-HT_3 recognition site, i.e., a precise three-dimensional arrangement of the essential chemical functional groups, common to many different molecules recognized at the 5-HT_3 recognition site.

Molecular Modeling Dataset of 5-HT_3 Antagonists

Our studies followed the classical approach to pharmacophore identification, described by Marshall (42) as the "active analog approach," also known as the "common template hypothesis" or the "common conformation hypothesis." This approach consists of superimposition of key features in the low-energy three-dimensional structures of diverse ligands. These low-energy structures are statistically populated to a large extent under physiological conditions. Of the many ligands that bind with high affinity to the 5-HT_3 recognition site (Figs. 1 and 2), we chose to study a structurally unique subset of five ligands: MDL 72222, ICS 205-930, LY 278584, BRL 43694 (granisetron), and zacopride. We assumed a common mode of binding for all five ligands for comparative purposes.

Glennon has previously identified, within the context of serotonergic receptor subtypes (22), several structure-affinity relationships for 5-HT_3 receptor ligands. In addition, we made the following general observations on both the standard ligands and the new 5-HT_3 antagonist structures (Fig. 2) (5–7, 10, 21, 37, 62, 68). The substitution pattern on the azabicyclo[3.2.1]octane and azabicyclo[3.3.1]nonane ring systems influences 5-HT_3 binding affinity: that is, the geometric isomer displaying 5-HT_3 antagonistic activity always has the alpha or endo substitution. Ligands that have beta substitution, such as cocaine, have consistently been reported to bind with extremely low affinity. The substitution pattern of the quinuclidyl ring also contributes to potency, with (S)-zacopride binding at least eight times tighter than (R)-zacopride (53). When the heteroaromatic ring system is indole, a carboxylic acid ester is present, e.g., ICS 205-930,

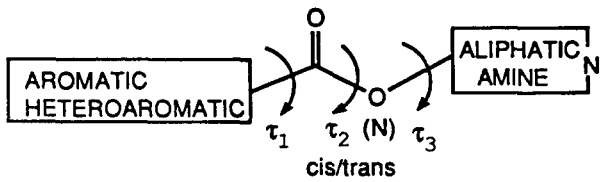

FIG. 3. Schematic illustrating the degrees of freedom identified for a systematic conformational analysis of 5-HT$_3$ antagonists: τ_1, [C(N)]=C−C−[O(N)]; τ_2, O=C−O−C or O=C−N−H; τ_3, C−[O(N)]−C−H.

whereas an amide is found in compounds containing an indazole ring, e.g., LY 278584. Simple benzamide-type structures contain an ortho-alkoxy group, e.g., zacopride, metoclopramide. Our set of five ligands was representative in that it contained ligands of unique structure and geometry, yet the ligands had common features and/or functional groups.

Computational Methods

The three-dimensional structure of MDL 72222 was obtained from X-ray crystallographic coordinates, extracted from the Cambridge Structural Database (CSD). The remaining molecules were constructed with standard bond lengths and angles, by using the SYBYL Molecular Modeling Software (V 5.33, Tripos), from fragments extracted from the CSD. For BRL 43694 (granisetron), the most energetically stable chair-chair conformation was used for the complex azabicyclo ring system (5,9). For zacopride, only the (S)-enantiomer was built, due to its enhanced affinity over the (R)-isomer (53,64).

Using the SYBYL software, systematic conformational searching was done over a set of rotatable bonds using a 5-degree stepwise increment for the dihedral angles over the range 0–359 degrees. Only those conformations that were 9999.9 kcal above the minimum-energy conformation were eliminated during the searching. This ensures that the conformational space is adequately sampled. The electrostatic term was omitted from the energy calculation, as recommended when using the Tripos 5.2 general force field (12). Along with the total energy of each conformation, two interatomic distances were measured.

Conformational Analysis Results

The flexibility inherent in each of the following five ligands was assessed from its respective energy surface, obtained by mapping the conformational energy as a function of bond rotation: MDL 72222, ICS 205-930, LY 278584, BRL 43694 (granisetron) and (S)-zacopride. Each ligand is comprised of four substructural fragments, linked via rotatable bonds, giving a total of three degrees of freedom (Fig. 3). The amide or ester linkages, however, were removed as a degree of freedom, based on their known conformational preferences (67): τ_2(O=C−O−C) of 0 degrees is preferred over 180 degrees; τ_2(O=C−N−H) of 180 degrees is preferred over 0 degrees. Interestingly, the trans ester does not seem to be the biologically important conformation, since a conformationally restricted analog of ICS 205-930, trans ester (τ_2 = 180 degrees) containing a spirofused tropanyl group, has failed to show 5-HT$_3$ serotonergic activity either in vitro or in vivo (41). A conformational analysis was therefore done for the two bonds labeled τ_1 and τ_3, with the ester or amide group locked into the cis or trans geometry, respectively.

The energy surfaces of the five ligands were very similar. A typical energy contour map is shown in Fig. 4 for LY 278584. Interestingly, the primary degree of freedom found for each ligand containing an azabicyclo ring system was τ_3, the bond from the carboxylic acid ester or amide to the aliphatic amine, containing what Peroutka had termed the "embedded nitrogen" (60). Rotation of this bond dictates the overall shape of the ligands and resulted in the low-energy conformations being clustered into two equienergetic families, corresponding approximately to a $+\Theta$ and a $-\Theta$ torsional angle value for τ_3. For example, ICS 205-930 had two energetically equivalent conformations, one at +45 degrees and the other at −45 degrees (τ_3), a consequence of the mirror-image symmetry of the aliphatic amine. (S)-Zacopride was an exception to the mirror-image symmetry, due to the presence of the chiral quinuclidine ring. Table 1 shows the torsional angles along with the corresponding relative energy for the local minima in each ligand.

TABLE 1

LOW ENERGY CONFORMATIONS OF 5-HT$_3$ ANTAGONISTS

Ligand	Relative Energy Above Minimum (kcal)	Dihedral Angle Θ for τ_3 (degrees)
MDL 72222	0	−45
	0.55	+45
ICS 205-930	0	−45
	0.27	+45
BRL 43694	0	+75
	0.97	−80
LY 278584	0	+60
	1.26	−60
(S)-Zacopride	0	+65
	0.15	−35

The carbonyl group was consistently in the plane of the aromatic/heteroaromatic ring, with the τ_1 = 0 degree conformation being more stable than the alternate τ_1 = 180 degree conformation by at least 9 kcal. Physical data support the former conformation and suggest that the stabilization might be due to the presence of a hydrogen bond between the amide proton and the ortho-alkoxy group in substituted benzamide structures, such as zacopride. Thus the crystallographic structure of metoclopramide, a flexible nonspecific ligand, contains an amide group coplanar with the aromatic ring and has a distance of 1.97 Å or 2.09 Å for NH . . . OCH_3 (two different rotamers, both obtained from crystal data), which is consistent with hydrogen bond formation. An intramolecular hydrogen bond between an amide hydrogen and a carbonyl group in the benzimidazolone DAU 6215 (Fig. 1) has also been confirmed by single-crystal X-ray diffraction analysis and infrared spectroscopic studies (62). In addition, recently disclosed benzotriazinones (Fig. 2), which are locked into the hydrogen-bonded "virtual ring" via a fused planar heterocyclic system, have been shown to be potent 5-HT$_3$ antagonists (37). The diminished activity of a 2-methyl indazole ligand has been rationalized by the folded conformation found in the X-ray structure, which shows a 120-degree out-of-plane rotation of the carbonyl group (18).

The superimposition of the minima from one of the two conformational classes defined by the above five ligands resulted in the identification of the three-dimensional pharmacophore (Fig. 5), that is, the interrelationship of the three functional groups postulated to be important for interaction with the recognition site: the aromatic ring, the carbonyl group, and the nitrogen

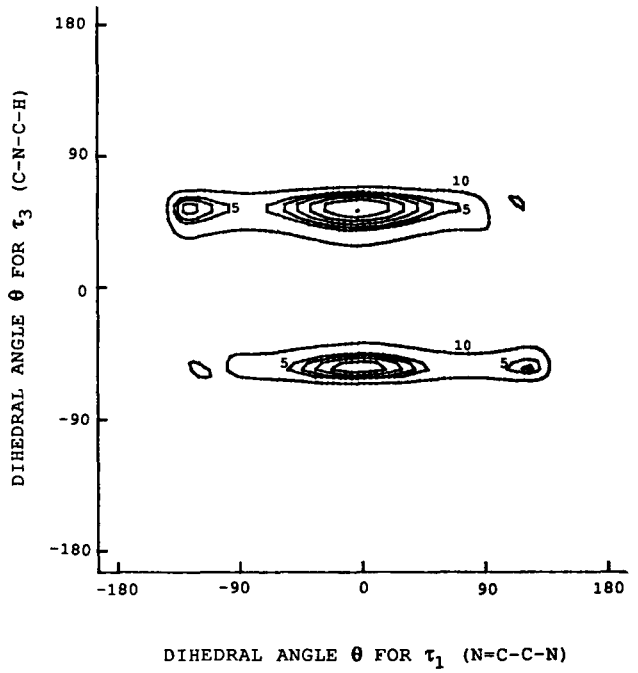

FIG. 4. Energy contour map of LY 278584 (dihedral angle in degrees): minimum-energy conformation is located at the dot; contours shown are at 1-kcal increments from the minimum up to the 5-kcal contour; outermost contour is 10 kcal from the minimum.

center. The structural elements used for superimposition were: the centroid of the five-membered ring of a fused aromatic/heteroaromatic ring system or the centroid of the single benzamide six-membered ring; the carbonyl oxygen atom (and/or the C=O vector, due to its planarity); and the nitrogen atom. The three-site pharmacophoric binding model contains heteroatom functionalities which would most likely be involved in electrostatic interactions with the receptor. Noteworthy is the fact that the dimethyl quaternary ammonium derivatives of ICS 205-930, LY 277359 (LY 191617), and metoclopramide bind to the 5-HT_3 receptor site with an affinity similar to their respective tertiary amines.

The geometric relationship among the recognition elements, i.e., the three functional groups comprising the pharmacophore of the ligands which bind to the 5-HT_3 recognition site, is given in Fig. 6. The average distance was obtained from the five superimposed molecules (Fig. 5), and the distance ranges were determined from the distance maps. The aromatic ring centroid to carbonyl oxygen atom distance remained fairly constant within each molecule, due to the restricted motion of τ_1. The remaining two distances, i.e., 1) the centroid of the aromatic ring to the aliphatic nitrogen and 2) the carbonyl oxygen to the aliphatic nitrogen, were analyzed as a function of both energy and bond rotation. Representative distance maps for these values are shown in Figs. 7 and 8 for ICS 205-930. The entire range in all conformations for the first distance (Fig. 7) is narrow, approximately 6.4–6.9 Å. However, the distance range in conformations within 5 kcal from the minimum-energy conformation is much tighter, 6.76–6.91 Å. The second distance (Fig. 8) shows the same trend. The entire distance range, 3.64–5.60 Å, is wider than above, but in conformations within 5 kcal from the minimum-energy conformation, the distances cluster in a narrow band at the higher end of the range, 5.14–5.60 Å.

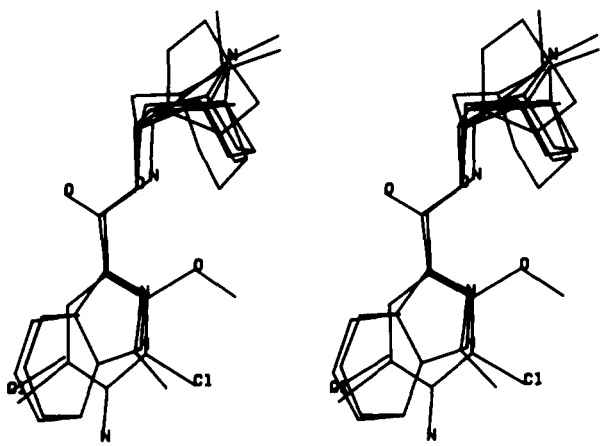

FIG. 5. Superimposition of MDL 72222, ICS 205-930, LY 278584, BRL 43694, (S)-zacopride in one of the low-energy conformational classes ($-\Theta$ rotation angle) (hydrogens removed, relaxed stereo view).

The geometric relationship among the recognition elements comprising the pharmacophore is similar in each of the two conformational classes. However, superimposing the two minima from one ligand, for example ICS 205-930 (Fig. 9), indicates that the two classes differ in overall three-dimensional character by the position of the terminal nitrogen. Overlapping the aromatic and carbonyl groups shows that the height of the nitrogen is either 2 Å above or below the plane containing these functional groups. Each resultant three-dimensional shape is distinct, hence one may be preferred by the 5-HT_3 receptor, which is presumably chiral in nature. In the ligands studied, both "binding shapes" are possible, since they arise from low-energy conformations which are related by rotation of a single bond (τ_3). In the future, rigid and/or chiral ligands, which can adopt one shape only, would help to identify the optimal three-site pharmacophoric arrangement adopted by ligands that bind to the 5-HT_3 receptor/recognition site.

Our results are consistent with molecular modeling studies of 5-HT_3 ligands which have appeared in the literature. Hibert and coworkers (28) have described a basic three-dimensional pharmacophore for 5-HT_3 antagonists which consists of an aromatic ring, a coplanar carbonyl group, and a basic center, interrelated by well-defined distances. This pharmacophore was obtained through a fitting procedure in which a molecular mechanics technique forces the chosen reference features to overlap at the

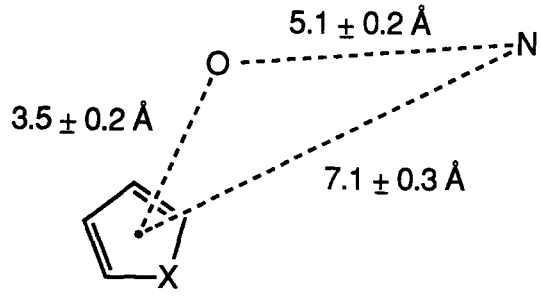

FIG. 6. Composite of interatomic distances in the 5-HT_3 receptor antagonist pharmacophore.

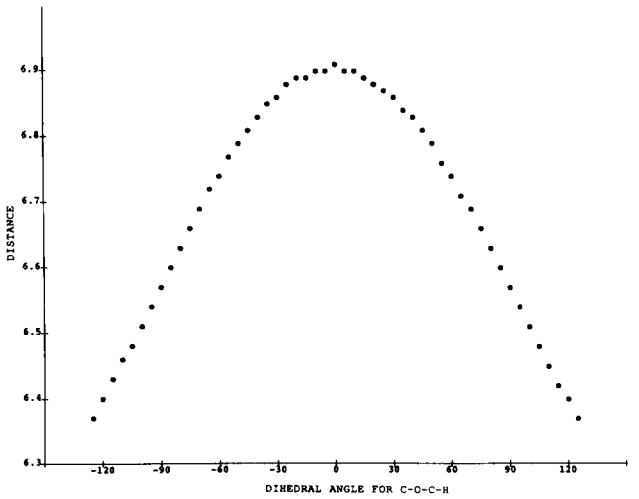

FIG. 7. Distance (Å) from the aromatic ring centroid to the nitrogen as a function of dihedral angle (degrees) for τ_3 in ICS 205-930: range for conformations within 5 kcal from the minimum is 6.76–6.91 Å.

expense of some conformational energy. Only a single superimposition of ligands was obtained, corresponding to one of our two conformational classes ("binding shapes"). The structural features that were chosen for superimposition were a 2-Å vector normal to the plane of the aromatic ring and centered on the aromatic ring centroid, the carbonyl group vector, and a 1-Å vector corresponding to the lone pair of electrons on the nitrogen center. The pharmacophore identified for 5-HT$_3$ antagonists by this technique has distances of 3.3 Å between the aromatic ring centroid and carbonyl oxygen, 5.2 Å between the oxygen and the nitrogen atom, and 6.7 Å between the nitrogen atom and the aromatic ring centroid. These distances are very close to the three corresponding distances of 3.5 Å, 5.1 Å, and 7.1 Å which we have obtained through conformational analysis (Fig. 6).

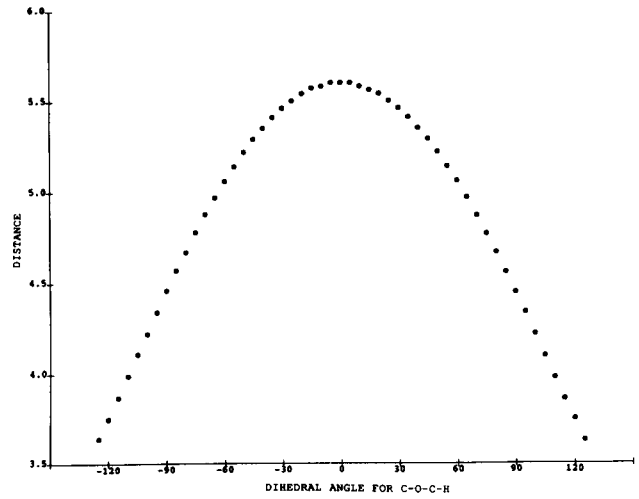

FIG. 8. Distance (Å) from the carbonyl group oxygen to the nitrogen as a function of dihedral angle (degrees) for τ_3 in ICS 205-930: range for conformations within 5 kcal from the minimum is 5.14–5.60 Å.

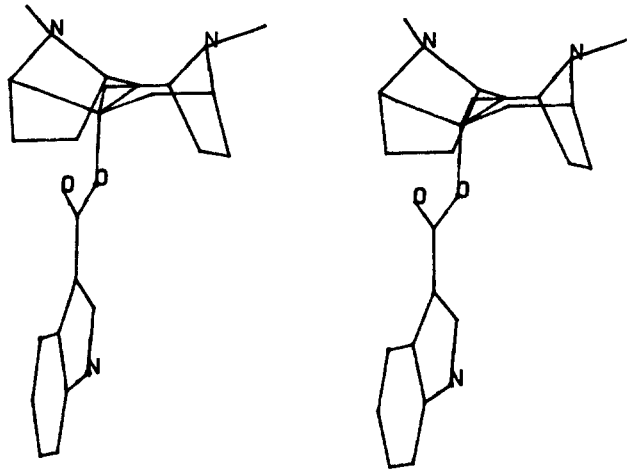

FIG. 9. The two equienergetic minima of ICS 205-930, viewed along the aromatic plane, with the aromatic carboxylic acid groups superimposed (hydrogens removed, relaxed stereo view).

Indeed, the preferred conformation of benzotriazinones has been shown to be in agreement with this three-dimensional pharmacophore (37). However, since Hibert and co-workers did not analyze the energy surface of the ligands, they did not detect the alternate conformational class.

ELECTROSTATIC MODEL FOR 5-HT$_3$ ANTAGONISTS

Rizzi and co-workers (55) have focused on electrostatic interactions necessary for binding to 5-HT$_3$ sites, using four ligands: ICS 205-930, zacopride, ondansetron, and a novel thiazole (Fig. 2) (56,57). Molecular nonbonded energy surfaces were generated for these ligands in the minimum-energy conformation by using probe atoms to represent the electrostatic nature of the receptor. The energy at each grid point on the Van der Waals surface was computed using a Lennard-Jones potential, an electrostatic potential, and a hydrogen-bonding potential. By inspecting favorable areas of interaction with the electrostatic probes, they identified a hydrogen bond-accepting (carbonyl) and a hydrogen bond-donating (nitrogen) region in each of the four ligands. The interaction of the carbonyl group with a donor in the receptor was divided into two areas, one for each of the two lone pairs of electrons associated with the carbonyl group. Surprisingly, only one area was common to all ligands. By superimposing the two common regions above, they arrived at a three-component pharmacophore: two electrostatic interactions (via carbonyl lone pair and protonated nitrogen), separated by approximately 7.7 Å, and a structural component, an aromatic region.

The electrostatic regions superimpose well, whereas the aromatic region is spread over a wide region of space. This study did not employ an atom-by-atom overlap, yet it proved to be a good strategy for comparing structurally diverse ligands. Again, only superimposition of the lowest-energy conformation, i.e., the global minimum-energy conformation, was done. The thiazole seems to function as a carbonyl isostere since it is unprotonated at physiological pH and, therefore, is expected to act as a weak proton acceptor, like the carbonyl group of carboxylic acid derivatives (46,56). Interestingly, the region occupied by the aromatic ring systems is very wide, in accord with the concept of an antagonist's ability to block approach of an agonist to the receptor. By contrast, our pharmacophore contains a narrow, fixed, superimposed aromatic region, resulting in a smaller distance

(5.1 Å) between the two postulated electrostatic components. Antagonists do not necessarily superimpose on each other in an exact way, and therefore Rizzi's model represents an alternate possibility for the mode of binding to 5-HT_3 sites. This pharmacophore might explain the structure-affinity relationships found for substituents of the different aromatic ring systems, although these are not mentioned.

CONCLUSIONS

The molecular modeling studies of 5-HT_3 receptor antagonists done by several workers, who have assumed a single mode of binding but have used various computational approaches, have led to insights into the pharmacophore for the 5-HT_3 recognition site. The studies have independently suggested the necessary chemical template required for binding, as inferred from the chemical similarities among antagonists of diverse structural classes. The three functional groups comprising this template are: an aromatic/heteroaromatic ring system, a coplanar carbonyl group (or chemical equivalent), and a nitrogen center (either tertiary or quaternary).

The present investigation has used conformation-activity relationships of a series of 5-HT_3 antagonists as a basis for defining the geometry of the pharmacophore. The common molecular features which are important for effective binding to the 5-HT_3 recognition site and which, therefore, comprise the pharmacophore are the same as those identified by Hibert and coworkers. The defined distance relationships between the functional groups in our study are also similar to those in the Hibert model: our pharmacophore has distances of 3.5 Å (compared to 3.3 Å) between the aromatic ring centroid and the carbonyl oxygen, 5.1 Å (compared to 5.2 Å) between the oxygen and the nitrogen atom, and 7.1 Å (compared to 6.7 Å) between the nitrogen atom and the aromatic ring centroid. Whether these functional groups comprise the essential set, or the minimal set, of functional groups for optimal interaction with the receptor must still be determined. Identification of the pharmacophore in ligands such as quipazine may clarify this later point, since quipazine lacks the requisite carbonyl oxygen features in the current pharmacophore, and yet binds with a K_i of ~1 nM. Quipazine, however, contains a quinoline nitrogen, which may mimic the electronic properties of a carbonyl group. Indeed, Hibert suggested a fit of quipazine to this pharmacophore by using the electron lone pair on the quinoline nitrogen as a substitute for the carbonyl oxygen, implying that the quinoline nitrogen may be acting as a bioisostere for the carbonyl group. Similarly, Rizzi and coworkers suggested that the nitrogen in a thiazole-containing series of antagonists may function as a bioisostere for the carbonyl oxygen center.

Hibert's model considered the global minimum (lowest-energy) structure of each ligand to be the biologically relevant species. The resultant composite for the binding conformation, therefore, closely resembled the lowest-energy structure for each ligand. In contrast, our study identified all of the low-energy structures for each ligand through a detailed conformational analysis. By considering superimposition and chemical similarity for more than one conformation, we have expanded the previous concept of the pharmacophore. We have identified two three-dimensional "binding shapes" or "active shapes" which can be adopted by each ligand in the set studied. Both may be described as "half-T" in overall shape, yet they differ in spatial orientation since they are related by rotation about a single bond, the bond to the group containing the nitrogen center (Fig. 10, shape corresponding to the $-\Theta$ conformational class). We have

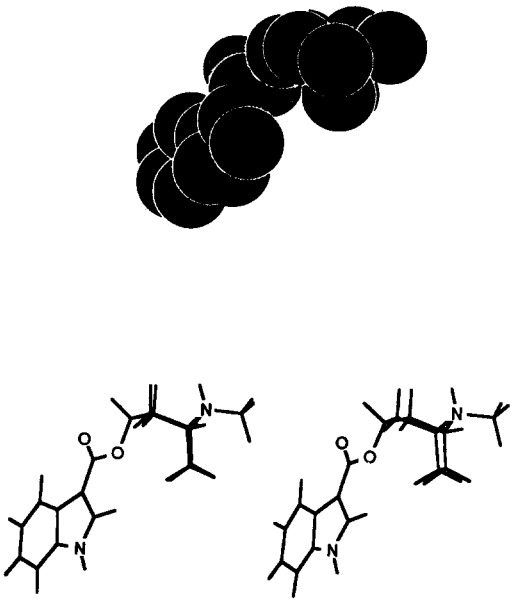

FIG. 10. Space-filling representation (hydrogens suppressed) of the pharmacophore for 5-HT_3 antagonists, along with stick representation (ICS 205-930 structure, $-\Theta$ conformational class).

thus been able to identify two different arrangements of the molecular features which are presented to the 5-HT_3 receptor, which implies one of two possible modes of binding and a potential "handedness" in the receptor.

In either three-dimensional shape, two electrostatic interactions with the receptor are possible, based on the two key heteroatom-containing functional groups of the pharmacophore. These agree with the hydrogen bond-accepting (electron-donating) and hydrogen bond-donating sites described by Rizzi and co-workers. The geometric relationship between these two sites differs from the Rizzi model, however, due to the different method used for superimposition of the ligands.

In the future, electrostatic characterization of the pharmacophore may need to be refined, not only for the electron-donating site but also for the nitrogen center, since the quaternary ammonium ligands, which lack a hydrogen bond donor but contain a positively charged nitrogen center, bind as well as the tertiary compounds. The minimal steric requirements for binding need to be determined, along with further exploration of alternate modes of binding. Both the electrostatic and steric components of the pharmacophore need to be combined into a unified hypothesis. Physicochemical properties which contribute to selectivity need to be bracketed, such as the pK_a of the nitrogen and total lipophilicity.

Thus, although several observations regarding the chemical template and "ligand occupied volume" of 5-HT_3 antagonists have been made, the unique pharmacophore presented to the 5-HT_3 receptor has not yet been identified. There exists a need to find novel, more selective 5-HT_3 serotonergic agents, e.g., the recent chiral ligand YM 060 (Fig. 2), whose R(−)-isomer is over two orders of magnitude more potent than its S(+)-enantiomer (48). Future molecular modeling efforts using these new ligands should illuminate the complementary 5-HT_3 receptor/recognition site surface, its essential volume, and its chirality.

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