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ACCELERATED COMMUNICATION

Three-Dimensional Steric Molecular Modeling of the 5-Hydroxytryptamine$_3$ Receptor Pharmacophore

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SUMMARY

A computer-based three-dimensional steric molecular model of the 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor pharmacophore was defined on the basis of radioligand binding data. Analysis of published data led to the identification of 19 different chemical structures that share only a single known pharmacological property, i.e., less than 10 nM affinity for the 5-HT$_3$ receptor. These 19 compounds were then categorized into seven chemical families, which derive from six main steric "core" structures. From the composite analysis of all 19 potent agents, nine steric chemical criteria were derived, which can be used to describe the 5-HT$_3$ receptor pharmacophore. This information was then used to explain the 5-HT$_3$ receptor inactivity of atropine, a compound that differs structurally from ICS 205-930 in the steric properties of only a single key atom. The steric chemical information was also used to predict the activity of serotonergic compounds that had never been analyzed at 5-HT$_3$ receptor binding sites. Two serotonergic drugs that meet all nine steric criteria were found to be active at the 5-HT$_3$ receptor binding site (i.e., pizotifen, K_i = 42 ± 10 nM, and clozapine, K_i = 52 ± 8 nM). By contrast, two serotonergic agents that do not meet the criteria were found to be inactive at the 5-HT$_3$ receptor binding site (i.e., ipsapirone and pirenperone, K_i values > 1000 nM). This computer-based steric molecular modeling approach allows for the analysis and identification of 5-HT$_3$ receptor-active agents with minimal dependence upon animals and radioactive compounds.

5-HT$_3$ receptors have been well characterized in the periphery, where they mediate the excitatory effects of 5-HT. Selective 5-HT$_3$ antagonists such as ICS 205-930 and MDL 72222 block these peripheral effects of 5-HT. Recently, 5-HT$_3$ binding sites have been identified in rat brain using [^{3}H]GR 65630 (1) and a variety of other radioligands (2–4). 5-HT$_3$ agonists such as 5-HT, 2-methyl-5-HT, and phenylbiguanide display moderate affinity (K_i values of approximately 150 nM) for this site, whereas 5-HT$_3$ antagonists such as granisetron (formerly called BRL 43694), zacopride, and ICS 205-930 display subnanomolar affinity (K_i values = 0.1–0.8 nM) for 5-HT$_3$ binding sites.

The identification of 3D structure-activity relationships between drugs and receptors has been attempted by a number of investigators (5). However, most of these endeavors have used only a small number of structures to define the receptor pharmacophore. By contrast, the present study attempted to determine the 3D steric chemical similarities between a wide variety of compounds that share high affinity for this single biogenic amine receptor. The goal of the present study was to define the 3D steric pharmacophore of the 5-HT$_3$ receptor. This information was then used to provide a possible explanation for the receptor inactivity of structural analogues and to identify previously unrecognized 5-HT$_3$ receptor-active agents.

Materials and Methods

Computer modeling techniques. 3D drug models were made on an IBM-PS/2 computer (Model 50Z) using the CAMSEQ/M Molecular

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ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 3D, three-dimensional; 2D, two-dimensional; DAU 6215, (3-α-tropanyl)1*H*-benzimidazolone-3-carboxamide; DAU 6287, (3-α-homotropanyl)-1*H*-benzimidazolone-3-carboxamide; GR 65630, (3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone; granisetron, (endo-*N*-(methyl-9-azabicyclo-[3.3.1]-non-3-yl)-1-methyl-indazol-3-carboxamide; ICS 205-930, (3-α-tropanyl)-1*H*-indole-3-carboxylic acid ester; LY 211000, (8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxylic acid ester; LY 258458, *N*-(8-methyl-8-azabicyclo[3.2.1.]oct-3-yl)-1*H*-indazole-3-carboxamide; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LY 278584, 1-methyl-*N*-(8-methyl-8-azabicyclo[3.2.1.]oct-3-yl)-1*H*-indazole-3-carboxamide; MDL 72222, 1-α*H*-3-α-5-α*H*-tropan-3-yl-3,5-dichlorobenzoate; *n*-methylquipazine, *N*-methyl-2-(1-piperazinyl)quinoline; *N*-propylquipazine, *N*-propyl-2-(1-piperazinyl)quinoline; ondansetron, (1,2,3,9-tetrahydro-9-methyl-3[(2-methyl-1*H*-imidazol-1-yl)methyl]-4-one; QICS 205-930, quaternized-(3-α-tropanyl)-1*H*-indole-3-carboxylic acid ester; quipazine, 2-(1-piperazinyl)quinoline; renzapride, [(+)-endo]-4-amino-5-chloro-2-methoxy-*N*-(1-azabicyclo[3.3.1.]non-yl)benzamide; SDZ 206-792, (*N*-desmethyl-3-α-homotropanyl)-1*H*-indole-3-carboxylic acid ester; SDZ 206-830, (3-α-homotropanyl)-1-methyl-5-fluoro-indole-3-carboxylic acid ester; SDZ 210-204, (−)-(1*R*,2*R*,4*S*)-1*H*-indole-3-carboxylic acid-7-methyl-7-azabicyclo-[2.2.1]hept-2-yl-ester; zacopride, 4-amino-*N*-(1-azabicyclo[2.2.2.]oct-3-yl)-5-chloro-2-methoxybenzamide.

Modeling System (Weintraub Software Design Associates, Cincinnati, OH). This software package creates 3D molecular structures, measures the distance between atoms, and overlays structures. 2D structures were made using WIMP software (Aldrich Chemical Co., Milwaukee, WI).

Radioligand binding studies of potential 5-HT$_3$ agents. Radioligand binding studies were performed as described previously (6). Briefly, rat cortices (Pel Freeze Biologicals; Rogers, AR) were homogenized in 20 volumes of 50 mM Tris·HCl buffer (pH 7.7 at 25°) and centrifuged at 49,000 × *g* for 10 min. The pellet was resuspended in fresh buffer and incubated at 37° for 10 min. After the final centrifugation (49,000 × *g* for 10 min), the pellet was resuspended in 80 volumes of assay buffer (25 mM HEPES, 180 mM NaCl, 5 mM KCl, 2.5 mM $CaCl_2$, and 1.2 mM $MgCl_2$; pH adjusted to 7.4). Tissue (10 mg of original wet weight) was added to assay tubes that contained 0.8 nM [^{3}H] quipazine and displacing drug or buffer in a final volume of 1 ml. Nonspecific binding was measured in the presence of 10^{-6} M ICS 205-930 and specific binding was defined as the total binding minus the nonspecific binding. After a 30-min incubation at room temperature, the tissue was rapidly filtered under vacuum through No. 32 glass fiber filters (Schleicher and Schuell, Keene, NH) and was rinsed twice with 5 ml of 50 mM Tris·HCl buffer (pH 7.7). The radioactivity retained on the filters was measured by scintillation counting in 2.5 ml of scintillation fluid (Biosafe II; Research Products International Corp., Mount Prospect, IL). All experiments were performed 3–6 times, each in triplicate.

Chemicals. Drugs were dissolved in 0.01% acetic acid (10^{-3} M solution) and dilutions were made in the radioligand binding assay buffer. Drug sources were as follows: [^{3}H]quipazine was the generous gift of Dr. Stephen Hurt (DuPont-New England Nuclear) (specific activity = 61.2 Ci/mmol); atropine, Sigma Chemical Co. (St. Louis, MO); clozapine, ICS 205-930, and pizotifen, Sandoz Pharmaceuticals (East Hanover, NJ); ipsapirone, Troponwerke (Cologne, Germany); and pirenperone, Janssen Pharmaceutica (Piscataway, NJ).

Results

Identification of potent 5-HT$_3$ receptor agents. A literature search was performed in order to identify drugs that interact with 5-HT$_3$ receptor binding sites. A total of 14 publications, containing 246 separate IC_{50} or K_i values, were then collated. Each of the drugs analyzed had been reported to display less than 10 nM affinity for the 5-HT$_3$ receptor binding site. The 2D structures of these 19 agents are shown in Fig. 1.

As shown in Table 1, these 19 agents can be subcategorized into seven different chemical families. The potent indole compounds include extensively studied agents such as ICS 205-930. Other chemical families that contain potent 5-HT$_3$ agents include indazoles (e.g., granisetron), benzimidazolones (e.g., DAU 6215 and DAU 6287), carbazoles (e.g., ondansetron, formerly called GR 38032F), benzoates (e.g., MDL 72222), benzamides (e.g., zacopride and renzapride, formerly called BRL 24924), and quinolines (e.g., quipazine).

Determination of the primary 5-HT$_3$ pharmacophore. The 19 potent 5-HT$_3$ agents may also be categorized on the basis of their steric "core" structures. The goal of this portion of the study was to identify similarities in the 3D steric configuration of all 19 structures. The basic assumption, based on the hypothesis of Lloyd and Andrews (16), was that the 5-HT$_3$

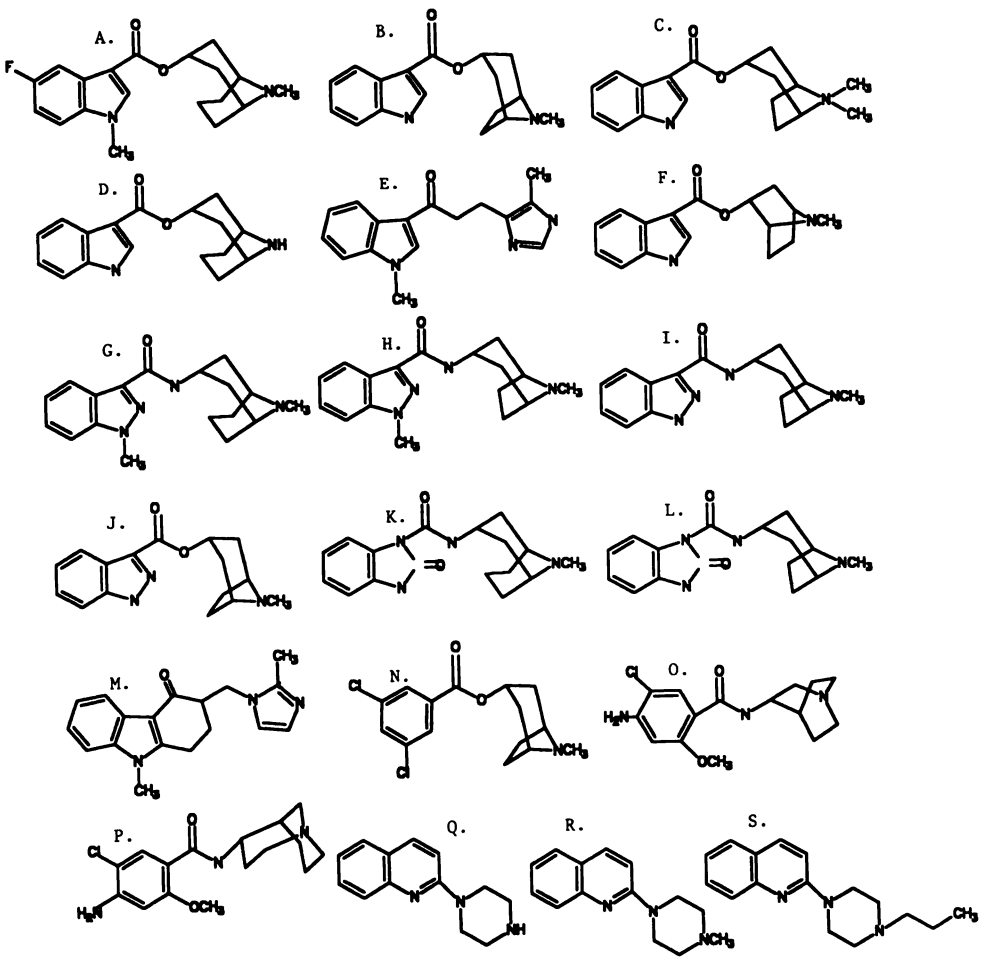


Fig. 1. 2D structures of potent 5-HT$_3$ agents. The *letters* shown over each 2D structure correspond to the compound names listed in Table 1.

TABLE 1
Reported receptor affinities of potent 5-HT$_3$ agents

Drug	Reported affinity range (K_i)	References
	nM	
Indoles		
A. SDZ 206-830	0.16–0.45	8, 9
B. ICS 205-930	0.20–12	1–4, 6–13, 15
C. QICS 205-930	0.45	4
D. SDZ 206-792	0.72–1.8	8, 9
E. GR 65630	1.4–1.6	1, 2
F. SDZ 210-204	5.0–7.8	8, 9
Indazoles		
G. Granisetron	0.30–4.1	1, 2, 6, 8, 9, 10, 12, 13, 15
H. LY 278584	0.52	6, 13
I. LY 258458	0.76	6
J. LY 211000	4.2	6
Benzimidazolones		
K. DAU 6287	1.5	15
L. DAU 6215	3.8	15
Carbazoles		
M. Odansetron	0.86–14	1, 2, 4, 8, 10, 12, 15
Benzoates		
N. MDL 72222	6.2–55	1, 3, 4, 6–9, 11–13, 15
Benzamides		
O. Zacopride	0.32–2.0	2, 6, 13, 15
P. Renzapride	3.2–8.7	8, 9
Quinolines		
Q. Quipazine	0.48–3.4	1, 3, 4, 6–9, 12, 14
R. *N*-Methylquipazine	3.1	14
S. *N*-Propylquipazine	8.7	14

pharmacophore consists of at least an aromatic ring and a nitrogen atom, which must be located at a specific distance from the ring.

3D steric models of each of the 19 compounds were made using the CAMSEQ/M Molecular Modeling System. Six distinct steric core structures were then identified (Fig. 2) and were configured to allow for maximal structural overlap. For example, ICS 205-930, QICS 205-930, SDZ 206-792, SDZ 206-830, granisetron, LY 211000, LY 258458, LY 278584, DAU 6215, and DAU 6287 are all derivatives of a single steric core structure (Fig. 2, structure **1**; Table 2). MDL 72222 and renzapride are derivatives of a second core structure (Fig. 2, structure **2**). Quipazine, *N*-methylquipazine, and *N*-propylquipazine are derivatives of a third core structure (Fig. 2, structure **3**), whereas GR 65630 and ondansetron are derivatives of a fourth core structure (Fig. 2, structure **4**). Zacopride is similar to the second core structure but differs in the linkage between the quinuclidine ring and the aromatic ring portion of the molecule (Fig. 2, structure **5**). SDZ 210-204 is similar to the first core structure in that it has an indole nucleus, but the nitrogen is embedded in a different ring structure (Fig. 2, structure **6**).

The distance from the center of the aromatic ring to the ring-embedded nitrogen, when the nitrogen is placed in the same plane as the aromatic ring, was determined using CAMSEQ/M. As shown in Table 2, this distance ranges from 6.0 (core structure **3**) to 7.8 Å (core structure **4**). The average distance from the center of the aromatic ring to the ring-embedded nitrogen is 6.9 ± 0.1 Å ($n = 19$).

Composite analysis of potent 5-HT$_3$ agents. A composite analysis of the six core structures was made, in order to allow for the determination of steric structural similarities that might be used to hypothesize a series of chemical "rules" for the 5-HT$_3$ pharmacophore. As shown in Fig. 3, the six core structures can be aligned so as to overlay the aromatic rings and to place the ring-embedded nitrogen atom in the same plane as the aromatic ring. The following steric rules, which apply to all 19 potent 5-HT$_3$ agents, were derived from this analysis.

1. The most obvious similarity between the six core structures is the fact that each contains an aromatic ring. This observation is in agreement with the hypothesis of Lloyd and Andrews (16), which states that all central nervous system-active drugs contain an aromatic ring.

2. A ring-embedded nitrogen is also present in each structure and, in the most direct pathway from the aromatic ring, is located at a distance of no more than seven atoms from the aromatic ring. The importance of this nitrogen is documented by the fact that N^4-deazaquipazine, an analog of quipazine in which this single nitrogen is replaced by a carbon atom, is inactive (i.e., $K_i > 1000$ nM) at the 5-HT$_3$ binding site (14).

3. When the nitrogen is aligned in the same plane as the aromatic ring, it can be placed at a distance of 6.0 (e.g., core structure **3**) to 7.8 Å (e.g., core structure **4**) from the center of the aromatic ring.

4. Substitutions are allowed on the nitrogen atom that is embedded in a ring structure but do not exceed a length of three atoms (as is observed with *n*-propylquipazine). Droperidol (K_i = 4,200 nM) (6), prazosin (K_i > 10,000 nM) (2), and ritanserin (K_i > 10,000 nM) (2) are examples of inactive compounds that otherwise meet the criteria for potent 5-HT$_3$ receptor-active agents.

5. The ring structure containing the nitrogen has no substitutions that exceed the size of a methyl group (e.g., ondansetron and GR 65630). Larger substitutions appear to significantly decrease affinity. For example, a carboxylic acid methyl substitution on the tropane ring of MDL 72222 (which creates cocaine) results in an approximately 3 orders of magnitude decrease in affinity (1, 2, 6, 11, 12).

6. In the most direct pathway to the ring-embedded nitrogen, steric similarities are present on the first three atoms from the ring (Table 3). For example, the first atom from the aromatic ring is never a tetrahedral carbon atom. This atom is invariably trigonal in configuration (Table 3).

7. In the most direct pathway to the ring-embedded nitrogen, the second atom from the aromatic ring is never a tetrahedral carbon but is either a trigonal carbon, a non-fully substituted nitrogen, or an oxygen molecule (Table 3). These observations suggest that the 5-HT$_3$ pharmacophore does not tolerate non-planar atoms within a distance of two bond lengths from the aromatic ring in the most direct pathway to the nitrogen.

8. In the most direct pathway to the ring-embedded nitrogen, the third atom from the ring may be a tetrahedral carbon but it is never fully saturated (Table 3).

9. Substitutions on the aromatic ring must be able to adopt a planar configuration within a distance of two atoms from the ring. Sulpiride is an example of a compound that meets each of the above nine criteria, yet its aromatic ring contains an aminosulfonyl substitution that cannot be planar. Sulpiride has been reported to be inactive (i.e., K_i > 10,000 nM) at the 5-HT$_3$ binding site (2).

3D molecular analysis of atropine, a structural analogue of ICS 205-930. An attempt was made to test the hypothetical 5-HT$_3$ pharmacophore model by analyzing a struc-

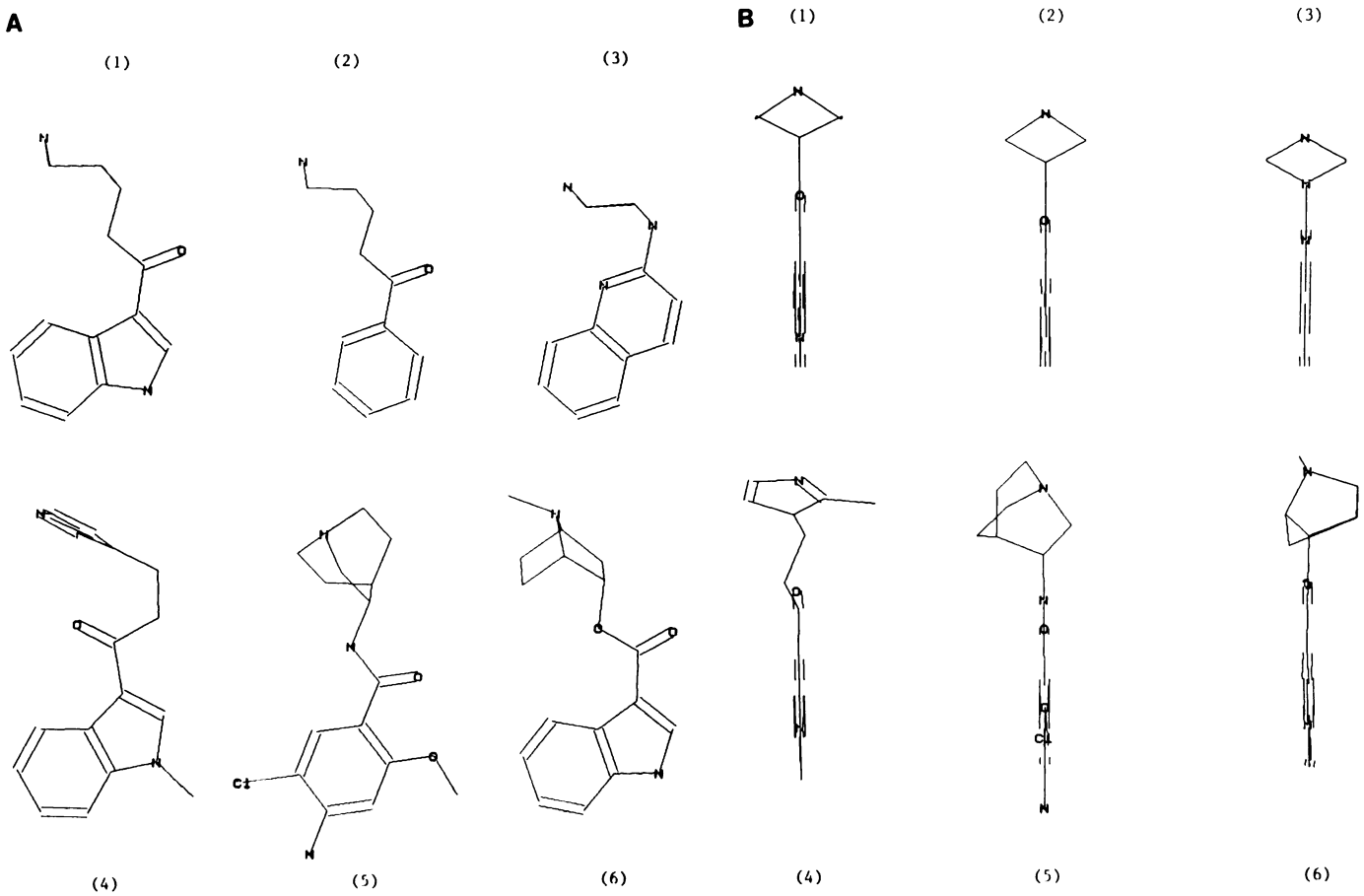


Fig. 2. 3D core structures of compounds that interact with the 5-HT_3 receptor. In A, the core structures are viewed with the aromatic ring in the plane of the figure. In B, the core structures are viewed with the aromatic ring placed perpendicular to the plane of the figure.

TABLE 2
Core structure classification for potent 5-HT_3 agents

Core structure	Drug	Distance from ring-embedded nitrogen to center of aromatic ring
		Å
Core 1	ICS 205-930	6.9
	QICS 205-930	
	SDZ 206-830	
	SDZ 206-792	
	Granisetron	
	LY 211000	
	LY 258458	
	LY 278584	
	DAU 6215	
	DAU 6287	
Core 2	MDL 72222	7.1
	Renzapride	
Core 3	Quipazine	6.0
	N-Methylquipazine	
	N-Propylquipazine	
Core 4	Ondansetron	7.8
	GR 65630	
Core 5	Zacopride	7.3
Core 6	SDZ 210-204	7.6

turally related compound that differed from a potent 5-HT_3 agent in the steric characteristics of only a single key atom. Atropine was selected for analysis because of its chemical similarity to ICS 205-930 (Table 4). The molecular weight, chemical formula, and 2D structure of atropine and ICS 205-930 are nearly identical. However, radioligand binding studies, using [^{3}H]quipazine to label 5-HT_3 binding sites, reveal that the affinity of ICS 205-930 (K_i = 0.49 ± 0.09 nM) is more than 1000 times higher than that of atropine (K_i > 1000 nM).

Based on our 3D model of the 5-HT_3 pharmacophore, this significant difference in affinity may be explained by the 3D analysis of the two compounds, as shown in Fig. 4. Atropine and ICS 205-930 are homologous in their 3D structures, except for the steric characteristics of the first atom in the most direct pathway from the aromatic ring to the ring-embedded nitrogen (Fig. 4A). ICS 205-930 contains a trigonal carbon, whereas the analogous carbon in atropine is tetrahedral. As a result, atropine cannot adopt a planar configuration within two bond lengths from the aromatic ring (Fig. 4B). This steric characteristic therefore violates criterion 6 of the 5-HT_3 pharmacophore model.

In addition, the fact that this carbon atom is tetrahedral leads to an inability to simultaneously overlap both the tropane (Fig. 4C) and aromatic ring (Fig. 4D) portions of the two molecules. As a result, the ring-embedded nitrogen in atropine is located at a distance of 5.8 Å from the center of the aromatic ring, a violation of criterion 3 of the 5-HT_3 pharmacophore model. These data support the crucial importance of the steric properties of the first atom from the aromatic ring in 5-HT_3 receptor-active agents.

Prediction of drug activity at 5-HT_3 binding sites based on the nine steric criteria that define the 5-HT_3 receptor pharmacophore. An attempt was made to predict

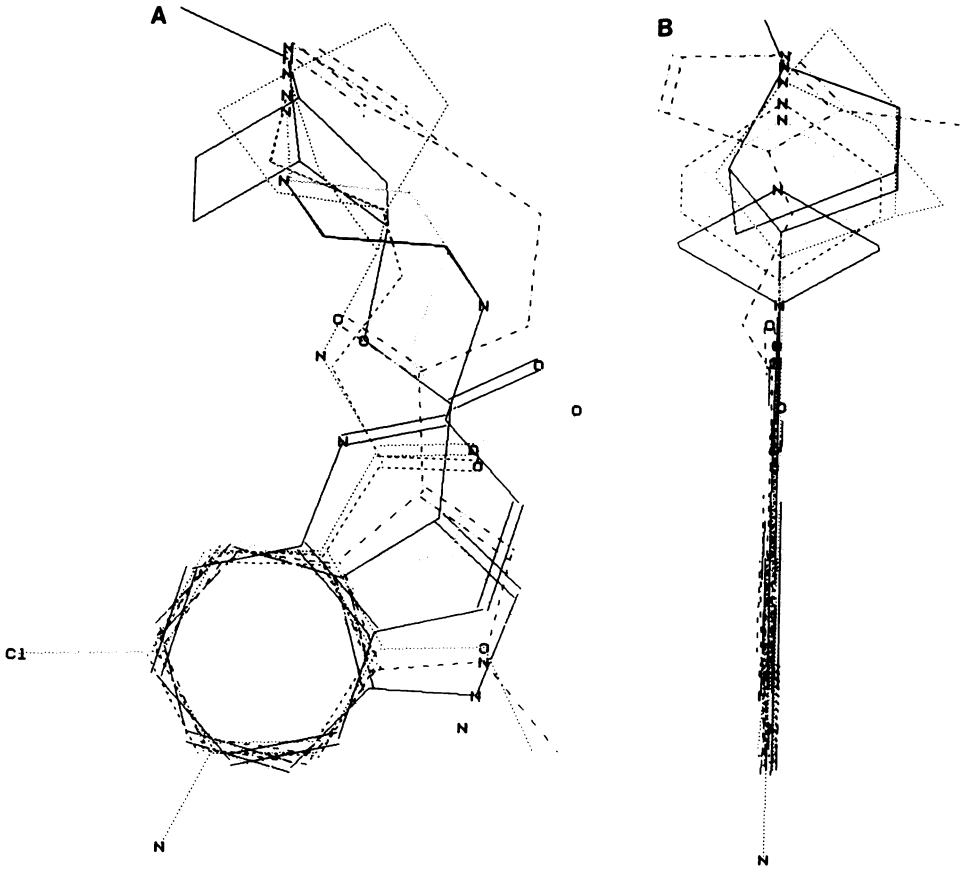


Fig. 3. Composite 3D structure analysis of the six 5-HT$_3$ receptor core structures. The 3D representation of the six 5-HT$_3$ core structures were overlaid using CAMSEQ/M. The composite structure is viewed with the aromatic ring in the plane of the figure (A) and with the aromatic ring placed perpendicular to the plane of the figure (B).

TABLE 3

Steric analysis of atoms in the most direct pathway to the ring-embedded nitrogen

	No. of compounds containing group		
	Atom 1	Atom 2	Atom 3
Saturated tetrahedral carbon	0	0	0
Nonsaturated tetrahedral carbon	0	0	5
Tetrahedral nitrogen	0	2	8
Oxygen	0	1	6
Trigonal carbon	14	16	0
Trigonal nitrogen	5	0	0

the activity of compounds that have not previously been reported to interact with 5-HT$_3$ receptors. Four serotonergic compounds were selected for analysis, based on the fact that each contains an aromatic ring and a nitrogen-embedded ring system. Clozapine and pizotifen meet all nine of the steric criteria for the hypothetical 5-HT$_3$ receptor pharmacophore, whereas ipsapirone and pirenperone do not. Therefore, clozapine and pizotifen were predicted to display activity at the 5-HT$_3$ receptor binding site, whereas ipsapirone and pirenperone were predicted to be inactive.

Radioligand studies were performed using [^{3}H]quipazine to label 5-HT$_3$ binding sites. Pizotifen and clozapine display moderate affinity for the 5-HT$_3$ binding site, with K_i values of 42 ± 10 and 52 ± 8 nM, respectively (Table 5). In marked contrast, ipsapirone and pirenperone are inactive at 5-HT$_3$ sites at concentrations below 1000 nM (Table 5).

Discussion

The major finding of the present study is that computer-based 3D steric chemical modeling can be used to identify novel classes of receptor-active agents. Analysis of published data led to the identification of 19 different chemical structures that share only a single known pharmacological property, i.e., nanomolar affinity for the 5-HT$_3$ receptor. These 19 compounds were then subcategorized into seven chemical families, which are members of six main core structures. The rules generated from the composite analysis of all 19 active agents were then applied to a close structural analogue of ICS 205-930 (i.e., atropine), which differs in the steric properties of a single key atom. Atropine failed to meet two of the nine hypothetical criteria and was also found to be inactive at the 5-HT$_3$ receptor binding site. By contrast, two previously unanalyzed serotonergic compounds were identified that meet all nine of the hypothetical criteria and were found to display significant activity at the 5-HT$_3$ binding site.

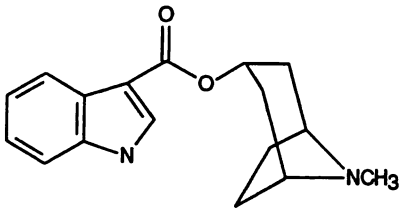
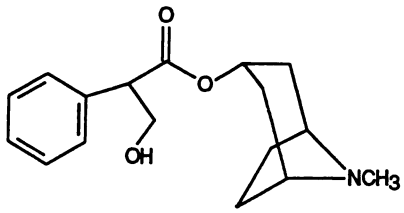
The results of this study are in agreement with the hypothesis of Lloyd and Andrews (16), which states the following.

1. There is a common structural basis for the activity of many different central nervous system-active drug classes.
2. An aromatic ring and a single nitrogen moiety are the primary binding groups whose topographical arrangement is fundamental to the activity of these drug classes.
3. It is the nature and placement of secondary binding groups that determine different classes of central nervous system drug activity.

The molecular modeling approach described in the present

TABLE 4

Comparison of the molecular characteristics of ICS 205-930 and atropine

	ICS 205-930	Atropine
Molecular weight	286	289
Chemical formula	$C_{17}H_{22}N_2O_2$	$C_{17}H_{23}NO_3$
2D structure		
5-HT_3 K_i value (nM)	0.49 ± 0.09	>1,000

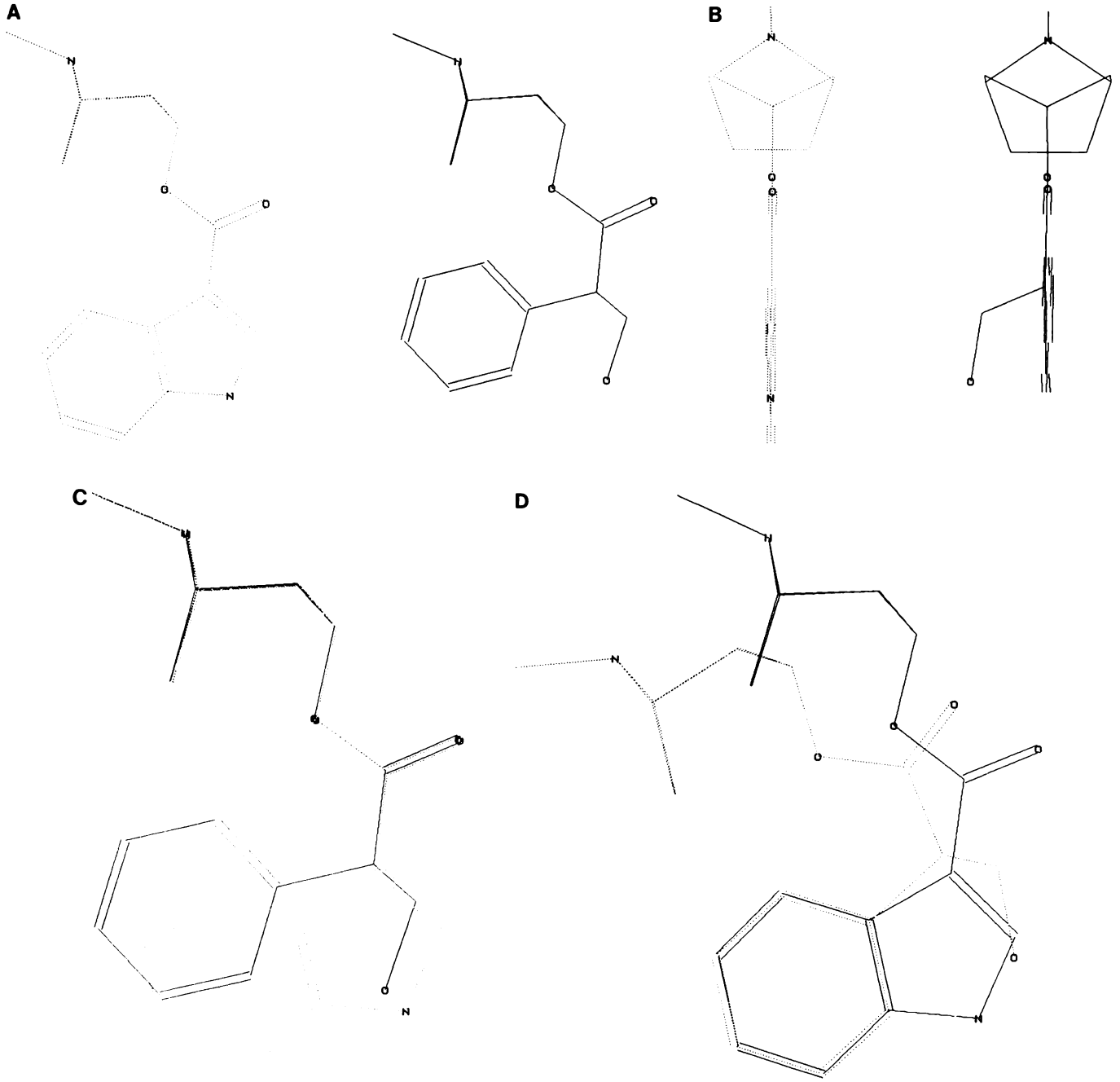
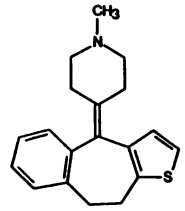
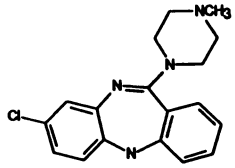
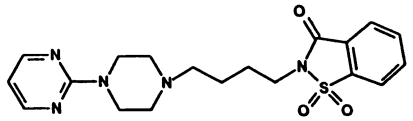
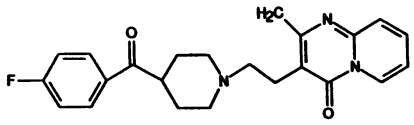


Fig. 4. Comparison of 3D molecular structures of atropine and ICS 205-930. The 3D representation of ICS 205-930 (*left*) and atropine (*right*) is shown in A with the aromatic ring in the plane of the figure and in B with the aromatic ring placed perpendicular to the plane of the figure. The tropane portion of the structures is overlaid in C and the aromatic rings in D.

TABLE 5

Drug interactions with [^{3}H]quipazine-labeled 5-HT$_3$ binding sites

Data shown are the 2D structures of the compounds and their K_i values for binding to the 5-HT$_3$ binding site labeled by [^{3}H]quipazine.

Drug	2D structure	K_i
		nM
Pizotifen		42 ± 10
Clozapine		52 ± 8
Ipsapirone		>1000
Pirenperone		>1000

study differed from previous studies of receptor pharmacophore analysis in two major ways. Firstly, the analysis in the present study was restricted to only nanomolar potent 5-HT$_3$ agents, as opposed to an analysis of "active" receptor agents regardless of potency (17). The ability to analyze 19 different structures that share only a single pharmacological property allowed for the generation of the nine chemical rules that were met by all 19 agents. These nine criteria, however, should be considered as a first attempt to define the 5-HT$_3$ receptor pharmacophore. It is to be expected that, as more data on the affinities and potencies of various compounds become available, this model is likely to be improved and refined.

Secondly, this study differs from many previous modeling approaches in that it does not take into account the electrostatic properties of the molecule. In general, previous receptor pharmacophore studies have focused on the "minimal energy state" of the molecules. By contrast, the minimal energy state was not determined in the present study. Although the energetic properties of the drugs are likely to be important factors in determining agonist versus antagonist properties, the present study suggests that steric properties alone may allow for the prediction of drug affinity for the receptor site. This fact is important, because most electronic drug databases consist solely of steric structural data. As a result, computer-based drug databases could be screened electronically for compounds that meet each of the steric rules defined in the present study.

Computer-based molecular modeling and drug screening offer a number of advantages over conventional radioligand binding and other drug-screening techniques. Firstly, a steric receptor pharmacophore model allows for the generation of chemical rules, which can be applied to electronic databases. This model can be continually tested and, as a result, refined and improved. Secondly, computer-based receptor pharmacophore modeling and screening does not require the use of animal tissues. Thirdly, the use of radioactivity is not required and, consequently, the technique carries no radiation risk to either the individuals performing the experiments or the environment. Furthermore, due to the cost of laboratory animals and radioligands, this technique offers substantial financial savings over traditional radioligand binding studies. In addition, the determination of the key steric requirements in a receptor pharmacophore may be useful to molecular biological studies of the "active site" of the receptor. Ultimately, the approach described in the present report might allow for the prediction of drug-receptor interactions, based upon the 3D steric characteristics of the chemical compound.

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