0026-895X/88/010042-12$02.00/0

MOLECULAR PHARMACOLOGY, 34:42–53

Molecular Determinants for Recognition of RU 24969 Analogs at Central 5-Hydroxytryptamine Recognition Sites: Use of a Bilinear Function and Substituent Volumes to Describe Steric Fit

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Received November 5, 1987; Accepted April 11, 1988

SUMMARY

The putative serotonin (5-HT) agonist RU 24969 [5-methoxy-3-(1,2,3,6-tetrahydropyridin-4-yl)indole; 5-MeO-THPI] has been extensively used in the study and classification of 5-HT receptors. In order to study molecular determinants for recognition of THPIs at central 5-HT recognition sites, about 25 additional THPI derivatives were synthesized, incorporating, among others, 16 different indole-5-substituents and three different pyridine-*N* substituents in various combinations. Two saturated derivatives (piperidin-4-ylindoles) and two 2-methyl analogs were also included. Binding affinities at 5-HT$_{1A}$, 5-HT$_2$, and total 5-HT$_1$ sites were obtained and the data were incorporated in quantitative structure-activity relationships (QSARs) using a combined linear free energy/molecular modeling approach. The QSAR analyses suggest distinct differences in the structural features that determine optimal potency at 5-HT$_{1A}$ sites versus those directing optimal potency for 5-HT$_2$ sites. The parameter of the indole-5 substituent that almost exclusively determines potency for 5-HT$_{1A}$ sites is volume, the optimal size being about 24 cubic angstroms (calculated by fitting the activity versus volume data to a bilinear function). This is approximately the size of a carboxamide group. In contrast, at the 5-HT$_2$ site both volume and hydrophobicity play major but opposing roles for the 5-substituent. A balance between the smallest possible volume and the greatest possible hydrophobicity is required for maximal 5-HT$_2$ potency. Benzyl groups on the indole-1 or pyridyl-1 positions also favor potency at the 5-HT$_2$ site (probably largely due to increased hydrophobic binding) while decreasing potency at the 5-HT$_{1A}$ site. A minor electronic contribution to the QSARs involving the charge on the indole 5-carbon is of opposite sign for 5-HT$_{1A}$ versus 5-HT$_2$ sites and thus may also be useful for selective drug design. The data are consistent with the possibility that the indole and pyridyl rings are in a coplanar configuration when binding at both 5-HT$_{1A}$ and 5-HT$_2$ sites, because the indole-2-methyl substituent, which provides a large energy barrier to the coplanar configuration, greatly reduces the potency of THPIs at both binding sites. Similarities in analog selectivity patterns suggest that the indolic portion of these compounds binds similarly to that of other indole derivatives such as tryptamines; thus, it is possible that optimally selective substituents predicted by these QSARs may be extrapolated to tryptamines and other indoles.

The tetrahydropyridylindoles RU 24969 and RU 28253 (Fig. 1) are of interest because of their actions at serotonergic systems and their structural similarities to serotonin (5-HT; Fig. 1). Structurally, they represent semi-rigid analogs of 5-HT and, as such, provide fewer possible conformations for recognition by receptors than does 5-HT with its relatively flexible aminoethyl side chain. This property is particularly desirable in studies in which the goal is to determine the structural features of compounds that determine high potency for and selectivity between the different subtypes of 5-HT receptors. In this paper, we have undertaken a quantitative structure-activity study of a series of RU 24969 analogs at central 5-HT recognition sites, partly in the hope that some of the information gained can be extrapolated to other types of indole derivatives (e.g., tryptamines).

RU 24969 is the most thoroughly investigated of a number of derivatives of THPI that have been previously examined for dopaminergic and 5-HT agonist activity (1–4). Its 5-HT agonist activity is documented by an impressive accumulation of binding (1, 2, 4–7), biochemical (1, 2, 8, 9), and behavioral (4, 10, 11) data. Binding studies suggest that RU 24969 is relatively selective for 5-HT$_1$ sites, with lesser activity at 5-HT$_2$ sites (5, 6). An earlier suggestion by Sills *et al.* (12) that it is selective for 5-HT$_{1B}$ sites has been refuted by subsequent binding studies that reveal an equally high affinity for 5-HT$_{1A}$ sites labeled with [^{3}H]8-OH-DPAT (5–7). RU 24969 has low affinities for putative 5-HT$_{1C}$ (5, 7) and 5-HT$_3$ sites (13).

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; THPI, 3-(1,2,3,6-tetrahydropyridin-4-yl)indole; QSAR, quantitative structure-activity relationship; *d*-LSD, *d*-lysergic acid diethylamide; 8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetralin.

This work was supported by National Institutes of Health Grants NS-16605 and NS-01009 and in part by NS-21715.

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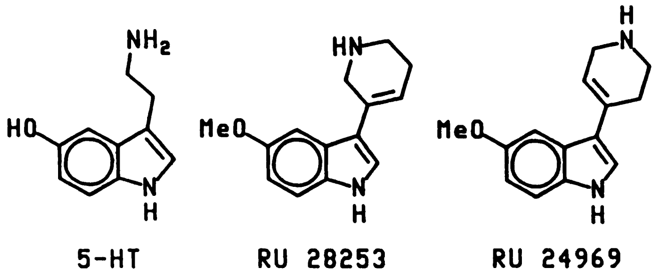


Fig. 1. Structures of serotonin and the two tetrahydropyridylindoles RU 28253 and RU 24969.

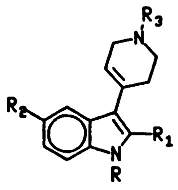
For the present study, over 25 analogs of RU 24969 were synthesized (Table 1) and examined at the 5-HT$_{1A}$ and 5-HT$_2$ recognition sites. Total 5-HT$_1$ binding was also determined for most of the compound; however, a QSAR analysis of the 5-HT$_1$ data was not performed, because this represents a heterogeneous group of sites (7, 14) and also because a high degree of correlation was found between the 5-HT$_1$ and 5-HT$_{1A}$ data. (See the Results section for a discussion of this correlation and also the rationale for omission of certain compounds from the QSAR analysis.)

The compounds were further studied by molecular modeling using the integrated system CHEMLAB (Molecular Design Ltd., San Leandro, CA) and the results were incorporated in the quantitative structure-activity analysis. The substituent position of primary interest was the indole-5 position, for which 16 different substituents were examined. Because of the comparative ease of synthesis, most of the compounds have a methyl group on the pyridine nitrogen; however, a few analogs are unsubstituted at this position (including RU 24969 itself). Additional compounds include several *N*-benzyl and two saturated derivatives (piperidinylindoles). Two 2-methyl derivatives were also included to study the effect of forcing the tetrahydropyridine ring out of the plane of the indole ring (15).

TABLE 1

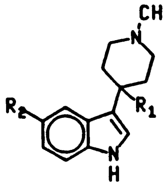
5-HT recognition site data for tetrahydropyridylindole derivatives

5-HT$_{1A}$ sites are defined by the binding of [^{3}H]8-OH-DPAT in rat cerebral cortical membranes, 5-HT$_2$ sites by the binding of [^{3}H]ketanserin in rat frontal cortical membranes, and total 5-HT$_1$ sites (1A + 1B + 1C) by the binding of [^{3}H]5-HT in rat cortical membranes. Compounds marked * in column 1 vary only in their indole-5-substituents and were considered separately in the preliminary QSAR analysis (see text). ND, not determined.



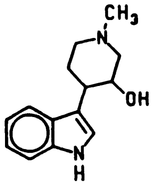
Structure 1

1a–1w



Structure 2

2a, R$_1$= H, R$_2$= H
2b, R$_1$= H, R$_2$= OCH$_3$
2c, R$_1$= 3-indolyl, R$_2$= H



Structure 3

3a

Compound		Substituents (Structure 1)				Apparent K_i Values^a			
Number	Name/Code	R	R$_1$	R$_2$	R$_3$	5-HT$_{1A}$	5-HT$_2$	5-HT$_1$	m.p.
							nM		°C
Part A: Compounds Treated in the QSAR Analysis									
1a	SN-18	H	H	H	H	189.4	204	ND	185–186
1b *	SN-1	H	H	H	CH$_3$	146	67	230	218–220
1c	BW-1	H	CH$_3$	H	CH$_3$	1,785	869	>5,000	132–134
1d	RU 24969	H	H	OCH$_3$	H	10.96	912	9.6	
1e *	SN-3	H	H	OCH$_3$	CH$_3$	21.1	897	45.4	220–224
1f	SN-10	H	CH$_3$	OCH$_3$	CH$_3$	345	4,329	1640	99–101
1g *	SN-2	H	H	Br	CH$_3$	15	47.9	44.3	255–257
1h *	SN-21	H	H	Cl	CH$_3$	26.2	51.1	ND	248–252
1i *	SN-14	H	H	F	CH$_3$	67.9	44.8	107	224–226
1j *	SN-26	H	H	CONH$_2$	CH$_3$	5.31	16,820	ND	288–290^b
1k *	SN-11	H	H	COOCH$_3$	CH$_3$	19.4	3,665	94.9	212–214
1l *	SN-7	H	H	COOC$_2$H$_5$	CH$_3$	36.1	4,365	88.7	209–210
1m *	SN-25	H	H	OOCCH$_3$	CH$_3$	80.5	557.5	ND	204–206
1n *	SN-4	H	H	CH$_3$	CH$_3$	31.8	125	75.5	228–232
1o	SN-24	H	H	NO$_2$	H	64.3	164.2	ND	290, dec.^{b}
1p *	SN-5	H	H	NO$_2$	CH$_3$	36.8	86.4	202	278–280
1q *	SN-17	H	H	CN	CH$_3$	55.0	956	ND	274–276
1r *	SN-20	H	H	OH	CH$_3$	61.7	356	ND	244–247
1s *	SN-6	H	H	OCH$_2$C$_6$H$_5$	CH$_3$	111	397	219	194–195
1t *	SN-15	H	H	phthalimido	CH$_3$	377	10,800	715	225, dec.
1u	SN-13	H	H	H	benzyl	923	43.6	2,608	154–155
2a	SN-22		See structure 2 above			514	19.3	ND	178–180
2b	SN-23		See structure 2 above			137	591	ND	134–136
Part B: Compounds not Treated in the QSAR Analysis									
1v	SN-27	H	H	NH$_2$	CH$_3$	24,620	>100,000	ND	225–227
1w	SN-12	benzyl	H	H	CH$_3$	>10,000	15.4	3,574	103–105
2c	SN-9		See structure 2 above			544	286.4	772	297–300
3a	SN-19		See structure 3 above			5,539	1,489	ND	224–226

[a] Each value is the mean of at least three separate experiments.
[b] HCl salt.

Although there have been a number of previous QSAR studies of tryptamines and hallucinogens, the pharmacologic data has usually been either hallucinogenic potency (16–20) or 5-HT receptor affinities in the rat stomach fundus preparation (21–24). However, one early study (25) was based on the [^{3}H]5-HT and [^{3}H]*d*-LSD binding data of Bennet and Snyder (26). To our knowledge no published QSAR studies of serotonergic compounds have used [^{3}H]8-OH-DPAT or [^{3}H]ketanserin binding data. Because it is widely accepted that hallucinogens exert their effects at least in part by actions at 5-HT receptors (Weinstein and Green and co-workers' "LSD/serotonin receptor;" Refs. 20 and 27), and perhaps the central 5-HT$_2$ receptor in particular (28–30), some of the physiochemical parameters found to significantly correlate with activity in these previous studies of hallucinogens were ones that we wished to examine for possible correlations in the present study. Among others, these include the log *P* (18, 19, 25), ionization potentials (19) (or the corresponding calculated parameter, the energy of the highest occupied molecular orbital, E_{HOMO}) (16, 17, 21), the resonance constant (*R*) (31) of the indole-5-substituents (21), and, for a series of 5-substituted tryptamines, the charge on the indole-5 carbon atom (23).

The more recent work of Weinstein and collaborators (27, 32, 33, and references therein), although not QSAR, is based on qualitative correlations (for a series consisting of tryptamine and four isomeric hydroxytryptamines) between activity and the orientation of the molecular electrostatic potential vector for the indole ring. This is the basis for a more sophisticated quantum chemical model involving a computer simulation of 5-HT activation of proton transfer (33). A proposed early event in this process is the binding of the protonated amino side chain to anchor the ligand so that the electrostatic fields of the receptor and indole ring can interact. Because no compounds lacking the basic amino group have been shown to have high affinity for serotonin receptors, something of this sort is no doubt a key event in 5-HT ligand binding. However, as pointed out in a recent review (34), there are alternative hypotheses for the sequence of events leading to charge and/or proton transfer. In any case, the amino group is certainly essential for activity; the current study primarily addresses the effects of alterations in other parts of the molecule.

During the course of the data analysis, it became apparent that with this series of compounds, at both the 5-HT$_{1A}$ and 5-HT$_2$ recognition sites, the most significant effects on affinity were primarily steric. This has traditionally been the most difficult function to parameterize in QSAR studies and also the least standardized. The largest available steric-related parameter set is that of molar refractivities, which are readily available (35, 36). However, as noted by Hansch *et al.* (36), this parameter is only a crude (but nonetheless useful) measure of "bulk" because it contains an electronic contribution, and those authors have regarded its use "as only a possible interim solution" (36). Parameters based on direct calculations of molecular shapes and volumes are generally regarded as preferable but have not been very feasible until the recent proliferation of computer software making such calculations routine. Differences in overlap volume are the basis of the method of "Minimum Steric Differences" (37) and the "3-dimensional molecular shape descriptor" developed by Motoc and collaborators (38); extensive use of "molecular shape descriptors" is also found in the QSAR work of Hopfinger and co-workers (e.g., Refs. 39–40). A fragmental system for the calculation of van der Waals volumes has recently been proposed by Motoc and Marshall (41). In the present study the most significant correlations were obtained using substituent volume as the steric parameter.

Materials and Methods

Chemicals and drugs. RU 24969 was donated by Roussel-Uclaf (Romainville, France), and methysergide, by Sandoz (East Hanover, NJ). [^{3}H]8-OH-DPAT was obtained from Research Products International (Mount Prospect, IL), and [^{3}H]5-HT and [^{3}H]ketanserin from New England Nuclear Corporation (Boston, MA). All other chemicals used were from standard commercial sources.

Syntheses of RU 24969 analogs. With a few exceptions noted below, all of the 3-(1,2,5,6-tetrahydro-1-methylpyridin-4-yl)indole derivatives were prepared by reacting 1-methyl-4-piperidone with the appropriate indole in glacial acetic acid at reflux with ammonium acetate as a buffer (see Fig. 2 for scheme). This synthesis is an improvement of the earlier procedure used by Freter (42, 43), which fails with deactivated indoles. Our method cannot be used to directly prepare derivatives lacking an *N*-1 pyridine substituent (e.g., **1a**). The latter was prepared by reacting 1-tosyl-4-piperidone with indole as above, followed by detosylation of the 3-(1,2,5,6-tetrahydro-1-tosylpyridin-4-yl)indole under Birch conditions (Na, liquid NH$_3$). Compound **1r** was obtained by reacting the *O*-*t*-butyldimethylsilyl derivative of 5-hydroxyindole with 1-methyl-4-piperidone followed by deprotection of the 5-OH group in aqueous acetic acid. The two saturated derivatives **2a** and **2b** were obtained by catalytic reduction of **1b** and **1e** using 10% palladium on charcoal as catalyst. The alcohol **3a** was prepared by hydroboration of **1b**. The 2:1 adduct **2c** was formed as a byproduct in the synthesis of **1**. Compounds **1o** and **1j** were obtained using a variant of the general procedure, by refluxing the appropriate indole derivative with either 4-piperidone hydrochloride or 1-methyl-4-piperidone in ethanolic hydrochloric acid. The 5-amino derivative **1v** was made according to the procedure of Guillaume *et al.* (4). Structures were confirmed by standard physical methods, including proton NMR and IR spectroscopy and comparison with known compounds. Melting points for all the synthesized compounds are included in Table 1.

5-HT receptor binding assays. These were performed as described previously (6). In brief, tissue was obtained from male Sprague-Dawley rats, which were killed by decapitation; the brains were then rapidly removed and dissected over ice. For the 5-HT$_1$ and 5-HT$_{1A}$ assays, the cortex dorsal to the rhinal sulcus was used; the 5-HT$_2$ assay was done using frontal cortex alone (44). Final tissue suspensions were in a buffer of 50 mM Tris at pH 7.6. For the 5-HT$_1$ assay, [^{3}H]5-HT to a final concentration of about 2 nM was used as ligand, and unlabeled 5-HT at 10 μM was used to define nonspecific binding. For the 5-HT$_{1A}$ assay, the ligand was [^{3}H]8-OH-DPAT at about 1 nM, and again 10 μM unlabeled 5-HT was used to define nonspecific binding. [^{3}H]Ketanserin to a final concentration of about 0.4 nM was used as the 5-HT$_2$ ligand, and nonspecific binding was defined using 1 μM methysergide. The assay tubes were incubated at 37° for 10 min (15 min for the 5-HT$_2$ assay) and filtered through Whatman GF/B filters using a Brandel cell harvester. For the 5-HT$_{1A}$ and 5-HT$_2$ assays, the GF/B filters were

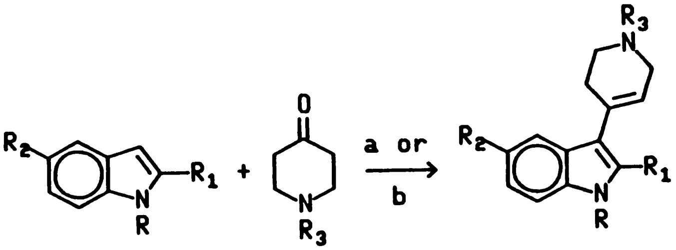


Fig. 2. General scheme for the synthesis of the THPI derivatives. Reagents: a, acetic acid, heat; b acetic acid/tetrahydrofuran, ammonium acetate, heat.

pretreated with a 0.1%, v/v, solution of polyethyleneimine for 2 hr and allowed to dry (this was found to reduce nonspecific binding to the filters). For a similar reason, the 5-HT_2 assays were performed in disposable polypropylene rather than glass tubes. For all three binding assays, potencies of inhibiting drugs are reported as apparent K_i values, calculated from inhibitor IC_{50} values using the Cheng-Prusoff equation (45). It must be emphasized that the apparent K_i for 5-HT_1 sites is a composite value, because [^{3}H]5-HT labels a heterogeneous group of sites (7, 14).We have chosen to report "apparent" K_i rather than IC_{50} values for the 5-HT_1 site for the sole purpose of making the comparison of affinities between the three ligands easier.

Molecular and quantum mechanics calculations. These were carried out exclusively with various modules of the program CHEMLAB (Molecular Design Ltd.) running on a VAX 11/780. The initial structures were generated with the DRAWMOL and PRXBLD options and further refined with MMFF. The first problem was to determine the appropriate orientation for the tetrahydropyridine ring to be used in the calculations. After parameterization according to Hopfinger and Pearlstein (46), use of the SCAN option suggested that the planar conformation with the vinylic proton *cis* to the indole-2 carbon was very close to the global minimum for all except the 2-methyl analogs. This conformation was chosen as the starting point in the molecular mechanics minimization of each molecule. A similar procedure was used for the 2-methyl analogs, except that during the SCAN both the pyridine ring and the 2-methyl group were driven through torsional angles of 0–360° to generate a three-dimensional map of total free-space energy versus conformation (see Fig. 7). The quantum chemical parameters were calculated with the CNDO2 option, except for the 5-bromo analog for which the atomic charges were estimated by a PCILO calculation.

Calculation of log *P* values. For the hydrophobic parameter, we chose to use the fragmental constant system of Rekker (47, 48) to calculate log *P* values, rather than using the "pi" substituent system of Hansch and Leo (35), partly because the latter would not have extended to our complete set of compounds. The Rekker system is used by CHEMLAB, but it must be noted that the implementation of this system by CHEMLAB is apparently imperfect, as the program gives very unreasonable values for some compounds (in comparison with available substituent constants), specifically those containing carbonyl functions. In contrast, our manually calculated values (based on Rekker's system) for 15 different 5-substitued compounds correlate very well with Hansch pi values ($r = 0.996$). For the 5-phthalimido group, which presents problems in terms of the many special rules in the Rekker system, a fragmental constant was calculated from the experimental log *P* for phthalimide (47). The fragmental constant from Rekker's extended data set (48) were used.

Approach used for the QSAR analysis. The approach used is an empirical linear regression analysis of the Hansch type. An alternate QSAR approach is the so-called "theoretical QSAR," which is based on quantum perturbation theory and attempts to explain drug activity in terms of purely quantum chemical indices and moments of inertia (22, 49, 50), rather than the traditional hydrophobic, electronic, and steric parameters. A drawback of the theoretical QSAR method is the requirement that each potential drug candidate be modeled in the computer and subjected to expensive calculations before its activity can be predicted; with the Hansch method, assuming that most of the activity can be explained by readily available (tabulated) or easily calculable parameters, predicted activities can be determined with relative ease. (For a discussion of the relative merits of these two QSAR methods, see pp. 115–125 of Franke's treatise on drug design methods; Ref. 51.) The approach we have taken incorporates the examination of some quantum chemical parameters within an empirical QSAR [As discussed previously, other non-QSAR quantum chemical approaches have been used to model the binding of tryptamines (27, 32, 33); possible relations between these studies and our results will be examined in the Discussion.]

An examination of the data in Table 1 revealed a complex pattern of differences in the selectivity between 5-HT_{1A} and 5-HT_2 sites for the various indole-5 substituents. Because this was the substituent position of primary interest, in order to simplify the problem initially a QSAR study of only the 15 different 5-substituted analogs (marked with asterisks in Table 1A) of 3-(1-methyl-1,2,3,6-tetrahydropyridin-4-yl)indole (**1b**) was undertaken. Many parameters were examined as possible variables; however, it became apparent that for this limited series of compounds over 75% of the variation in pK_i could be accounted for in terms of only two parameters, log *P* and size of the indole-5 substituent (V_5), defined as the substituent volume (in cubic angstroms) in excess of that of the unsubstituted reference compound (**1b**). Note that by this definition, V_5 for the 5-H compounds (R_2 = H) is zero.

After deriving equations capable of predicting the variation in activity due to changes of the 5-substituent alone (see Results), more inclusive equations describing all of the compounds in Table 1A were derived by adding the following steric and shape descriptors:

1) The volume of the pyridyl nitrogen substituent, V_N, defined as the *N*-substituent volume (in cubic angstroms) in excess of that of the unsubstituted reference compound (**1b**). Again, note that by this definition, V_N for the unsubstituted secondary amines (R_3 = H) is zero.

2) An "indicator variable" (52), I_1, describing the presence ($I_1 = 1$) or absence ($I_1 = 0$) of the indole 2-methyl group. (Substituent volume could have been used here with essentially identical results for this data set but, because no substituents other than methyl and hydrogen were studied, use of volume as the descriptor might be misleading if the variation in pK_i is not linearly related to substituent size for this position.)

3) An indicator variable I_2 describing the presence ($I_2 = 1$) of a fully saturated pyridine ring (compounds **2a** and **2b**) as opposed to a tetrahydropyridine ring ($I_2 = 0$).

The use of bilinear model for steric fit. When a variable *x* such as substituent volume or log *P* has an optimal (or least optimal) value, a linear correlation with *x* may not be observed. To deal with this situation within the framework of the multiple regression model, the method of introducing an x^2 term into the correlation was pioneered by Leo and Hansch for hydrophobicity (53). This method (introducing a quadratic term) has also been recommended for modeling steric fit (37); this would correspond to the situation shown as Fig. 3, *plot A*. The same authors also suggest an alternate model using a single term equal to the absolute value of the difference between the actual and

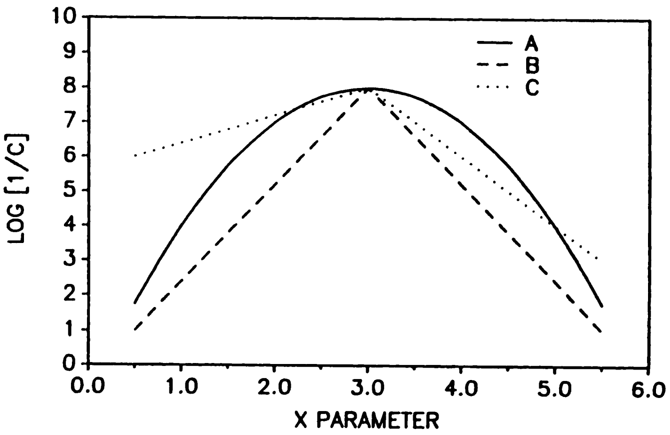


Fig. 3. Models for fitting a parameter *x* having an optimal value x_o in a multiple regression analysis (in this hypothetical case, $x_o = 3$). *C* is a concentration producing a standard biological effect, e.g., K_i. By inversion of the curves, a least optimal value of *x* can also be described. *Plot A*; the quadratic model, described by $\log(1/C) = ax^2 + bx + c$; *plot B*, symmetric model, described by $\log(1/C) = a\,|\,x{-}x_o\,|_x + b$; *plot C*, bilinear model, described by $\log(1/C) = a\cdot\log(\beta\cdot 10^x + 1) + cx + d$, or the mathematically equivalent $\log(1/C) = a\cdot\log(10^{x-b} + 1) + cx + d$. Models A and C have been used for fitting hydrophobicity, models A and B for steric fit. In this paper we use model C to describe steric fit.

TABLE 2

Database of some experimental and calculated parameters examined in the QSAR analysis

$pK_i = -\log K_i$ from data in Table 1. V_5, volume of indole-5-substituent, V_N, volume of pyridine *N*-substituent (for hydrogen, both V_5 and V_N are zero by definition); Q_4 and Q_5 are net electronic charge on the indole-4 and -5 carbons, respectively; E_{HOMO} and E_{LUMO} are the energies of the highest occupied and lowest unoccupied molecular orbitals (eV electron volts); *R* is the resonance constant of the indole-5-substituent. Data for the 5-amino compound **1v** are included although it is an outlier in the QSAR analysis (see text). The indicator variables I_1 and I_2 (not in this table) are defined in the text.

Compound	5-HT$_{1A}$ pK_i	5-HT$_2$ pK_i	log *P*	V_5	V_N	Q_4	Q_5	E_{HOMO}	E_{LUMO}	*R*
				Å^3		*e*		eV		
1a	6.723	6.691	2.408	0	0	0.0316	−0.0075	−8.998	3.358	0
1b	6.836	7.174	2.838	0	16.8	0.0315	−0.0073	−9.024	3.324	0
1c	5.748	6.061	3.357	0	16.8	0.0319	−0.0090	−8.989	3.133	0
1d	7.960	6.040	2.488	25	0	−0.0445	0.1943	−8.933	3.325	−0.51
1e	7.676	6.047	2.918	25	16.8	−0.0445	0.1946	−8.963	3.294	−0.51
1f	6.462	5.364	3.437	25	16.8	−0.0438	0.1929	−8.942	3.107	−0.51
1g	7.824	7.320	3.772	21	16.8	0.0676	0.0590			−0.17
1h	7.582	7.292	3.580	14.8	16.8	0.0159	0.0509	−9.909	2.355	−0.15
1i	7.168	7.349	3.047	2.7	16.8	−0.0522	0.1977	−9.822	2.528	−0.34
1j	8.276	4.774	1.548	23.5	16.8	0.0580	−0.0880	−9.193	2.908	0.14
1k	7.712	5.436	2.973	38.5	16.8	0.0618	−0.0877	−9.310	2.657	0.15
1l	7.442	5.360	3.492	56.1	16.8	0.0613	−0.0876	−9.286	2.694	0.15
1m	7.094	6.254	2.198	38.1	16.8	−0.1164	0.2021	−8.803	3.446	−0.07
1n	7.498	6.903	3.357	17.8	16.8	0.0190	0.0091	−8.986	3.285	−0.13
1o	7.192	6.785	2.173	17.2	0	0.0595	0.0103	−9.725	0.620	0.16
1p	7.434	7.063	2.603	17.2	16.8	0.0593	0.0106	−9.748	0.610	0.16
1q	7.260	6.020	2.482	9.3	16.8	0.0372	−0.0030	−9.228	2.881	0.19
1r	7.210	6.449	2.342	6.9	16.8	−0.0519	0.2024	−8.928	3.309	−0.64
1s	6.955	6.401	4.576	88.6	16.8	−0.0461	0.1950	−8.911	3.342	−0.47^a
1t	6.424	4.967	3.335	92.6	16.8	−0.0376	0.1755	−8.781	1.782	−0.07^a
1u	6.035	7.361	4.496	0	79.1	0.0317	−0.0074	−8.985	3.326	0
2a	6.289	7.714	2.913	0	16.8	0.0246	−0.0180	−9.765	4.050	0
2b	6.862	6.228	2.993	25	16.8	−0.0565	0.1921	−9.588	3.911	−0.51
1v	4.609	<4	1.608	10.7	16.8	−0.0682	0.1680	−8.430	3.535	−0.68

[a] *R* values for the benzyloxy and phthalimido groups were not readily available; these values are estimates calculated from available σ values (30).

optimal steric parameter values. This would correspond to a situation in which potency decreased in a linear fashion on either side of the optimal value x_o (Fig. 3, *plot B*). Note that with this model, the value of x_o must be estimated first (perhaps by using the quadratic model) and then refined by an iterative method.

A significant advance in the empirical fitting of log *P* data was the recent development of the bilinear model (54, 55). Functions of this type can be derived from theoretical pharmacokinetic models, and the bilinear model has been shown to give a significantly better fit for many data sets that had previously been analyzed by the quadratic model. In a purely empirical fashion, we have applied a bilinear function to this set of compounds to more accurately describe the steric fit for the indole 5-substituent. This model allows for situations such as that shown as Fig. 3, *plot C*, in which the linear relationships on either side of x_o have different slopes. The availability of such a model is useful in this type of empirical study, because there are no *a priori* reasons to expect that steric fit should be symmetric above and below an optimal (or least optimal) value.

The bilinear function we have used to model the steric fit for the indole-5-substituent is (assuming nonsteric effects are constant):

$$y = a \log(10^{x-b} + 1) + cx + d \qquad (1)$$

where y is the traditional $\log(1/C)$ (in this case pK_i), x, the steric parameter (in this case V_5), and b is the only nonlinear coefficient. Once b is known, the constants a, c, and d can be determined by linear regression.

It is easily shown that when $x \ll b$, Eq. 1 reduces to $y = cx + d$, the left linear part of the curve, and if $x \gg b$, it reduces to $y = (a + c)x - ab + d$, the right linear part of the curve. These two equations are useful for obtaining preliminary estimates of a, c, and d for the nonlinear analysis; for example, c is the slope of the left linear part of the curve. Once the value of b has been determined, it is incorporated into the regression analysis as a $\log(10x-b + 1)$ term, in the same way that an x^2 term is incorporated in the quadratic model. The exact value of x_o is obtained by differentiating with respect to x and equating to zero; solving for x gives:

$$x_o = b + \log(-c/(a + c)) \qquad (2)$$

Note that b is mathematically equivalent to the negative log of β in the bilinear model that is now commonly used to fit hydrophobicity data (e.g., Ref. 56). We used the program Eureka (Borland International) on an IBM-PC to calculate b; the program PC-NONLIN (Statistical Consultants Inc.) gave identical results.

Results

The binding data for the RU 24969 analogs are presented in Table 1 A and B; only the compounds in Table 1A were included in the QSAR because it is desirable to have at least two compounds substituted at a given position before adding a steric or indicator variable to the equation. The compounds in Table 1B are those of which we only had one example of a different substituent at that position (e.g., the *N*-1 benzyl of **1w**) and also the 5-amino compound **1v**, which is an outlier in the 5-HT$_{1A}$ QSAR (by almost 3 orders of magnitude) and too impotent at 5-HT$_2$ for an IC$_{50}$ to be measured; possible reasons for this will be considered later. A high degree of correlation was found between the affinities for the 5-HT$_1$ and 5-HT$_{1A}$ recognition sites; for the compounds in Table 1A for which 5-HT$_1$ binding was measured, $r^2 = 0.886$, $p < 0.001$. Because of this correlation and the known heterogeneity of 5-HT$_1$ sites, we did not undertake a QSAR analysis of the total 5-HT$_1$ binding data.

Selected calculated physicochemical parameters and substituent constants for all the included compounds plus compound **1v** are given in Table 2. A few variables of interest that were not statistically significant in the QSAR (E_{HOMO}, E_{LUMO}, Q_4) have been included in the table. The indicator variables I_1 and I_2 (defined previously) are not shown in Table 2 because they merely have the values of 1 or 0 depending on the presence or absence of the 2-methyl group or the piperidine/tetrahydropyridine ring.

For all of the following QSAR equations, Tables 3 and 5 contain multiple regression summaries, which include the standard errors and probability levels for the regression coefficients and also sequential r^2 values. Tables 4 and 6 contain actual versus predicted values and residuals for the most significant regression equations.

QSAR of 5-substituted, 1′-methylpyridyl analogs of RU 24969. For the 15 compounds in Table 1A differing only in their 5-substituent (all marked with an asterisk in the table), a scatter plot of V_5 versus pK_i at the 5-HT$_{1A}$ site (Fig. 4) indicates a quadratic or bilinear fit with an optimal 5-substituent volume of about 24 cubic angstroms calculated by the bilinear fit and 39 cubic angstroms by the quadratic. This is reflected in the regression equations. For the quadratic fit:

$$\mathrm{p}K_i = 7.0706 + 0.0324V_5 - 0.000411V_5^2 \quad (3)$$

($n = 15$, $s = 0.298$, $r^2 = 0.6204$; F ratio $= 9.8$, $p < 0.005$.)

For the bilinear fit:

$$\mathrm{p}K_i = 6.9169 + 0.0400V_5 - 0.0573\log(10^{(V_5 - b)} + 1) \quad (4)$$

($b = 23.2$; $n = 15$, $s = 0.241$, $r^2 = 0.7520$; F ratio $= 18.2$, $p < 0.001$)

It is noteworthy that hydrophobicity does not make a statistically significant contribution to these equations. Thus, at the 5-HT$_{1A}$ site, 5-substituent size alone accounts for 75% of the observed variability, based on the bilinear fit of Eq. 3. A comparison of r^2 values and F ratios from Eqs. 3 and 4 shows that this model fits the data significantly better than the quadratic model, which can only account for 62% of the variability in the data. The quadratic model also gives a distorted fit of the data at the peak of the parabola, because differentiation of Eq. 3 gives a predicted value for the optimal V_5 of 39.4 cubic angstroms, which can be seen to be a high estimate by a visual inspection of Fig. 4. Using Eq. 2, the bilinear fit of Eq. 4 predicts the optimal V_5 to be 23.6 cubic angstroms, which is very close to the V_5 for the most potent compound, the 5-carboxamido analog **1j**.

The best equation obtained predicting 5-HT$_{1A}$ affinities for this set of compounds includes the electronic parameters Q_5 (the charge on the indole 5-carbon) and R, the resonance constant (31) for the indole 5-substituent:

$$\mathrm{p}K_i = 7.0092 + 0.0364V_5 - 0.0506\log(10^{(V_5 - b)} + 1) - 2.546Q_5 - 0.833R \quad (5)$$

($b = 23.4$; $n = 15$, $s = 0.,183$, $r^2 = 0.8806$; F ratio $=$ 18.44, $p < 0.001$.)

The 5-HT$_2$ data for the same 15 compounds can be used to generate the equation:

$$\mathrm{p}K_i = 4.4575 - 0.0281V_5 + 0.9022(\log P) \quad (6)$$

($n = 15$, $s = 0.485$, $r^2 = 0.7376$; F ratio $= 16.87$, $p < 0.001$.)

For V_5 alone versus the 5-HT$_2$ data, $r^2 = 0.3019$; thus, substituent volume and hydrophobicity each account for about 30–40% of the observed variability. The scatter plots in Figs. 5 and 6 were generated by using Eq. 6 to selectively eliminate the predicted log P (Fig. 5) and V_5 (Fig. 6) contributions to the individual pK_i values to show the resulting linear relations for each of the two variables. This analysis suggests that a small 5-substituent with maximal hydrophobicity is required for optimal 5-HT$_2$ affinity.

An interesting finding was that, as for the 5-HT$_{1A}$ data, the best equation obtained predicting 5-HT$_2$ affinities for this set of compounds includes the electronic parameters Q_5 and R for the indole 5-substituent:

$$\mathrm{p}K_i = 4.1233 + 1.007(\log P) - 0.0332V_5 + 5.0946Q_5 + 1.47R \quad (7)$$

($n = 15$, $s = 0.361$, $r^2 = 0.8788$; F ratio $= 18.12$, $p < 0.001$.)

TABLE 3

Multiple regression summary for Eqs. 3–7

Dependent Variable	Independent Variables	Parameter Estimate	Standard Error	t Value	Probability Level[a]	Sequential r^2
pK_i at 5-HT$_{1A}$ (Eq. 3)	Intercept	7.0706	0.1680	42.10	0.0000	
	V_5	0.0324	0.0104	3.11	0.0090	0.1535
	V_5^2	−0.000411	0.000107	−3.84	0.0023	0.6204
pK_i at 5-HT$_{1A}$ (Eq. 4)	Intercept	6.9169	0.1486	46.55	0.0000	
	V_5	0.0400	0.0089	4.51	0.0007	0.1535
	$\log(10^{(V_5-b)} + 1)$	−0.0573	0.0107	−5.38	0.0002	0.7520
pK_i at 5-HT$_{1A}$ (Eq. 5)	Intercept	7.0092	0.1231	56.93	0.0000	
	V_5	0.03641	0.00693	5.25	0.0004	0.1535
	$\log(10^{(V_5-b)} + 1)$	−0.0506	0.00851	−5.95	0.0001	0.7519
	Q_5	−2.546	0.776	−3.28	0.0083	0.8001
	R	−0.8325	0.3206	−2.60	0.0266	0.8806
pK_i at 5-HT$_2$ (Eq. 6)	Intercept	4.4575	0.5622	7.93	0.0000	
	V_5	−0.0281	0.0052	−5.42	0.0002	0.3019
	log P	0.9022	0.2021	4.46	0.0008	0.7376
pK_i at 5-HT$_2$ (Eq. 7)	Intercept	4.123	0.450	9.16	0.0000	
	V_5	−0.0332	0.0043	−7.74	0.0000	0.3019
	log P	1.007	0.167	6.03	0.0001	0.7376
	Q_5	5.046	1.560	3.23	0.0090	0.8250
	R	1.465	0.696	2.11	0.0614	0.8788

[a] These probability levels apply to the significance of the individual variables within each equation. The F ratios and probability levels for the equations, as well as the value of the nonlinear coefficient b in Eqs. 4 and 5, are given in Results.

TABLE 4

Residual analysis: actual versus predicted values for Eqs. 5 and 7

Compound (5-substituent)	5-HT$_{1A}$ pK_i Values: Eq. 5			5-HT$_2$ pK_i Values: Eq. 7		
	Actual pK_i (5HT$_{1A}$)	Predicted value	Residual	Actual pK_i (5HT$_2$)	Predicted value	Residual
1b (H)	6.836	7.028	−0.192	7.174	6.945	0.229
1e (MeO)	7.676	7.767	−0.091	6.047	6.467	−0.420
1g (Br)	7.824	7.765	0.059	7.320	7.274	0.046
1h (Cl)	7.582	7.543	0.039	7.292	7.275	0.017
1i (F)	7.168	6.887	0.281	7.349	7.602	−0.253
1j (CONH$_2$)	8.276	7.955	0.321	4.774	4.657	0.117
1k (COOMe)	7.712	7.745	−0.033	5.436	5.617	−0.181
1l (COOEt)	7.442	7.495	−0.053	5.360	5.557	−0.197
1m (OOCMe)	7.094	7.196	−0.102	6.254	5.990	0.264
1n (Me)	7.498	7.742	−0.244	6.903	6.769	0.134
1p (NO$_2$)	7.434	7.475	−0.041	7.063	6.462	0.601
1q (CN)	7.260	7.197	0.063	6.020	6.578	−0.557
1r (OH)	7.210	7.278	−0.068	6.449	6.336	0.113
1s (benzyloxy)	6.955	6.829	0.126	6.401	6.088	0.313
1t (phthalimido)	6.424	6.489	−0.065	4.967	5.193	−0.226

TABLE 5

Multiple regression summary for Eqs. 8 and 9

Dependent Variable	Independent Variables	Parameter Estimate	Standard Error	t Value	Probability Level[b]	Sequential r^2
pK_i at 5-HT$_{1A}$ (Eq. 8)[a]	Intercept	7.0530	0.1247	56.55	0.0000	
	V_5	0.04005	0.00572	7.00	0.0000	0.0122
	$\log(10^{(V_5-b)}+1)$	−0.0533	0.0072	−7.38	0.0000	0.4771
	V_N	−0.0108	0.0034	−3.13	0.0069	0.5134
	I_1	−1.223	0.173	−7.09	0.0000	0.7602
	I_2	−0.765	0.173	−4.43	0.0005	0.8626
	Q_5	−2.718	0.915	−2.97	0.0095	0.8732
	R	−0.972	0.367	−2.65	0.0182	0.9137
pK_i at 5-HT$_2$ (Eq. 9)	Intercept	4.4147	0.3796	11.63	0.0000	
	V_5	−0.0352	0.0039	−9.01	0.0000	0.3060
	$\log P$	1.0704	0.1608	6.65	0.0000	0.4921
	V_N	−0.0224	0.00707	−3.17	0.0059	0.5164
	I_1	−1.528	0.284	−5.38	0.0001	0.7561
	Q_5	5.331	1.528	3.49	0.0030	0.7802
	R	1.897	0.619	3.06	0.0074	0.8615

[a] In Eq. 8, the nonlinear coefficient b is 23.4.

[b] See Table 3, footnote *a*.

QSAR for all compounds in Table 1A. By including the variables V_N, I_1, and I_2 (discussed in Materials and Methods), more inclusive equations predicting 5-HT$_{1A}$ and 5-HT$_2$ affinities for all the compounds in Table 1A were derived. For the 5-HT$_{1A}$ data, the best equation found was:

$$\mathrm{p}K_i = 7.0530 + 0.0401V_5 - 0.0533\log(10^{(V_5 - b)} + 1) - 0.0108V_N - 1.223I_1 - 0.7653I_2 - 2.72Q_5 - 0.972R \quad (8)$$

($b = 23.4$; $n = 23$, $s = 0.225$, $r^2 = 0.9137$; F ratio $= 22.68$, $p < 0.001$.)

For the 5-HT$_2$ data, the I_2 variable was not necessary:

$$\mathrm{p}K_i = 4.4147 + 1.07(\log P) - 0.0352V_5 - 0.0224V_N - 1.528I_1 + 5.33Q_5 + 1.90R \quad (9)$$

($n = 23$, $s = 0.361$, $r^2 = 0.8615$; F ratio $= 16.58$, $p < 0.001$.)

Table 7 contains the correlation matrix for all the variables used in the QSAR equations, calculated using data for the 23 compounds in Table 1A.

Molecular mechanics study of conformational properties of compounds 1b and 1c. The 2-methyl analogs (**1c** and **1f**) were synthesized with the expectation that this substituent would tend to force the tetrahydropyridyl ring out of coplanarity with the indole ring. Molecular mechanics calculations were performed using the SCAN option of CHEMLAB to generate a three-dimensional energy surface for various conformations of **1c** (Fig. 7A). The two torsional angles (Fig. 7B) define rotation of the pyridine ring (τ_1) and methyl group (τ_2). Energy is plotted inversely on the y-axis, with maxima shown projecting below the plateaus of minimum energy conformations.

The two plateaus correspond to the nonplanar conformations ($\tau_1 \neq 0$ or 180°). For each planar conformation massive energy barriers (about 80 kcal) are presented by the three values of τ_2 at which a methyl proton is *trans* to the indole-1 nitrogen. The planar conformation shown as Fig. 7B corresponds to a saddle point on Fig. 7A (marked with an asterisk) where a methyl proton is *cis* to the indole-1 nitrogen. This is the only type of 2-methyl conformation that could allow rotation of the pyridine ring between the two plateau areas representing energy minima; although its energy is only about 5 kcal/mole above the global energy minimum, it is clearly an unstable conformation.

Compared with the highest energy barriers of Fig. 7A, in the absence of the 2-methyl group (compound **1b**; R$_1$ = H) there is only a comparatively slight energy barrier (≈6 kcal above baseline; plotted in Fig. 8) at $\tau_1 = 0°$, corresponding to a steric

TABLE 6
Residual analysis: actual versus predicted values for Eqs. 8 and 9

Compound	5-HT$_{1A}$ pK_i Values: Eq. 8			5-HT$_2$ pK_i Values: Eq. 9		
	Actual pK_i (5HT$_{1A}$)	Predicted Value	Residual	Actual pK_i (5HT$_2$)	Predicted Value	Residual
1a	6.723	7.073	−0.350	6.691	6.952	−0.261
1b	6.836	6.892	−0.056	7.174	7.037	0.137
1c	5.748	5.674	0.074	6.061	6.055	0.006
1d	7.960	7.936	0.025	6.040	6.267	−0.227
1e	7.676	7.754	−0.078	6.047	6.352	−0.305
1f	6.462	6.536	−0.074	5.364	5.370	−0.006
1g	7.824	7.718	0.106	7.320	7.329	−0.009
1h	7.582	7.472	0.110	7.292	7.336	−0.044
1i	7.168	6.773	0.395	7.349	7.613	−0.264
1j	8.276	7.897	0.379	4.774	4.665	0.109
1k	7.712	7.701	0.011	5.436	5.684	−0.248
1l	7.442	7.468	−0.026	5.360	5.621	−0.261
1m	7.094	7.133	−0.039	6.254	5.996	0.258
1n	7.498	7.687	−0.189	6.903	6.807	0.096
1o	7.192	7.558	−0.366	6.785	6.494	0.291
1p	7.434	7.377	0.057	7.063	6.580	0.483
1q	7.260	7.068	0.192	6.020	6.712	−0.692
1r	7.210	7.220	−0.010	6.449	6.167	0.282
1s	6.955	6.870	0.085	6.401	5.968	0.433
1t	6.424	6.482	−0.058	4.967	5.154	−0.187
1u	6.035	6.222	−0.187	7.361	7.414	−0.053
2a	6.289	6.156	0.133	7.714	7.060	0.654
2b	6.862	6.995	−0.133	6.228	6.419	−0.191

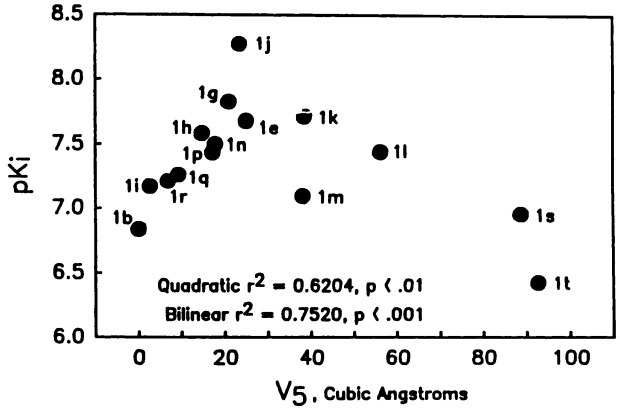


Fig. 4. Scatter plot of pK_i versus V_5 (5-substituent volume) with the 5-HT$_{1A}$ binding data for the 15 compounds differing only in their indole-5-substituent (marked * in Table 1A). Note the distinct bilinear distribution: Although the 5-acetoxy compound **1m** appears to be somewhat of an outlier, the QSAR analysis suggests that this compound has an unusually large electronic contribution; when this is taken into account (Eq. 7), its pK_i residual is only −0.102.

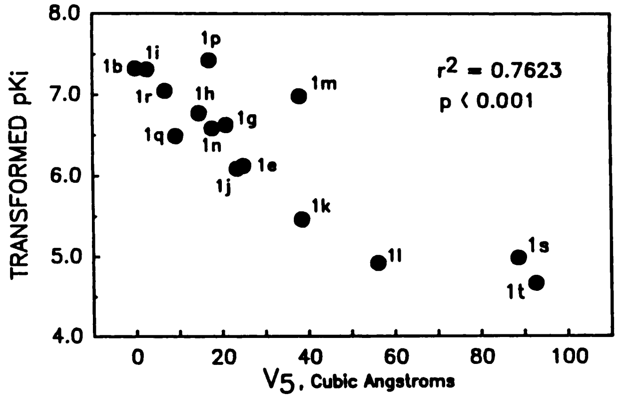


Fig. 5. Scatter plot of transformed 5-HT$_2$ pK_i versus V_5 (5-substituent volume) for the 15 compounds differing only in their indole-5-substituent (marked * in Table 1A). The log P contribution to the 5-HT$_2$ pK_i predicted by Eq. 6 was subtracted from the actual pK_i values to unmask the residual V_5 contribution, i.e., transformed pK_i = actual pK_i − 0.9022(log P − 3). This transformation predicts what the pK_i values would be if all the compounds had a log P of 3. A linear decrease in potency with increasing V_5 is seen.

interaction between the vinylic and indole-4 protons. On the scale of Fig. 7A, the other minor peaks of Fig. 8 would be negligible. The highest energy conformation of the 2-H compound (Fig. 8; $\tau_1 = 0°$) is approximately as far above the global energy minimum as the lowest energy planar conformation of the 2-methyl compound (the saddle point of Fig. 7A). Based on this analysis, the 2-methyl substituent provides a significant energy barrier to planar configurations, compared with the 2-H substituent.

Discussion

The QSAR analyses suggest distinct differences in the structural features that determine optimal potency at 5-HT$_{1A}$ sites versus those determining optimal potency for 5-HT$_2$ sites. The separate analysis of the 15 different N-methylpyridyl analogs differing only in their 5-substituent (Eqs. 3–7) resulted in the final Eqs. 5 and 7, which should be useful for optimizing the choice of indole-5-substituent to achieve either 5-HT$_{1A}$ or 5-HT$_2$ selectivity. The 5-substituent characteristics required for optimal potency at these two recognition sites can be briefly summarized as follows:

1) The parameter that almost exclusively determines potency for 5-HT$_{1A}$ sites is volume, the optimal size being about 24 cubic angstroms as calculated from the bilinear fit.

2) In contrast, at the 5-HT$_2$ site both volume and hydrophobicity play major but opposing roles for the 5-substituent. A balance between the smallest possible volume and the greatest possible hydrophobicity is required for maximal 5-HT$_2$ potency.

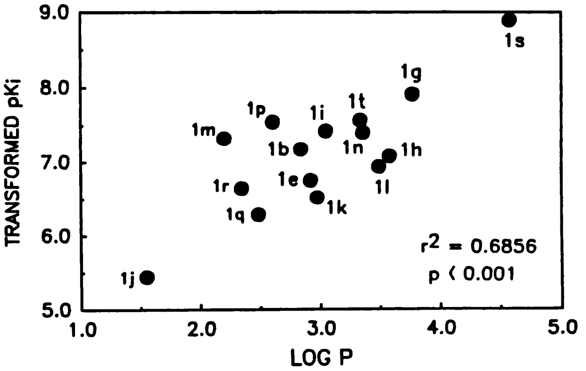


Fig. 6. Scatter plot of transformed 5-HT_2 pK_i versus log *P* for the 15 compounds differing only in their indole-5-substituent (marked * in Table 1A). The V_5 (5-substituent volume) contribution to the 5-HT_2 pK_i predicted by Eq. 6 was subtracted from the actual pK_i values to unmask the residual V_5 contribution, i.e., transformed pK_i = actual pK_i + 0.0281V_5. This transformation predicts what the pK_i values would be if all the compounds had 5-substituents the size of hydrogen (of course, it is physically impossible to attain such high log *P* values with such a small substituent). A linear increase in potency with increasing log *P* is seen.

3) A comparatively minor electronic contribution to the QSARs (Eqs. 5 and 7) involving the charge on the indole 5-carbon (Q_5) and the 5-substituent resonance constant *R* is of opposite sign for 5-HT_{1A} versus 5-HT_2 sites and thus may be useful for selective drug design.

Although we did not find any significant correlations with E_{HOMO}, the observed correlation with Q_5 is consistent with the results of a previous study of 5-substituted tryptamines using rat stomach fundus data (23). A correlation of 5-HT receptor affinity with the Swain and Lupton resonance constant *R* has also been previously reported (21). The significance of the electronic effect in our equations must be interpreted with caution, because there are correlations between some of the electronic parameters. For the set of 15 5-substituted compounds, there is a negative correlation between Q_5 and *R* (r^2 = 0.635) and also between Q_4 and Q_5 (r^2 = 0.811) and thus a positive correlation between Q_4 and *R* (r^2 = 0.479). The r^2 values from Table 7 are slightly different because they were calculated for the larger set of 23 compounds.) These correlations are of course somewhat to be expected, because a −R group should release electrons, particularly to the *ortho*-4-position. In these equations, the charge itself is the most significant electronic parameter, with Q_4 being slightly less so than Q_5 (thus equations involving Q_4 were not reported); addition of *R* to the correlations has the effect of making the entire electronic contribution more statistically significant. Although the probability level for *R* in Eq. 7 is not quite <0.05 (Table 3, Eq. 7; for *R*, $p = 0.0614$), *R* becomes considerably more significant when the rest of the compounds are included (Table 5, Eq. 9; for *R*, $p = 0.0074$). Thus, we have included *R* in Eq. 7 despite this borderline *p* value, particularly because addition of *R* to the equation increases the significance of Q_5 substantially.

In regard to the electronic contributions, it is noteworthy that the 5-acetoxy compound **1m**, which appears to be somewhat of an outlier in the steric fit of V_5 to 5-HT_{1A} data (Fig. 4), has an unusually large electronic contribution and falls well into line when Q_5 and *R* are added to the correlation (Table 4, Eq. 5; the residual value = −0.102.). The only true outlier is the 5-amino compound **1v**, which is so impotent that it was left out of the QSARs. This compound exhibited unusually high water solubility at neutral pH, which could be consistent with the existence of a tautomeric form, which would be expected to have a significantly different pK_a, and, more significantly, an altered electronic structure that could be very different from that of the other compounds. Such tautomerism has been reported for some 2-aminoindoles, for which a zwitterionic resonance structure has been postulated (57). Alternatively, because pK_a was not explicitly examined as a parameter, the low potency of this compound may be related to the (relative) basicity of the amino substituent. Whatever the reason, this compound was such an outlier that it could not be meaningfully included in the QSARs. This also suggests the caveat that one should be cautious about extrapolating QSAR equations to compounds whose substituent properties lie outside the range of those used to derive the equations.

Although we have found that steric and hydrophobic effects predominate over the relatively small electronic contribution to these QSARs, it is interesting that this appears to be related to the charges on the indole 4- or 5-carbons, because Weinstein *et al.* (27) find that the optimal orientation of the electrostatic vector for 5-HT passes between these two atoms. Thus, our findings are certainly not inconsistent with the Osman-Weinstein model (33). The predominance of nonelectronic determinants in our analysis may merely reflect a certain uniformity in the electronic structure of our compounds (none of which are substituted at the indole 4, 6, or 7 positions). Similarly, the lack of a role for steric and hydrophobic effects in their model is not surprising, because it is based on a set of compounds (tryptamine and four isomers of hydroxytryptamine) having rather minor steric and hydrophobicity differences.

The 5-substituent steric, hydrophobic, and electronic contributions to pK_i predicted by Eqs. 5 and 7 are substantiated by the results obtained with the inclusion of other types of substitutions in the QSAR, i.e., Eqs. 8 and 9. The coefficients of the terms related to the 5-substituent are very similar for Eq. 5

TABLE 7

Correlation matrix for all dependent variables used in Eqs. 3–9

The matrix was calculated using all compounds in Table 1A, with data from Table 2 and indicator variable values as defined in Materials and Methods; the other variables are defined in Table 2. Although not in the reported equations, Q_4 is included to illustrate collinearity with Q_5 and *R* (see Discussion).

	V_5	V_N	I_1	I_2	Q_4	Q_5	*R*	log *P*
V_5	1.0000	−0.1202	−0.1378	−0.1378	−0.2706	0.2842	−0.1085	0.3002
V_N		1.0000	−0.0111	−0.0111	0.0676	−0.1303	0.0865	0.5408
I_1			1.0000	−0.0952	−0.0614	0.0683	−0.1395	0.1678
I_2				1.0000	−0.1216	0.0542	−0.1395	−0.0268
Q_4					1.0000	−0.9096	0.7408	−0.0007
Q_5						1.0000	−0.8523	0.0998
R							1.0000	−0.2037
log *P*								1.0000

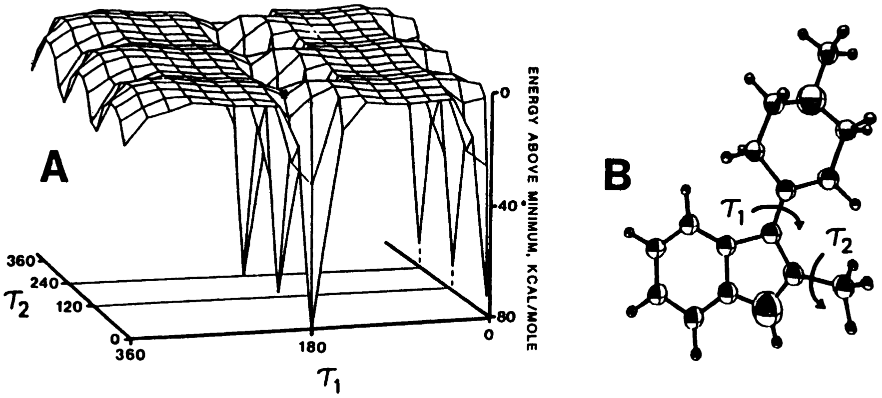


Fig. 7. A; Three dimensional energy surface for the 2-methyl THPI analog **1c**. Energy is plotted inversely on the *y* axis, so the two plateau areas represent energy minima. See text for discussion. B; Ortep structure of **1c** showing torsional angles τ_1 and τ_2 used to calculate energies in A. This planar conformation is not a minimum energy conformation, but corresponds to a saddle point indicated by an *asterisk* in A.

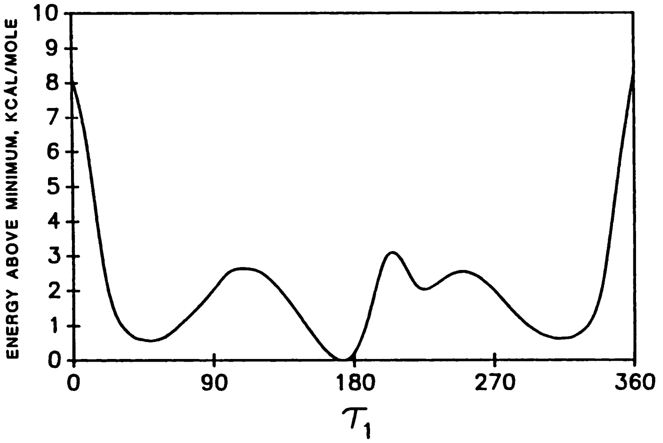


Fig. 8. Two-dimensional plot of energy versus conformation for the 2-H analog **1b**. The scale of the *y* axis is about one tenth that of Fig. 7A. The only significant energy barrier is the one at $\tau_1 = 0°$, corresponding to a steric interaction of the vinylic and indole-4 protons.

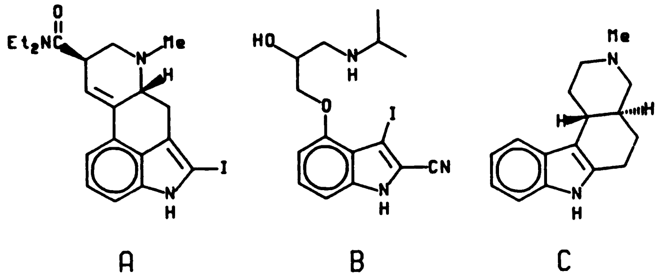


Fig. 9. A; The 5-HT$_2$ ligand [^{125}I]2-iodo-*d*-LSD; B; the 5-HT$_{1B}$ ligand (−)-[^{125}I]iodocyanopindolol; C; *trans*-1,2,3,4,4a,5,6,11c-octahydro-3-methyl-7*H*-pyrido[3,4-c]carbazole (see Discussion). All three of these compounds have indole-2-substituents roughly equivalent in size to the 2-methyl group, yet still possess relatively high affinity for at least one of the 5-HT receptor subtypes. Unlike the THPIs, in these compounds the 2-substituent is unable to perturb the geometry of the amino group; in the case of compound **C**, the 2-substituent actually helps to constrain the nitrogen in a near-coplanar position.

versus 8 and 7 versus 9. The coefficients of the additional parameters in Eqs. 8 and 9 suggest the following conclusions:

4) The inclusion of the 2-methyl substituent induces a loss in potency of about 1 order of magnitude at both 5-HT$_{1A}$ and 5-HT$_2$ sites.

5) At both sites, increase in pyridyl *N*-substituent volume *per se* causes a loss in affinity, but at the 5-HT$_2$ site it is important to note that this effect is largely offset by an increase in affinity due to increased hydrophobicity contributed by the *N*-substituent; thus, the *N*-benzyl analog **1u** is actually more potent than **1a** and **1b** at 5-HT$_2$ sites. The 1-benzyl analog **1w** (omitted from the QSAR for reasons discussed previously) is highly 5-HT$_2$ selective; its extremely low potency at the 5-HT$_{1A}$ site suggests a possible steric block at this position.

6) The saturation of the tetrahydropyridine double bond consistently reduces affinity for 5-HT$_{1A}$ sites; however, this alteration produces variable effects at 5-HT$_2$ sites, which were not well handled by the QSAR analysis, because I_2 was not sufficiently significant to be included in Eq. 9, presumably due to the exceptional potency of compound **2a**.

Reduction of the double bond results in increased flexibility of the piperidine ring and consequent altered geometry of the amine nitrogen; this is probably a major factor in the observed differences in activity between the unsaturated and reduced compounds. Because all of the THPIs have a relatively fixed geometry for the amino group, no major conclusions regarding its optimal configuration (e.g., relative to ergolines) can be drawn from this study. However, it is probable that the position of the amino nitrogen in these compounds is close to optimal for both 5-HT$_{1A}$ and 5-HT$_2$ sites, because as a class these compounds are remarkably potent by comparison with other indoleamines (15).

The data are consistent with the possibility that the indole and pyridine rings are in a coplanar configuration when binding at 5-HT$_{1A}$ and 5-HT$_2$ sites, inasmuch as the indole-2-methyl substituent, which provides a large energy barrier to the coplanar configuration, greatly reduces the potency of THPIs at all 5-HT recognition sites. A similar effect has been reported for 2-methyl serotonin at 5-HT$_1$ and 5-HT$_2$ sites (13). It is unlikely that this is due to a steric block at the indole 2-position, because high affinity 5-HT ligands exist that possess indole-2-substituents approximately equal in size to the 2-methyl group (Fig. 9, A–C). These include the 5-HT$_2$ ligand [^{125}H]2-iodo-*d*-LSD (58), the 5-HT$_{1B}$ ligand (−)-[^{125}I]iodocyanopindolol (59), and a relatively planar 2,3-bridge tryptamine homolog (60); the racemic *trans* isomer of the latter shows enhanced potency (about 10-fold) relative to tryptamine at the 5-HT$_2$ site.[2] It is significant that in all three of these compounds the indole-2-substituent cannot perturb the geometry of the amino nitrogen (in the compound shown in Fig. 9C, the 2-substituent actually helps constrain the nitrogen in a near-coplanar position). Thus, there is reason to suspect that the loss of affinity produced by 2-methylation for the THPIs and

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[2] M. Herslof, D. L. Nelson, and A. R. Martin, unpublished observations.

5-HT is primarily due to the effects on conformation of the 3-substituent and not a direct steric block at the 2-position itself.

One of the goals of this research is to obtain information useful for the design of selective 5-HT ligands. These might include other classes of compounds such as tryptamines or 2-aminotetralins. An encouraging result of the current study is that similarities in analog selectivity patterns can be observed between these THPI derivatives and tryptamines. For example, the exceptionally high potency of 5-carboxamidotryptamine for 5-HT$_{1A}$ sites and low affinity for 5-HT$_2$ sites is precisely paralleled by the 5-carboxamido THPI derivative **1j**, the 5-HT$_{1A}$ selectivity of which is clearly predicted by these QSARs (see Tables 4 and 6). Such parallels suggest that the indolic portion of these compounds binds similarly to that of other indole derivatives; thus, it is possible that optimally selective substituents predicted by these QSARs may be extrapolated to tryptamines and other indoles. We offer these equations to those desiring to do so.

Acknowledgments

The authors would like to thank Brian Weck for contributing the 2-methyl compound **1c**; Suketu Desai, for technical assistance; Dr. Philip Monroe, for assistance with Sigmaplot; and Dr. Ioan Motoc, for his valuable comments on the manuscript.

References

1. Euvrard, C. R., and J. R. Boissier. Biochemical assessment of the central 5-hydroxytryptamine agonist activity of RU 24969, a piperidinyl indole. *Eur. J. Pharmacol.* **63**:65–72 (1980).
2. Hunt, P., L. Nedelec, C. Euvrard, and J. R. Boissier. Tetrahydropyridinyl indole derivatives as serotonin analogues which may differentiate between two distinct receptor types. *Int. Congr. Pharmacol.* **8**:659, abstr. 1434 (1981).
3. Hunt, P., and C. Oberlander. The interaction of indole derivatives with the serotonin receptor and non-dopaminergic circling behavior, in *Serotonin—Current Aspects of Neurochemistry and Function* (B. Haber, ed.). Plenum Press, New York 547–562 (1981).
4. Guillaume, J., C. Dumont, J. Laurent, and L. Nedelec. (Tetrahydro-1,2,3,6-pyridinyl-4)-3-1*H* indoles: synthese, propriétés serotonergique et anti-dopaminergiques. *Eur. J. Med. Chem.* **22**:33–43 (1987).
5. Hoyer, D., G. Engel, and H. O. Kalkman. Molecular pharmacology of 5-HT$_1$ and 5-HT$_2$ recognition sites in rat and pig brain membranes: radioligand binding studies with $[^3H]$5-HT, $[^3H]$8-OH-DPAT, (−)$[^{125}I]$iodocyanopindolol, $[^3H]$mesulergine and $[^3H]$ketanserin. *Eur. J. Pharmacol.* **118**:13–23 (1985).
6. Taylor, E. W., S. P. Duckles, and D. L. Nelson. Dissociation constants of serotonin agonists in the canine basilar artery correlate to K_i values at the 5-HT$_{1A}$ binding site. *J. Pharmacol. Exp. Ther.* **236**:118–125 (1986).
7. Peroutka, S. J. Pharmacological differentiation and characterization of 5-HT$_{1A}$, 5-HT$_{1B}$ and 5-HT$_{1C}$ binding sites in rat frontal cortex. *J. Neurochem.* **47**:529–540 (1986).
8. Middlemiss, D. N. The putative 5-HT$_1$ receptor agonist, RU 24969, inhibits the efflux of 5-hydroxytryptamine from rat frontal cortex slices by stimulation of the 5-HT autoreceptor. *J. Pharm. Pharmacol.* **37**:434–437 (1985).
9. Brazell, M. P., C. A. Marsden, A. P. Nisbet, and C. Routledge. The 5-HT$_1$ receptor agonist RU 24969 decreases 5-HT release and metabolism in the rat frontal cortex *in vitro* and *in vivo*. *Br. J. Pharmacol.* **86**:209–216 (1985).
10. Green, A. R., A. P. Guy, and C. R. Gardner. The behavioral effects of RU 24969, a suggested 5-HT$_1$ agonist, in rodents and the effect on the behavior of treatment with antidepressants. *Neuropharmacology* **23**:655–662 (1984).
11. Tricklebank, M. D., D. N. Middlemiss, and J. Neill. Pharmacological analysis of behavioral and thermoregulatory effects of the putative 5-HT$_1$ receptor agonist, RU 24969, in the rat. *Neuropharmacology* **25**:877–886 (1986).
12. Sills, M. A., B. B. Wolfe, and A. Frazer. Determination of selective and non-selective compounds for the 5-HT$_{1A}$ and 5-HT$_{1B}$ receptor subtypes in rat frontal cortex. *J. Pharmacol. Exp. Ther.* **231**:480–487 (1984).
13. Richardson, B. P., G. Engel, P. Donatsch, and P. A. Stadler. Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. *Nature (Lond.)* **316**:126–131 (1985).
14. Pedigo, N. W., H. I. Yamamura, and D. L. Nelson. Discrimination of multiple $[^3H]$5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. *J. Neurochem.* **36**:220–226 (1981).
15. Taylor, E. W., S. Nikam, B. Weck, A. R. Martin, and D. L. Nelson. Relative selectivity of some conformationally constrained tryptamine analogs at 5-HT$_1$, 5-HT$_{1A}$ and 5-HT$_2$ recognition sites. *Life Sci.* **41**:1961–1969 (1987).
16. Kang, S., and J. P. Green. Steric and electronic relationships among some hallucinogenic compounds. *Proc. Natl. Acad. Sci. USA* **67**:62–67 (1970).
17. Kang, S., and J. P. Green. Correlation between activity and electronic state of hallucinogenic amphetamines *Nature (Lond.)* **226**:645 (1970).
18. Nichols, D. E., A. T. Shulgin, and D. C. Dyer. Directional lipophilic character in a series of psychotomimetic phenethylamine derivatives. *Life Sci.* **21**:569–575 (1977).
19. Domelsmith, L. N., and K. N. Houk. Photoelectron spectroscopic studies of hallucinogens: the use of ionization potentials in QSAR. *Res. Monogr. Natl. Inst. Drug Abuse Ser.* **22**:423–440 (1978).
20. Weinstein, H., J. P. Green, R. Osman, and W. D. Edwards. Recognition and activation mechanisms on the LSD/serotonin receptor: the molecular basis of structure-activity relationships. *Res. Monogr. Natl. Inst. Drug Abuse Ser.* **22**:333–358 (1978).
21. Green, J. P., and S. Kang. The correlation of electronic structures of indole derivatives with their biological activities, in *Molecular Orbital Studies in Chemical Pharmacology* (L. B. Kier, ed.). Springer-Verlag, New York 105–120 (1970).
22. Gomez-Jeria, J. S., and D. R. Morales-Lagos. Quantum chemical approach to the relationship between molecular structure and serotonin receptor binding affinity. *J. Pharm. Sci.* **73**:1725–1728 (1984).
23. Gomez-Jeria, J. S., D. Morales-Lagos, J. I. Rodriguez-Gatica, and J. C. Saavedra-Aguilar. Quantum-chemical study of the relation between electronic structure and pA$_2$ in a series of 5-substituted tryptamines. *Int. J. Quantum Chem.* **28**:421–428 (1985).
24. Gomez-Jeria, J. S., D. Morales-Lagos, B. K. Cassels, and J. C. Saavedra-Aguilar. Electronic structure and serotonin receptor binding affinity of 7-substituted tryptamines: QSAR of 7-substituted tryptamines. *Quant. Struct.-Act. Relat.* **5**:153–157 (1986).
25. Chan, Y. L., E. J. Lien, and J. C. Shih. QSAR of agents involved in the serotonin and LSD binding sites. *Res. Monogr. Natl. Inst. Drug Abuse Ser.* **22**:103–113 (1978).
26. Bennett, J. P., and S. H. Snyder. Serotonin and lysergic acid diethylamide binding in rat brain membranes: relationship to postsynaptic serotonin receptors. *Mol. Pharmacol.* **12**:373–389 (1976).
27. Weinstein, H., D. Chou, S. Kang, C. L. Johnson, and J. P. Green. Reactivity characteristics of large molecules and their biological activity: indolealkylamines on the LSD/serotonin receptor. *Int. J. Quantum Chem.: Quantum Biol. Symp.* **3**:135–150 (1976).
28. Shannon, M., G. Battaglia, R. A. Glennon, and M. Titeler. 5-HT$_1$ and 5-HT$_2$ binding properties of the hallucinogen 1-(2,5-dimethoxyphenyl)-2-aminopropane (2,5-DMA). Eur. J. Pharmacol. **102**:23–29 (1984).
29. Titeler, M., K. Herrick, R. A. Lyon, J. D. McKenney, and R. A. Glennon. $[^3H]$DOB: a specific agonist radioligand for 5-HT$_2$ serotonin receptors. *Eur. J. Pharmacol.* **117**:145–146 (1985).
30. Glennon, R. A., J. D. McKenney, R. A. Lyon, and M. Titeler. 5-HT$_1$ and 5-HT$_2$ binding characteristics of 1-(2,5-dimethoxy-4-bromophenyl)-2-aminopropane analogues. *J. Med. Chem.* **29**:194–199 (1986).
31. Swain, C. G., and E. C. Lupton. Field and resonance components of substituent effects. *J. Am. Chem. Soc.* **90**:4328–4337 (1968).
32. Weinstein, H., R. Osman, W. D. Edwards, and J. P. Green. Theoretical models for molecular mechanisms in biological systems: tryptamine congeners acting on an LSD-serotonin receptor. *Int. J. Quantum Chem.: Quantum Biol. Symp.* **5**:449–461 (1978).
33. Osman, R., S. Topiol, L. Rubenstein, and H. Weinstein. A molecular model for activation of a 5-hydroxytryptamine receptor. *Mol. Pharmacol.* **32**:699–705 (1987).
34. Kolb, V. M. Electron-transfer and charge-transfer clastic binding hypotheses for drug-receptor interactions. *Pharm. Res.* **4**:450–456 (1987).
35. Hansch, C., and A. Leo. *Substituent Constants for Correlation and Analysis in Chemistry and Biology.* Wiley-Interscience, New York (1979).
36. Hansch, C., A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien. "Aromatic" substituent constants for structure-activity correlations. *J. Med. Chem.* **16**:1207–1216 (1973).
37. Balaban, A. T., A. Chiriac, I. Motoc, and Z. Simon. Steric fit in quantitative structure-activity relationships. *Lecture Notes in Chemistry* (G. Berthier *et al.* eds.), Vol. 15. Springer-Verlag, New York (1980).
38. Motoc, I., G. R. Marshall, R. A. Dammkoehler, and J. Labanowski. Molecular shape descriptors. 1. Three-dimensional molecular shape descriptors. Z. Naturforsch Teil *A Physics Phys. Chem. Cosmic Phys.* **40**:1108–1113 (1985).
39. Hopfinger, A. J. A QSAR investigation of dihydrofolate reductase inhibition by Baker triazines based upon molecular shape analysis. *J. Am. Chem. Soc.* **102**:7196–7206 (1980).
40. Mabilia, M., R. A. Pearlstein, and A. J. Hopfinger. Molecular shape analysis and energetics-based intermolecular modelling of benzylpyrimidine dihydrofolate reductase inhibitors. *Eur. J. Med. Chem.* **20**:163–174 (1985).
41. Motoc, I., and G. R. Marshall. Van der Waals volume fragmental constants. *Chem. Phys. Lett.* **116**:415–419 (1985).
42. Freter, K. 3-Cycloalkenylindoles. *J. Org. Chem.* **40**:2525–2529 (1975).
43. Freter, K., and V. Fuchs. Synthesis of pyrido[3,4-b]pyrano[3,4-b]indoles. *J. Heterocycl. Chem.* **19**:377–379 (1982).
44. Leysen, J. E., J. N. Neimegeers, J. M. V. Neuten, and P. M. Laduron. $[^3H]$ Ketanserin, a selective ^3H-ligand for serotonin$_2$ receptor binding sites. *Mol. Pharmacol.* **21**:301–314 (1982).
45. Cheng, Y., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration which causes 50 percent inhibition (IC$_{50}$) of an enzymatic reaction. *Biochem. Pharmacol.* **22**:3099–3108 (1973).

46. Hopfinger, A. J., and R. A. Pearlstein. Molecular mechanics force-field parameterization procedures. *J. Comput. Chem.* **5**:486–499 (1984).
47. Rekker, R. F. The hydrophobic fragmental constant: its derivation and application, a means of characterizing membrane systems. Elsevier Publishing, New York (1977).
48. Rekker, R. F. The hydrophobic fragmental constant: extension to a 1000 point data set. *Eur. J. Med. Chem.* **14**:479–488 (1979).
49. Cammarata, A. Quantum perturbation theory and linear free energy relationships in the study of drug action, in *Molecular Orbital Studies in Chemical Pharmacology* (L. Kier, ed.). Springer-Verlag, New York, 156–190 (1970).
50. Gomez-Jeria, J. S. On some problems in quantum pharmacology. I. the partition functions. *Int. J. Quantum Chem.* **23**:1969–1972 (1983).
51. Franke, R. *Theoretical Drug Design Methods.* Pharmacochemistry Library Series, Vol. 7, Elsevier, New York (1984).
52. Free, S. M., and J. W. Wilson. A mathematical contribution to structure-activity studies. *J. Med. Chem.* **7**:395–399 (1964).
53. Leo, A., and C. Hansch. Linear free-energy relationships between partitioning solvent systems. *J. Org. Chem.* **36**:1539–1544 (1971).
54. Kubinyi, H. Quantitative structure-activity relationships: non-linear dependence of biological activity on hydrophobic character: a new model. *Arzneim.-Forsch.* **26**:1991–1997 (1976).
55. Kubinyi, H. Quantitative structure-activity relationships. 7. The bilinear model, a new model for nonlinear dependence of biological activity on hydrophobic character. *J. Med. Chem.* **20**:625–629 (1977).
56. Hansch, C., R. Li, J. Blaney, and R. Langridge. Comparison of the inhibition of *E. Coli* and *L. Casei* dihydrofolate reductase by 2,4-diamino-5-(substituted-benzyl)pyrimidines: QSAR, X-ray crystallography, and computer graphics in structure-activity analysis. *J. Med. Chem.* **25**:777–784 (1982).
57. Elguero, J., C. Marzin, A. R. Katritzky, and P. Linda. *The Tautomerism of Heterocycles.* Academic Press, New York (1976).
58. Hartig, P. R., M. J. Kadan, M. J. Evans, and A. M. Krohn. ^{125}I-LSD, a high sensitivity ligand for serotonin receptors. *Eur. J. Pharmacol.* **89**:321–322 (1983).
59. Hoyer, D., G. Engel, and H. O. Kalkman. Characterization of the 5-HT$_{1B}$ recognition site in rat brain: binding studies with (−)[^{125}I]iodocyanopindolol. *Eur. J. Pharmacol.* **118**:1–12 (1985).
60. Herslof, M., and A. Martin. Synthesis of a new conformationally defined serotonin homologue by intramolecular [4 + 2] cycloaddition. *Tetrahedron Lett.* **28**:3423–3426 (1987).

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