0026-895X/90/040511-06$03.00/0

MOLECULAR PHARMACOLOGY, 38:511-516

Quantitative Molecular Analysis Predicts 5-Hydroxytryptamine$_3$ Receptor Binding Affinity

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Received March 15, 1990; Accepted July 2, 1990

SUMMARY

A quantitative molecular model was derived to predict drug affinities for 5-hydroxytryptamine$_3$ (5-HT$_3$) receptors. The model was based on the molecular characteristics of a "learning set" of 40 pharmacological agents that had been analyzed previously in radioligand binding studies. Molecules were analyzed for various structural features, i.e., the presence of a benzenoid ring and nitrogen atom, substitutions on the benzenoid ring, the location of the substitutions on the nitrogen, and the molecular characteristics of the most direct pathway from the benzenoid ring to the nitrogen. Weighting factors, based on published 5-HT$_3$ receptor affinity data, were then assigned to each of 10 molecular characteristics. The derived computational model predicts accurately the affinities of the learning set for the 5-HT$_3$ receptor ($r = 0.98$; $p < 0.001$). The computational model was then used to predict the receptor affinities of a "test set" of 40 pharmacological agents. The predicted values for these agents also correlate significantly ($r = 0.83$; $p < 0.001$) with drug affinities for the 5-HT$_3$ receptor, as determined by radioligand binding assays. This first line screening approach allows for the accurate prediction of drug affinities based on molecular characteristics with minimal dependence upon animal tissues or radioactivity.

The determination of structure-activity relationships is a basic goal of pharmacological research. Traditionally, structure-activity relationship studies have been based on drug "activity," as defined in various pharmacological, biochemical, and/or physiological assays. In the 1970s, molecular pharmacological techniques such as radioligand binding studies were developed that allow for the accurate and relatively rapid screening of drugs at their putative receptor binding sites (1). The development of these techniques led to a molecular pharmacological definition of drug "activity" based on the ability of agents to interact with membrane recognition sites. Theoretically, the ability of drugs to interact with membrane recognition sites results from a combination of steric and electronic chemical features.

This work was supported in part by National Institutes of Health Grants 23560-03 and 12151-15 and the Stanley Foundation.

The goal of the present study was to determine whether the affinity of a drug for a specific neurotransmitter receptor binding site could be predicted accurately based on an analysis of the molecular properties of the compound. The potential utility of a molecular analysis, in the absence of an electronic analysis, is that it allows for the rapid screening of chemical structures stored in computerized drug databases. The 5-HT$_3$ receptor was chosen for analysis because we have recently completed an extensive steric evaluation of its drug pharmacophore (2).

Materials and Methods

Computer modeling techniques. Three-dimensional drug models were made on an IBM-PS/2 computer (model 50Z), using the CAMSEQ/M molecular modeling system (Weintraub Software Design Associates, Cincinnati, OH). This software package creates three-dimensional molecular structures, measures the distance between atoms, and overlays structures. Two-dimensional structures were made using

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 5,6-DHT, 5,6-dihydroxytryptamine; 5,7-DHT, 5,7-dihydroxytryptamine; (−)-DOB, (−)-4-bromo-2,5-dimethoxyamphetamine; GR 65630, 3-(5-methyl-1*H*-imidazol-4-yl)-1-(1-methyl-1*H*-indol-3-yl)-1-propanone; 8-OH-DPAT, 8-hydroxy-2(di-*N*-propylamino)tetralin; ICI 169,369, 2-(2-dimethylaminoethylthio)-3-phenylquinoline; 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; LY 278584, 1-methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-1*H*-indazole-3-carboxamide; LY 278989, 2-methyl-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)-2*H*-indazole-3-carboxamide; 5-MeT, 5-methyl-tryptamine; MK 212, 6-chloro-2-(1-piperazinyl)pyrazine; MDL 72222, (1-α*H*-3α-5-α*H*-tropan-3-yl-3,5-dichlorobenzoate; RU 24969, 5-methoxy-3-1(1,2,3,6-tetrahydro-4-pyridinyl)-1*H*-indole; SCH 23390, (*R*)-(+)-8-chloro-3-methyl-5-phenyl-7-ol-benzazepine; SDZ 206-792, (*N*-desmethyl-3-α-homotropanyl)-1*H*-indole-3-carboxylic acid ester; SDZ 206-830, (3α-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; WB 4101, 2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; LSD, lysergic acid diethylamide; 5-HTP, 5-hydroxytryptophan; GABA, γ-aminobutyric acid; 5-CT, 5-carboxyamidotryptamine; *m*CPP, *m*-chlorophenylpiperazine; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TFMPP, Triflouromethylpiperazine.

WIMP software (Aldrich Chemical Co., Milwaukee, WI). Forty compounds that make up the "learning set" were analyzed for the presence or absence of various features based on our previous pharmacophore analysis (2).

Determination of weighting factors. Weighting factors were derived, when possible, from drugs that lacked only a single molecular feature, in comparison with the potent 5-HT_3 compounds. Weighting factors were derived from published affinity values. For example, as described in greater detail below, four drugs (5-HT, metoclopramide, 2-methyl-5-HT, and phenylbiguanide) comply with all molecular criteria, with the exception that they lack a ring-enclosed nitrogen. A weighting factor of 400× is assigned to drugs that lack a ring-enclosed nitrogen, because 400 nM is the geometric average of the reported affinity values in the literature for 5-HT (average reported K_i value = 260 nM), metoclopramide (average reported K_i value = 260 nM), 2-methyl-5-HT (average reported K_i value = 490 nM), and phenylbiguanide (average reported K_i value = 490 nM).

Radioligand binding studies to 5-HT_3 receptor binding sites. Radioligand binding studies in rat brain were performed according to the methods in Ref. 3. 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 minutes), the pellet was resuspended in 80 volumes of Krebs-HEPES buffer (25 mM HEPES, 118 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 containing 0.8 nM [^{3}H]quipazine and displacing drug or buffer in a final volume of 1 ml. Nonspecific binding was defined using 1 μM zacopride. 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 rinsed twice with 5 ml of 50 mM Tris·HCl buffer (pH 7.7). Radioactivity was quantified by liquid scintillation counting. All experiments were performed three to six times, each in triplicate.

Chemicals. Drug sources were as follows: [^{3}H]quipazine, the generous gift of Dr. Stephen Hurt (DuPont/New England Nuclear, Boston, MA); amitriptyline, atropine, chlordiazepoxide, cyproheptadine, desipramine, 5,6-DHT, 5,7-DHT, dopamine, imipramine, 5-MeT, noradrenaline, thioridazine, WB 4101, and yohimbine, Sigma Chemical Co. (St. Louis, MO); buspirone, Bristol-Myers Co. (Evansville, IN); chlorimipramine and phentolamine, Ciba-Geigy Co. (Summit, NJ); cinanserin, E.R. Squibb & Sons (Princeton, NJ); clozapine and pizotifen, Sandoz Pharmaceuticals (East Hanover, NJ); doxepin, prazosin, and sertraline, Pfizer Central Research (Groton, CT); (−)-DOB, National Institute on Drug Abuse (Bethesda, MA); fluoxetine, LY 211000, LY 278584, LY 258458, LY 278989, and nortriptyline, Eli Lilly Co. (Indianapolis, IN); ICI 169,369, Imperial Chemical Industries PLC (Cheshire, England); idazoxan, Reckitt & Colman (Hull, England); indalpine, Rhone-Poulenc Sante (Vitry sur Seine, France); ipsapirone, Miles Pharmaceuticals (West Haven, CT); metergoline, Farmitalia Carlo Erba (Milan, Italy); MK 212, Merck Sharpe & Dohme Research Laboratory (West Point, PA); MPTP and TFMPP, Research Biochemicals Inc. (Natick, MA); paroxetine, Beecham Pharmaceuticals (Surrey, England); pirenpirone, Janssen Pharmaceutica (Beerse, Belgium); zacopride, A. H. Robins Co. (Richmond, VA); and zimelidine, Astra (Sodertalje, Sweden).

Results

Analysis of general molecular features. The 40 members of the learning set were analyzed for the presence or absence of various features, based on our previous pharmacophore analysis (2). If the reported affinities of agents in a single category varied by more than 1 order of magnitude, then an attempt was made to identify other molecular features that could be used to differentiate the molecules. For example, in the learning set, GABA displays an average reported K_i value of >10,000 nM (4, 5) and is the only agent that lacks a benzenoid ring and/or a nitrogen atom. Therefore, a weighting factor of >10,000×, a value that represents the mean affinity value reported in the literature, was assigned to drugs that lack a benzenoid ring and/or a nitrogen atom. This weighting factor is in keeping with the hypothesis of Lloyd and Andrews (6) that a benzenoid ring and a nitrogen atom are obligatory components of all potent central nervous system-active drugs.

Analysis of benzenoid ring properties. Our initial evaluation of potent 5-HT_3 agents indicated that the size of the free substitutions on the benzenoid ring is limited to 0–2 atoms (2). "Free" substitutions were defined as those atoms attached to the benzenoid ring that are not contiguous to the most direct pathway to the key nitrogen atom. Three agents in the learning set were identified that contained a substitution greater than 2 atoms but that assume a planar configuration: 5-CT, pindolol, and propranolol. A weighting factor of 10× was assigned to this molecular configuration. The assigned weighting value of 10× represents the geometric average (after the consideration of other weighting factors, discussed below) of the reported affinities of propranolol (average reported K_i value = 12,000 nM, divided by 400× due to lack of a ring-enclosed nitrogen, divided by 7 due to atom 1 being a tetrahedron = 4.3×), pindolol (average reported K_i value = 29,000 nM, divided by 400× due to lack of a ring-enclosed nitrogen, divided by 7× due to atom 1 being a tetrahedron = 10×), and 5-CT (average reported K_i value = 12,000 nM, divided by 400× due to lack of a ring-enclosed nitrogen = 30×).

In comparison with potent agents such as ICS 205-930 (Fig. 1A), a single drug in the learning set, sulpiride (Fig. 1B), suggested that relatively large nonplanar substitutions on the benzenoid ring significantly reduce 5-HT_3 receptor affinity. Based on the reported affinity of sulpiride (7), a weighting factor of 20,000× was assigned to this molecular feature. However, it should be noted that the actual weighting factor may be higher, because published values using the ">" designation were not taken into consideration in determining this weighting factor.

Analysis of nitrogen properties. One of the most obvious features identified in our initial analysis of potent 5-HT_3 agents was the fact that each potent 5-HT_3 agent contains a ring-enclosed nitrogen (2). This feature was present in 26 members of the learning set. In contrast, 14 drugs lacked a ring-enclosed nitrogen. 5-HT (Fig. 1C; average reported K_i value = 260 nM), metoclopramide (average reported K_i value = 260 nM), 2-methyl-5-HT (average reported K_i value = 490 nM), and phenylbiguanide (average reported K_i value = 490 nM) comply with all 5-HT_3 pharmacophore criteria except for the lack of a ring-enclosed nitrogen. Based on the mean K_i values of these four agents, a weighting factor of 400× was assigned to this feature. The 400× weighting factor was also applied to the other agents in the learning set that lack a ring-enclosed nitrogen.

For agents lacking a ring-enclosed nitrogen, an evaluation was made of the free substitutions on the nitrogen. Substitutions of 0–3 atoms (n = 11) do not appear to affect receptor affinity significantly. In contrast, larger substitutions are present on LSD, 5-HTP dipeptide, and methysergide. When the other weighted molecular features of these agents are taken into consideration, a weighting factor of 80× is assigned to nitrogen substitutions greater than 3 atoms.

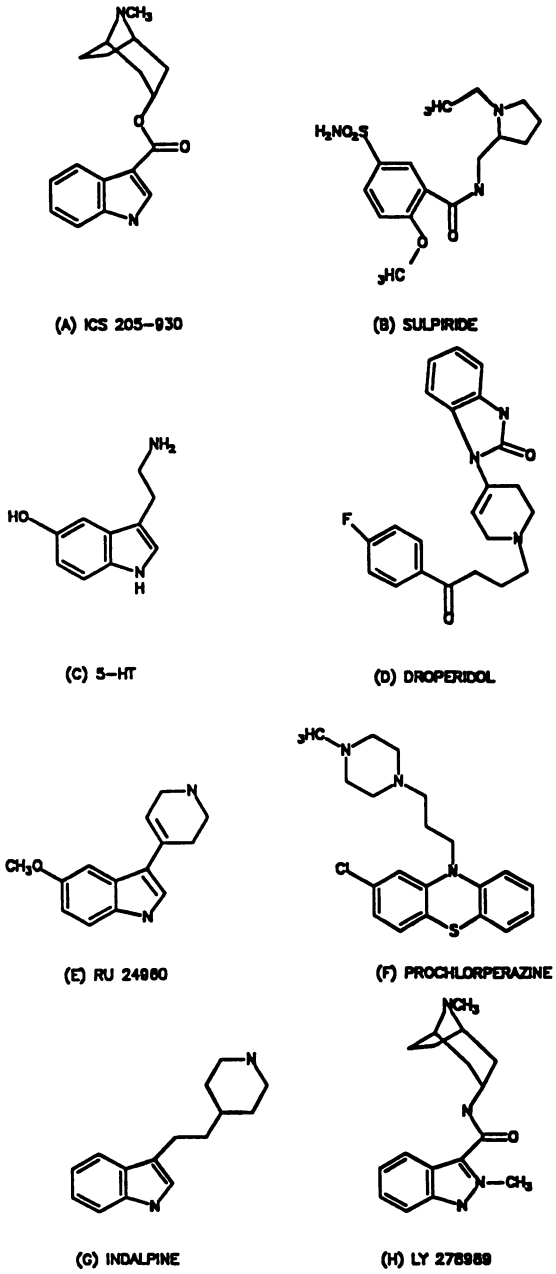

Fig. 1. Chemical structures of drugs that interact with 5-HT$_3$ receptor binding sites.

The free substitutions on the ring-enclosed nitrogen component of the molecule were also analyzed in the 26 drugs containing this feature. No affinity decrease is observed in molecules with 0–3-atom substitutions (n = 18). Agents with 4–6-atom substitutions (n = 2) show a decrease in affinity of approximately 600× (SCH 23390: reported K_i value = 17,000 nM, divided by 30× due to 0 atoms between the rings = 570×; cocaine: average reported K_i value = 3,400 nM, divided by 5× due to 6 atoms to nitrogen = 680×).

In addition, a weighting factor of 10,000× was assigned to molecules with substitutions greater than 6 atoms on the nitrogen-enclosed ring system, based on the following five agents: droperidol (Fig. 1D; average reported K_i value = 4,200 nM), domperidone (average reported K_i value = 6,200 nM), ritanserin (average reported K_i value = 7,300 nM), spiperone (average reported K_i value = 39,000 nM), and ketanserin (average reported K_i value = 55,000 nM). However, it should be noted that the actual weighting factor for this feature may be higher, because published values using the ">" designation were not included in the calculation of the weighting factor.

Analysis of the most direct pathway from the benzenoid ring to the nitrogen. The pathway from the benzenoid ring to the nitrogen was analyzed separately for drugs with and without a ring-enclosed nitrogen. In molecules containing a ring-enclosed nitrogen (n = 26), the number of atoms between the two ring systems appears to have an effect on the affinity value. For example, both mianserin (average reported K_i value = 34 nM) and mCPP (average reported K_i value = 34 nM) comply with all criteria except that they have 0 atoms between the two ring systems. A weighting factor of 30× is, therefore, assigned to this feature.

In contrast, RU 24969 (Fig. 1E; average reported K_i value = 280 nM) and methiothepin (average reported K_i value = 340 nM) meet all criteria except that 1 atom is present between the two ring systems, resulting in a weighting factor of 300×. More than 1 atom between the two ring systems has no apparent effect on affinity, based on compounds in the learning set.

The distance to the ring-enclosed nitrogen also affects the affinity value. There are four agents that comply with all other molecular criteria but have a ring-enclosed nitrogen located 6 atoms from the benzenoid ring, quipazine (average reported K_i value = 1.3 nM), renzapride (average reported K_i value = 5.3 nM), SDZ 210-204 (average reported K_i value = 6.2 nM), and MDL 72222 (average reported K_i value = 13 nM). A weighting factor of 5× is assigned to this molecular feature, based on the published affinities of these four agents.

The geometry of the first atom in the most direct pathway from the benzenoid ring to the nitrogen appears to play a critical role in the determination of affinity. In our previous study of potent 5-HT$_3$ agents, the first atom from the benzenoid ring was observed to be trigonal in all 19 potent 5-HT$_3$ agents analyzed (2). Prochlorperazine (Fig. 1F; average reported K_i value = 1,800 nM) and fluphenazine (reported K_i value = 17,000 nM) meet all 5-HT$_3$ pharmacophore criteria except for this apparently critical feature, to which a weighting factor of 6,000× is assigned.

The geometry of the first atom in the most direct pathway from the benzenoid ring to the nitrogen also plays a role in affinity determination in drugs lacking a ring-enclosed nitrogen. However, in this class of agents this factor appears to be less significant than in compounds that contain ring-enclosed nitrogens. For example, propranolol (average reported K_i value = 12,000 nM, divided by 400× due to the lack of a ring-enclosed nitrogen, divided by 10 due to greater than 2 planar substitutions on the benzenoid ring = 3.0×), chlorpromazine (average reported K_i value = 1,300 nM, divided by 400× due to the lack of a ring-enclosed nitrogen = 3.3×), pindolol (average reported K_i value = 29,000 nM, divided by 400× due to the lack of a ring-enclosed nitrogen, divided by 10 due to greater than 2 planar substitutions on the benzenoid ring = 7.3×), mepyramine (average reported K_i value = 5,000 nM, divided by 400× due to the lack of a ring-enclosed nitrogen = 13×), and 8-OH-DPAT (average reported K_i value = 7,300 nM, divided by 400× due to the lack of a ring-enclosed nitrogen = 18×) are examples of drugs in which the first atom from the benzenoid ring to the nitrogen is tetrahedral. A weighting factor of 7× can be assigned

TABLE 1

5-HT$_3$ computational model

Assume that x (i.e., estimated K_i value) = 1 nM, then evaluate molecule as follows.

Analysis	Weighting factors
1. Analysis of general molecular features (n = 40)	
Is a benzenoid ring and nitrogen atom present?	
A. Yes (n = 39)	1×
B. No (n = 1)	>10,000×
(Analysis completed)	
2. Analysis of benzenoid ring properties (n = 39)	
Evaluate the free substitutions on the benzenoid ring that are not contiguous to the most direct pathway to the nitrogen.	
A. 0–2 atoms (n = 35)	1×
B. >2 planar atoms (n = 3)	10×
C. >2 nonplanar atoms (n = 1)	20,000×
3. Analysis of nitrogen properties	
A. Evaluate for presence of a ring-enclosed nitrogen.	
1. Present (=n = 26)	1×
(Go to Section 3C1)	
2. Absent (n = 13)	400×
B. Evaluate the free substitutions on the nitrogen.	
1. 0–3 atoms (n = 10)	1×
2. >3 atoms (n = 3)	80×
(Go to Section 4D1)	
C. Evaluate the free substitutions on the ring system containing the nitrogen.	
1. 0–3 atoms (n = 18)	1×
2. 4–6 atoms (n = 2)	600×
3. >6 atoms (n = 6)	10,000×
4. Analysis of the most direct pathway from the benzenoid ring to the nitrogen	
A. Evaluate the number of atoms between the two ring systems.	
1. 0 atoms (n = 4)	30×
(Analysis completed)	
2. 1 atom (n = 2)	300×
(Analysis completed)	
3. >1 atom (n = 20)	1×
B. Evaluate the distance to the nitrogen from the benzenoid ring.	
1. <6 atoms (n = 1)	1×
2. 6 atoms (n = 5)	5×
3. >6 atoms (n = 20)	1×
C. Analyze the first atom in the most direct pathway from the benzenoid ring.	
1. Trigonal (n = 24)	1×
2. Tetrahedral (n = 2)	6,000×
(Analysis completed)	
D. Analyze the first atom in the most direct pathway from the benzenoid ring to the nitrogen (not ring enclosed).	
1. Trigonal (n = 8)	1×
2. Tetrahedral (n = 5)	7×
(Analysis completed)	

to this feature, after other weighting factors are taken into consideration. The computational model is summarized in Table 1.

Comparison of computationally derived 5-HT$_3$ receptor affinity values with experimentally determined K_i values. The mean reported affinities, the computationally predicted affinities, and the criteria violations of the learning set are summarized in Table 2. As shown in Fig. 2A, a significant correlation exists between the mean reported affinities for

TABLE 2

Reported and predicted receptor affinities at 5-HT$_3$ binding sites for the learning set

Reported affinity values are the mean log values taken from Refs. 3–5, 7, and 10–20.

Drug	Reported affinity (K_i)	Predicted affinity (K_i)	Criteria violations
	nM	nM	
1 SDZ 206-830	0.27	1	
2 Zacopride	0.41	1	
3 Granisetron	1.1	1	
4 SDZ 206-792	1.1	1	
5 ICS 205-930	1.2	1	
6 Quipazine	1.3	5	4B2
7 GR 65630	1.5	1	
8 Ondansetron	2.9	1	
9 Renzapride	5.3	5	4B2
10 SDZ 210-204	6.2	5	4B2
11 MDL 72222	13	5	4B2
12 Mianserin	34	30	4A1
13 *m*CPP	34	30	4A1
14 Metoclopramide	260	400	3A2
15 5-HT	260	400	3A2
16 RU 24969	280	300	4A2
17 Methiothepin	340	300	4A2
18 2-Methyl-3-HT	490	400	3A2
19 Phenylbiguanide	490	400	3A2
20 Chlorpromazine	1,300	2,800	3A2, 4D2
21 Prochlorperazine	1,800	6,000	4C2
22 Cocaine	3,400	3,000	3C2, 4B2
23 Droperidol	4,200	10,000	3C3
24 Mepyramine	5,000	2,800	3A2, 4D2
25 Domperidone	6,200	10,000	3C3
26 8-OH-DPAT	7,300	2,800	3A2, 4D2
27 Ritanserin	7,300	10,000	3C3
28 5-CT	12,000	4,000	2B, 3A2
29 Propranolol	12,000	28,000	2B, 3A2, 4D2
30 Sulpiride	15,000	20,000	2C
31 Fluphenazine	17,000	6,000	4C2
32 SCH 23390	17,000	18,000	3C2, 4A1
33 5-HTP dipeptide	21,000	32,000	3A2, 3B2
34 Pindolol	29,000	28,000	2B, 3A2, 4D2
35 Methysergide	35,000	32,000	3A2, 3B2
36 Spiperone	39,000	10,000	3C3
37 LSD	40,000	32,000	3A2, 3B2
38 Mesulergine	50,000	>100,000	3C3, 4A1
39 Ketanserin	55,000	10,000	3C3
40 GABA	>10,000	>10,000	1B

the learning set and the affinity values predicted by the computational model (r = 0.98; $p < 0.001$; n = 39; SE of regression = 0.33). The computational model was then applied to a "test set" of 40 pharmacological agents, in an attempt to predict 5-HT$_3$ receptor binding affinities for drugs that had not been used to define the model. The 40 agents that make up the test set represent a structurally diverse group of compounds, most of which are known to interact potently with one or more 5-HT receptor subtype (8). Radioligand binding studies using [^{3}H]quipazine were then performed in rat cortical membranes and affinity values were determined for each drug. These data, as well as the computationally predicted affinities and the molecular structural violations, are provided in Table 3. As shown in Fig. 2B, a significant correlation exists between drug affinities determined in radioligand binding assays and those predicted by the computational model (r = 0.83; $p < 0.001$; n = 35; SE of regression = 0.75).

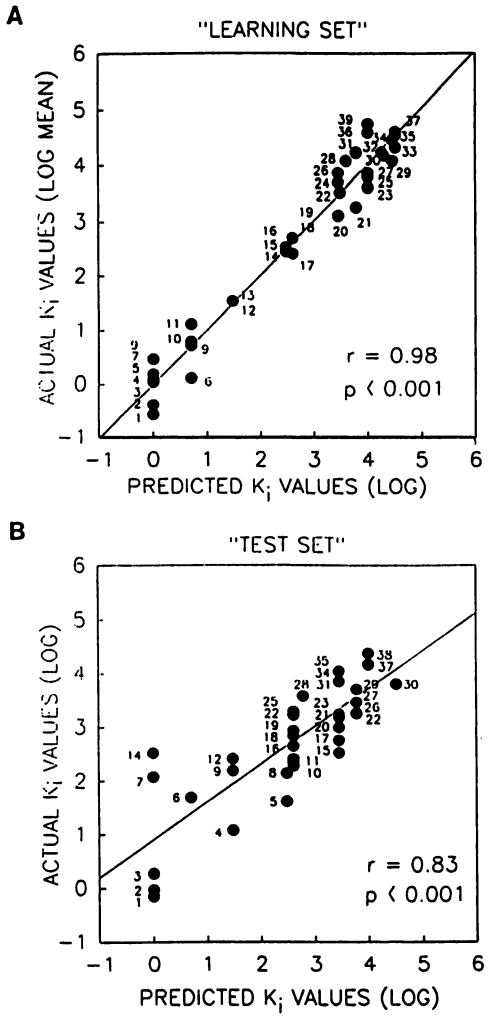

Fig. 2. Correlation of experimentally determined K_i values and K_i values predicted by the computational model. A, The correlation of predicted versus reported drug affinities for the 38 agents in the learning set. The *numbers* correspond to the compounds in Table 2. B, The correlation of predicted versus experimentally determined affinity values for the 35 agents in the test set. The *numbers* correspond to the compounds in Table 3.

TABLE 3

Experimentally determined and predicted receptor affinities at 5-HT$_3$ binding sites for the test set

Radioligand binding experiments using [^{3}H]quipazine to label 5-HT$_3$ receptor binding sites were performed as described in Materials and Methods. IC_{50} values were determined by log-logit analysis of drug competition data. K_i values were calculated using the Cheng-Prusoff equation. Data given are the means ± standard errors of three to six experiments, each performed in triplicate.

Drug	Actual affinity (K_i)	Predicted affinity (K_i)	Criteria violations
	nM	nM	
1 LY 278584	0.71 ± 0.06	1	
2 LY 258458	0.94 ± 0.04	1	
3 LY 211000	1.9 ± 0.1	1	
4 MK 212	12 ± 0.6	30	4A1
5 Pizotifen	42 ± 10	300	4A2
6 Clozapine	49 ± 10	5	4B2
7 Indalpine	120 ± 30	1	
8 Cyproheptadine	140 ± 50	300	4A2
9 TFMPP	160 ± 30	30	4A1
10 5,7-DHT	190 ± 40	400	3A2
11 5,6-DHT	220 ± 30	400	3A2
12 MPTP	260 ± 40	30	4A1
13 Amitriptyline	260 ± 40	400	3A2
14 LY 278989	330 ± 70	1	
15 Chlorimipramine	340 ± 30	2,800	3A2, 4D2
16 Doxepin	450 ± 30	400	3A2
17 Imipramine	570 ± 20	2,800	3A2, 4D2
18 ICI 169,369	680 ± 20	400	3A2
19 Nortriptyline	850 ± 200	400	3A2
20 Sertraline	1,000 ± 200	2,800	3A2, 4D2
21 Desipramine	1,500 ± 90	2,800	3A2, 4D2
22 Zimelidine	1,700 ± 500	400	3A2
23 Fluoxetine	1,700 ± 400	2,800	3A2, 4D2
24 Thioridazine	1,800 ± 300	6,000	4C2
25 5-MeT	1,900 ± 500	400	3A2
26 Idazoxan	2,900 ± 800	6,000	4C2
27 Phentolamine	2,900 ± 600	6,000	4C2
28 Ipsapirone	3,800 ± 1,000	600	3C2
29 Atropine	5,100 ± 800	6,000	4C2
30 Cinanserin	6,500 ± 2,000	32,000	3A2, 4D2
31 Dopamine	7,300 ± 1,000	2,800	3A2, 4D2
32 Buspirone	10,000 ± 3,000	>100,000	3C3, 4A1
33 WB 4101	10,000 ± 2,000	>100,000	3A2, 3B2, 4D2
34 Noradrenaline	11,000 ± 2,000	2,800	3A2, 4D2
35 (−)-DOB	11,000 ± 3,000	2,800	3A2, 4D2
36 Metergoline	11,000 ± 3,000	>100,000	3C3, 4A1
37 Pirenpirone	15,000 ± 2,000	10,000	3C3
38 Prazosin	24,000 ± 7,000	10,000	3C3
39 Yohimbine	26,000 ± 5,000	>100,000	3C3, 4A1
40 Chlordiazepoxide	>100,000	>100,000	3C3, 4A1

Discussion

The major finding of the present study is that the molecular properties of a chemical compound can be used to predict the affinity of the drug for a neurotransmitter receptor. The approach described in the present report allows for the derivation of a computational model that can be used to accurately predict, with a high degree of statistical significance ($p < 0.001$), receptor binding site affinity based on molecular information. The quantitative model, summarized in Table 1, is based on the identification of molecular features that consistently decrease drug affinity for the 5-HT$_3$ receptor. This approach relies on published affinity data and does not require the use of animal tissues or radioactivity.

Because this approach is based on the identification of "inactivating" molecular features and does not directly assess the electronic properties of the molecule, it is likely that the quantitative model would be more likely to identify false positive agents rather than false negative drugs. Indeed, of the 35 agents in the test set for which receptor affinities below 100,000 nM were predicted, 33 drugs were found to display affinities for the receptor that were within 1 order of magnitude of the computationally predicted value. Two agents, indalpine and LY 278989, display experimentally determined binding affinities that are more than 2 orders of magnitude less potent than their computationally predicted values. For example, indalpine (Fig. 1G) was predicted to have a K_i value of 1 nM, but the experimentally determined value is 120 ± 30 nM. In comparison with the 5-HT$_3$ agents that display K_i values below 10 nM, the ring-enclosed nitrogen in indalpine is not located in a constrained ring system (i.e., in either a benzenoid ring as in ondansetron, a bicyclic ring structure as in ICS 205-930, or a ring that is attached directly to a rigid structure as in quipazine). The relatively low affinity of indalpine at the 5-HT$_3$ receptor binding site suggests that constraints on the ring system containing the nitrogen may be an important feature of potent 5-HT$_3$ agents. Moreover, the computational model can now be refined

by the incorporation of an additional weighting factor for this molecular feature.

In contrast, no obvious molecular feature differentiates LY 278989 (Fig. 1H) from the most potent 5-HT_3 agents. LY 278989 has a predicted K_i value of 1 nM, but the experimentally determined K_i value is 330 ± 70 nM. LY 278989 has a methyl substitution on the 2-position of the indazole ring. Based on X-ray crystallographic data, the methyl group in this position appears to force the carbonyl group to assume a nonplanar configuration (9). Our previous analysis of the 5-HT_3 pharmacophore suggests that this region of the molecule is coplanar with the benzenoid ring in all potent 5-HT_3 agents (2). Theoretically, the loss of planarity in this region is the basis for the relative inactivity of LY 278989.

The unexpected inactivity of LY 278989 does illustrate a potential major weakness of the present approach; no attempt was made to directly assess the electronic properties of the drug. However, the addition of force field and quantum chemical calculations to the existing molecular screening system would significantly increase the analysis time as well as the computer power needed to analyze structures. Because the quantitative model is based on known molecular features that decrease receptor affinity, the model is most likely to predict a higher affinity than the actual value for drugs in which electronic features play a significant role. In addition, another weakness of the present approach is that stereochemical considerations are not taken into account. As a result, it is likely that a detailed analysis of electronic and stereochemical properties would be more likely to identify additional molecular features that would also decrease affinity rather than to identify molecular features that would counteract known inactivating molecular characteristics. In the case of LY 278989, the crystallographic data support the hypothesis that coplanarity of the atoms near the benzenoid ring is critical for high affinity 5-HT_3 receptor binding.

The utility of the molecular analysis, in the absence of an electronic analysis, is that it allows for the rapid screening of chemical structures stored in computerized databases. All published chemical structures (approximately 10,000,000 compounds) are stored in a computer-based system by the American Chemical Society. Substructure searching of the American Chemical Society database can be performed at the rate of approximately 32,000 agents/sec.[1] The computational model described in the present report could, theoretically, be used to electronically screen these compounds in order to predict their affinity for 5-HT_3 receptors. The identified drugs that are predicted to display activity in the computerized screen can then be analyzed experimentally in radioligand binding assays. Therefore, the ability to electronically screen drug structures with a relatively simple set of molecular criteria should allow for the rapid and sensitive identification of potentially receptor-active agents.

[1] A. W. Schmidt and S. J. Peroutka, unpublished observations.

Acknowledgments

We thank Mary T. Keller and Jean M. Peroutka for their excellent editorial assistance and Peter Murray for computer assistance.

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