Table IV. Force Field Parameters[a]

Natural Bond Length, Stretching Force Constant, and Bond Moment

bond	l_0, Å	K_s, mdyn/Å	moment, D
N:-N	1.33	5.6	0.0

Natural Bond Angles and Bonding Constants

angle	θ_0, deg	k_θ, mdyn Å/rad^2
C-N:-N	115.0	0.43
N-N:-LP	122.5	0.50
C'-N-N:	120.0	0.43
H-N-N:	113.0	0.36
out of plane bending		
N:-N		0.05
N-N:		0.05

Torsional Constants, kcal/mol

angle	V_1	V_2	V_3
A-N:-N-B	0.0	1.825	0.0
N:-N-C'=O	0.0	5.0	0.0
N:-N-C'-C''	0.0	5.0	0.0
N-N:-C-D	0.0	10.0	0.0
C''-C''-C-N:	-0.044	0.24	0.06

[a] C (C_{sp2}, alkene, imine); C' (C_{sp2}, carbonyl); C'' (C_{sp3}); LP (lone pair); N: (imine), N (amide); A (C, LP); B (H, C'); D (C, C'').

constant values of compound **3a** have been evaluated with a first-order approach. Some signals in the spectra of **4a** and **5a** were partially overlapped; a spin system simulation using the PANIC program of Brüker allowed the determination of the spectral parameters and a straightforward assignment of all the signals.

Molecular mechanics calculations were performed with the MM2(85)-PC program[4] obtained from QCPE. The parameter set was updated with the parameter list MM2(1987)-VAX kindly furnished by Prof. Allinger. Moreover, in the KOMEGA.FOR subroutine, three lines above line 135, the instruction IPOMG(NTPI) = IOMG(NTPI) was modified to IPOMG(NTPI) = IOMG(I). This correction was necessary in order to properly calculate molecules containing both an unsaturated and a saturated moiety; without the modification the V_2 torsional parameter for the bonds in the conjugated moiety are not correctly reduced as a function of the bond order. The program was then extensively tested, particularly on the molecules whose calculations are reported in ref 4, and gave satisfactory results.

A value of 4.7 for the dielectric constant was used during the calculations. The six phenyl carbon atoms and C and N imine atoms were considered π atoms. The parameters not included in the parameter list were chosen by analogy with similar atomic arrangements and are reported in Table IV. The natural bond length of the N1-N2 bond as well as the V_2 torsional parameters involving the same bond were chosen on the basis of MNDO calculations[12,13] performed by us on the model compound CH_2=NNHCHO. In order to test the influence of the values of these parameters on the calculations, they were varied over a reasonable range; these variations do not significantly affect the results.

The conformational space relative to the rotation of the phenyl ring of compounds **1a** and **2a** with respect to the dihydropyridazinone ring was explored by using the MM2 dihedral driver (NDRIVE = -1, 5° incremental changes). By examination of molecular models the candidate starting geometries of compounds **3a**, **4a**, and **5a** were identified and the energy was minimized. Moreover, extensive application of the dihedral driver (NDRIVE = 1, 5° incremental changes) to the bonds in rings II and III allowed the full exploration of their conformational space. Several local minima were found for compounds **4a** (three minima) and **5a** (eight minima) and the most significant are reported in Table II.

Vicinal ^{1}H NMR coupling constants were calculated with the Haasnoot et al.[5] modification of the Karplus equation utilizing the 3JHHPC program.[14]

Acknowledgment. We thank Dr. Pierangela Ciuffreda for the 500-MHz ^{1}H NMR spectra and Ministero della Pubblica Istruzione (Rome) for financial support.

Registry No. **1a**, 1011-46-7; **2a**, 110766-33-1; **3a**, 69099-74-7; **3b**, 103422-53-3; **3c**, 103422-54-4; **4a**, 25823-48-7; **4b**, 103422-66-8; **4c**, 103422-56-6; **5a**, 25742-87-4; **5b**, 103603-08-3; **5c**, 103603-09-4.

Supplementary Material Available: MM2-calculated atomic coordinates of the minimum energy conformers of compounds **1a**, **2a**, **3a**, **4a**, and **5a** (9 pages). Ordering information is given on any current masthead page.

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(13) QCPE program no. QCMP002.
(14) QCPE program no. QCMP025.

Conformation-Activity Relationship Study of 5-HT$_3$ Receptor Antagonists and a Definition of a Model for This Receptor Site

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A conformation-activity relationship study of 5-HT$_3$ receptor antagonists was used to define a pharmacophore and receptor map to qualitatively account for their activity. The design and synthesis of specific keto-amino-indole derivatives that are potent 5-HT$_3$ receptor antagonists gave some support to the model.

There is now substantial evidence for the existence of multiple 5-HT receptor subtypes, recently classified into three major categories designated "5-HT$_1$-like", 5-HT$_2$, and 5-HT$_3$.[1] The remarkable recent advances in our understanding of 5-HT neurotransmission reflect, in large part, the increasing availability of compounds with selectivity and potency for individual 5-HT receptor subtypes,[2] which led to proposals of models for the corresponding recognition sites.[3-5]

It is perhaps in the 5-HT$_3$ receptor area where the most spectacular developments have occurred.[6] In particular,

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Table I. Functional and Binding Activity of 5-HT_3 Receptor Antagonists

compd	pA_2 afferent rabbit vagus[a]	pA_2 autonomic rabbit heart[b]	pA_2 enteric guinea pig ileum[c]	pK_D:[16] [^{3}H]-7, mouse N1E 115 cells[d]	pK_I [^{3}H]-11,[9] rat cortex[e]	pK_I [^{3}H]-15,[12] rat cortex[f]
3	-	7.7[8]	-	-	-	-
4		7.2[8]	-	-	-	-
1	-	7.3[8]	-	6.65	6.46	6.7
5	7.9[14]	9.3[15]	<6[15]	8.21	7.26	-
6	-	9.0	-	-	-	-
14	-	9.8	-	-	-	-
7	10.2[17]	10.6[17]	7.9[17]	9.09	8.50	9.22
9	-	-	-	9.80	-	-
8	-	-	-	9.14	-	-
12	9.9[8]	10.7[19]	8.1[19]	8.85	9.23	8.04
13	8.5[8]	8.9[18]	7.6[18]	8.50	-	-
11	-	-	-	-	8.79	-
(***R***)-**10**	9.4[8]	10.1[8]	7.3[8]	7.9	8.48	-
(***S***)-**10**	-	-	-	-	8.27	-
15	-	-	-	8.69	8.85	9.0
22		8.3	-	-	-	-
23		9.2	-	-	-	-

[a] Potency of 5-HT antagonists on the desheated rabbit vagus nerve.[8,14,17] [b] Potency at neuronal 5-HT receptors in the rabbit heart.[8,15,17-19] [c] Potency at enteric 5-HT receptors in the guinea pig ileum.[8,15,17-19] [d-f] Binding assays using [^{3}H]-7, [^{3}H]-11, and [^{3}H]-15 as radioligands, respectively.[9,12,16]

a number of potent and selective antagonists have been developed that may represent important drugs since several of them are currently being evaluated in clinics as antiemetic, antischizophrenic, and anxiolytic agents.

We report here a conformation–activity relationship study of the most representative 5-HT_3 receptor antagonists and a graphics computer-generated model of the 5-HT_3 recognition site which qualitatively accounts for the activity of all the known 5-HT_3 ligands.

Material and Methods

Receptor Mapping. The general approach that has been followed was defined by Marshall[7] and can be outlined as follows: (i) critical examination of compounds active or inactive at the target receptor, (ii) graphics computer-aided definition of a pharmacophore, (iii) three-dimensional-graphics computer-assisted mapping of the recognition site, and (iv) use of the previously defined pharmacophore and receptor map to design original ligands whose biological activity would validate the model. In spite of its obvious limitations, this approach proved to be very efficient in the case of the 5-HT_{1A} recognition site,[3] and the same strategy has been applied to 5-HT_3 receptors as will be discussed below.

(i) Evaluation of Pharmacological Activity. For several years the activity of 5-HT_3 receptor antagonists has been evaluated in a number of functional assays such as the excitatory effects of 5-HT on afferent autonomic (rabbit vagus, rabbit heart) or enteric (guinea pig ileum) neurones[8] (Table I). More recently, binding sites which seem to correspond to the 5-HT_3 receptor have been identified both in brain[9] and cell lines[10] with tritiated **11** ([^{3}H] GR 65630) and **7** ([^{3}H]ICS 205-930), respectively, as ligands. A number of other radioligands have since been used, including [^{3}H]zacopride,[11] [^{3}H]quipazine,[12] and [^{3}H]GR 67330.[13] The affinities of several compounds for functional 5-HT_3 receptors and for these ligand-binding sites are summarized in Table I.

There is relatively good consistency between the results obtained in the different binding assays, suggesting that the compounds from Table I interact at the same recognition site. There is rather more variability in antagonist affinities determined in functional experiments and although this may result from slightly different experimental protocols or indicate the existence of subtypes of 5-HT_3

Table II. Conformation–Activity Relationship among 5-HT_3 Receptor Antagonists

compd	increment,[a] deg	E_{min},[b] kcal mol^{-1}	E_{act},[c] kcal mol^{-1}	ΔE,[d] kcal mol^{-1}	RMS,[e] Å
1	3	-1.70	2.39	4.09	0.41
5	3	5.56	5.64	0.08	0.14
6	3	5.70	5.87	0.17	0.14
14	3	20.70	21.03	0.33	0.00
7	3	21.43	21.89	0.46	0.05
9	3	21.24	21.68	0.44	0.05
12	3	24.14	24.49	0.35	0.07
10	3	41.15	41.37	0.22	0.11
15	1	-1.26	-0.99[f]	0.27	0.15[f]
5-HT	3	15.98	16.00	0.02	1.02
22	3	14.76	16.09	1.33	0.30
23	3	12.08	14.62	2.54	0.22

[a] Increment chose to rotate stepwise around the flexible bonds to perform the conformational analysis. [b] Global minimum energy: the energy represents the sum of bond, bond angle, torsional angle and van der Waals energy terms.[28] [c] Total energy of the putative active conformation. [d] Difference between the active conformer energy and the global minimum energy. [e] Root mean square index measured by using six reference points. [f] The lone pair of electrons of the quinoline nitrogen atom has been considered.

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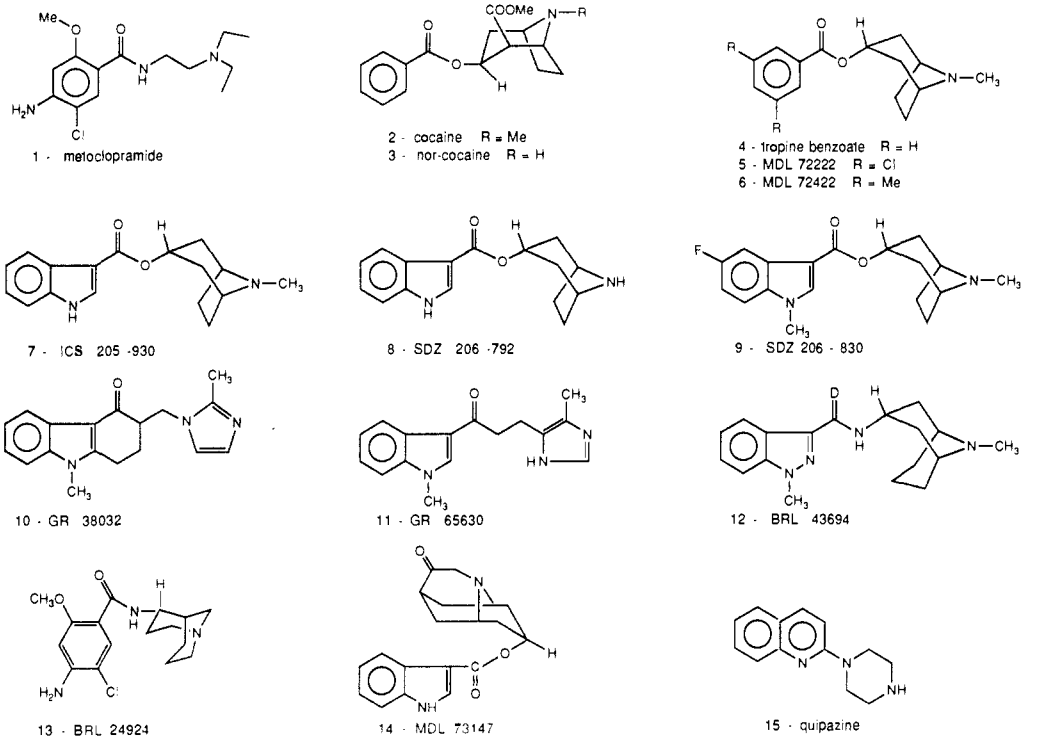
Chart I



receptors,[20,21] it probably means that other activities of these compounds, related to effects on ion channels,[22,23] interfere with the functional responses of the tissues used. Taking these limitations into account, it seems likely that there is only a single 5-HT_3 recognition site.

(ii) Definition of a Pharmacophore. As can be seen from Chart I, some of the 5-HT_3 receptor antagonists form a group of closely related structures [**3**, **5**, **7**, **12** (norcocaine, MDL 72222, ICS 205-930, BRL 43694, respectively)], while other compounds such as **1**, **10**, and **15** (metoclopramide, GR 38032, quipazine, respectively) are not closely related to them or to each other.

Examination of the structures led us to hypothesize that three reference structural features defining the pharmacophore could be the aromatic ring, the carbonyl group, and a basic atom or center that they all have in common, if one excepts **15**. Topographical characterization of the pharmacophore was attempted as follows: models of the most representative antagonists (Table II) were built with a μVAXII computer, Evans and Sutherland PS 390 terminal, and the SYBYL 5.2 molecular modeling package (Tripos).[24] Except for **5**, where the crystallographic structure was used,[25] the molecules were built from fragments extracted from the Cambridge crystallographic databank, and their geometry was optimized by using a combination of first and nonderivative methods (Maximin 2[26-30]).

Before looking for the major features that the considered group of compounds had in common, their energetically permissible conformations were ascertained. Thus, for each compound, rotatable bonds were assigned and a conformational search was made, allowing the bonds to rotate with a 3° stepwise increment of the dihedral angles. The electrostatic term was purposely not considered at this early stage of the study since we thought it would have represented an additional source of approximation rather than an improvement. Thus, we consider as irrelevant any method of charge calculation in such a case where nothing is known about the receptor and the microenvironment of the ligand in its binding site (e.g. nature of the counterions, ligand binding in protonated or nonprotonated form, etc.). The same search criteria have been applied to all the molecules investigated. No conformation was eliminated from the search based on energy. Angle files were thus produced and the internal energy corresponding to each conformation was evaluated (Tripos force field[27,28]). For each compound we then optimized the energy of the most stable conformer obtained by using a combination of first and nonderivative methods.[26-30] We thus obtained for each molecule an estimation of the energy of the most stable conformer (E_{min}, Table II) within the limits of the method.

The fitting attempt was made with the MAXIMIM MULTIFIT program of SYBYL 5.2. This method, based on molecular mechanics, will force the chosen reference molecular features into an optimized fit at the cost of some conformational energy.[30,32] The molecules are then relaxed to

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(25) Carpy, A.; Lemrabett, A.; Colleter, J. C. *Acta Crystallogr.*, in press.
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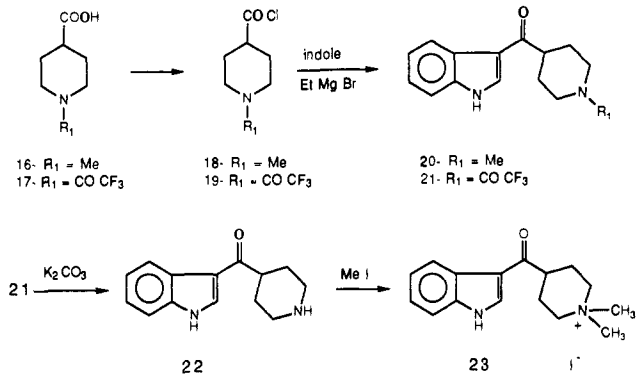
Scheme I



the closest minima, which obviously may not coincide with the global minimum energy. The structural features of the molecules that have been considered in the matching process are normal to the aromatic ring (2 Å long vector centered on the aromatic ring centroid), the vector corresponding to the carbonyl group of the compounds and a 1 Å long vector corresponding to the lone pair of electrons carried by the basic nitrogen atom.

In the particular case of **10**, we considered that the electrons or the positive charge would be essentially delocalized between the two nitrogen atoms of the imidazole ring. For this molecule, instead of the nitrogen atom lone pair, we took as molecular determinant a 2-Å vector normal to the imidazole ring centered on the N–C–N centroid. The two most constrained compounds, **10** and **5**, have first been submitted to the flexible fit. Each individual compound has then been fitted with the features thus obtained. The quality of the superimposition was measured by the room mean square (RMS) index which was defined by using six points corresponding to the extremities of the three reference vectors. The cost in internal energy (ΔE) was estimated for each molecule by comparing the energy of the putative receptor-bound conformation (E_{act}) to the energy of the most stable conformer (E_{min}) characterized as described above. We considered this difference in energy only as an indication of the possibility for a molecule to exist in a considered conformation in the binding site.

(iii) Receptor Mapping. With use of the superimposed putative receptor-bound conformers, a van der Waals surface corresponding to their envelope was defined. This generated a volume which can be considered as a zone accessible to ligands that are capable of preventing activation of the 5-HT$_3$ receptor recognition site.

(iv) Drug Design. In order to test the receptor model a compound was designed that retained the basic molecular determinants in a structure as simple as possible: an indole moiety, which, according to the SAR study, seemed to be preferable to a simple benzene ring; a ketone group, in order to evaluate the sufficiency of a ketone carbonyl compared to an ester or amide function; and a tertiary amine incorporated into a simple cyclic system.

Compound **22** was thus prepared in a straightforward manner according to Scheme I. A check prior to synthesis indicated that this compound fits the pharmacophore (RMS = 0.3 Å) in a stable conformation (ΔE = 1.3 kcal mol^{-1}).

The quaternary ammonium derivative of this molecule was also prepared in order to evaluate the activity of 5-HT$_3$ receptor antagonists in their protonated form as compared to that of the free base.

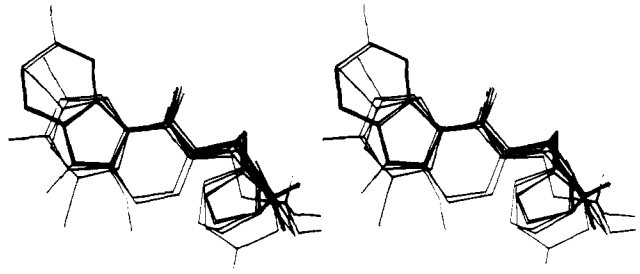


Figure 1. Superimposition of **1**, **5**, **6**, **7**, **9**, **10**, **12**, **14** and **15** in their putative 5-HT$_3$ receptor-bound conformation (relaxed stereo view).

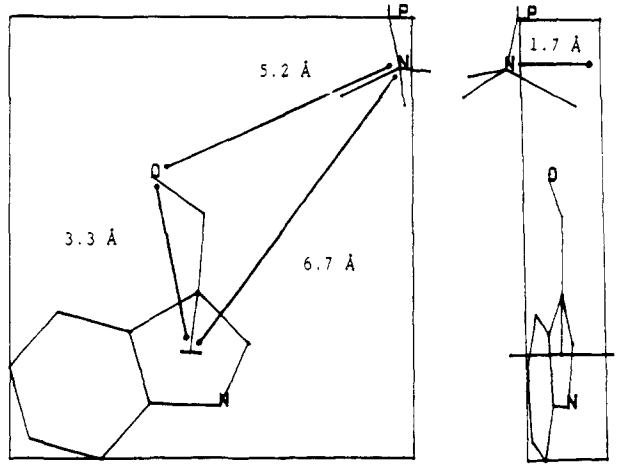


Figure 2. Basic pharmacophore of the 5-HT$_3$ receptor antagonist.

Results and Discussion

As demonstrated by previous works,[33] the low-energy conformations (global or secondary energy minima calculated in gas phase) are not necessarily the active forms of a given ligand. Moreover, the binding process of a ligand to its receptor can involve compensation as high as 30 kcal mol^{-1} for the required increase in conformational energy.[33] Therefore it seems reasonable to consider that, for all the molecules studied, it was possible to find stable conformations ($\Delta E \leq 4$ kcal mol^{-1}) where the structural determinants were superimposed (RMS $\leq$ 0.4; Table II, Figure 1). Thus aromatic rings, carbonyl functions, and basic centers of the different molecules can occupy the same relative position in space and probably interact with the same residues of the 5-HT$_3$ recognition site. If the working hypothesis is correct, then these structural features in the relative spatial position illustrated in Figure 2 can be considered to represent the 5-HT$_3$ receptor pharmacophore and crucial molecular determinants in this series of ligands. Obviously, all the other structural features of each individual compound also contribute to their affinity and selectivity.

This superimposition of structures derived from the conformational analysis prompts some comments: It seems that the benzene rings of **1**, **5**, and **6** are playing the role of the pyrrole ring contained in the indole nucleus of the other antagonists. Interestingly, there is a perfect match between the chloro substituents of **1** and **5**. It seems very likely that there exists an intramolecular hydrogen bond

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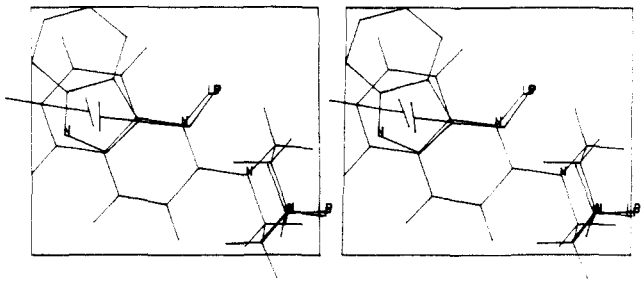

Figure 3. Putative 5-HT_3 receptor-bound conformation of quipazine.

between the amide hydrogen and the *o*-methoxy oxygen atom of **1** that stabilizes a conformation where the carbonyl group is coplanar to the aromatic ring. Our model suggests that this stabilized conformation corresponds to the preferred conformation for binding to the receptor.

Interestingly, the position of the second chloro substituent of **5** corresponds very closely to the nitrogen atom of the indole nucleus. From this it might be concluded that there is a beneficial electrostatic effect due to the presence of electronegative atoms in this particular region of the recognition site. However, **6**, in which a methyl replaces the chlorine substituent of **5**, was as potent as **5** (Table I). Thus a methyl group appears to be as effective functionally as a chlorine in this position.

As emphasized above, the locations of the carbonyl groups of the different molecules match perfectly well, suggesting that this group is responsible for a strong interaction with the receptor and contributes significantly to the binding process. It is noteworthy that this carbonyl group is perfectly coplanar with the adjacent aromatic ring, indicating that the receptor-bound conformation corresponds to one of the most stable conformations of this group in the flexible compounds. The presence of the carbonyl moiety seems to be an important feature since corresponding ketones, carboxylic esters, or amides lead to compounds with similar potency (e.g. in **11**, **7**, and **12**, respectively).

The basic component of the diverse molecules considered is very interesting. For some years, it seemed that a strong base such as a tertiary amine, a tropane, or a quaternary ammonium was required for activity. With the most recent disclosure of the compounds **10**, **11**, and **14**,[34] it is evident that much weaker bases (pK_a values of around 7) can also display a high affinity for 5-HT_3 recognition sites. It seems nevertheless very likely that the compounds bind in their protonated forms since quaternary derivatives are of similar potency (see below). Our model indicates that it is possible to achieve an extremely good fit between 1-Å vectors carried by the nitrogen atoms, which may represent the lone pair of electrons or the N^+H moiety. As discussed above, the imidazole group of **10** fits the model perfectly well if one assumes that the free electrons or the positive charge are delocalized.

The case of **15**, which has recently been described as a potent 5-HT_3 antagonist, has been considered separately.[9,12,16] This compound presents a structure different from the other antagonists since it does not contain a carbonyl function and has a piperazine moiety not found in any of the others. An attempt was made to fit this molecule to the pharmacophore by considering the two aromatic rings and the most basic nitrogen atom of the piperazine ring. As illustrated in Figure 3, it is indeed possible to match the nitrogen atom lone pair of electrons with the corresponding feature of the other antagonists and have the homocyclic aromatic ring of quinoline match the aromatic ring of the model pharmacophore. In this configuration, for instance, the heterocyclic ring of quinoline fits the saturated ring of **10**. Interestingly, the position of the nitrogen atom of quinoline then corresponds very well to that of the carbonyl moiety of the pharmacophore, suggesting that these two molecular features can play equivalent roles during the recognition process. Adequate molecules need to be designed to test this particular point.

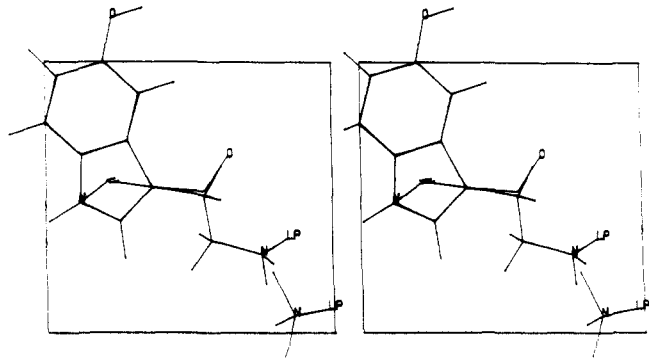

Figure 4. Comparison of 5-HT with the 5-HT_3 receptor antagonist pharmacophore.

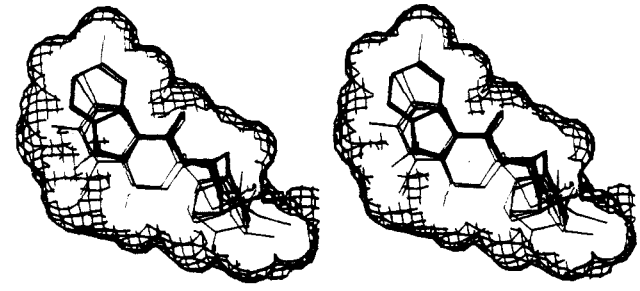

Figure 5. Volume accessible to ligands defined as the envelope of the superimposed 5-HT_3 receptor antagonists active conformers.

We have also checked if 5-HT, the endogenous 5-HT_3 receptor ligand, could satisfy the criteria defined by this SAR study. If one assumes that the indole nuclei in 5-HT, **7**, and **11** play the same role in the ligand–receptor complex, then the 5-HT nitrogen atom cannot occupy the ideal position defined by this study. The difference between the two features is at least 2.2 Å (Table II and Figure 4). This suggests that the activated and inactivated conformations of the 5-HT_3 receptor might have different structural requirements, at least at the level of the two molecular determinants considered. This idea seems to be in contradiction with the rationale which has been reported by Richardson et al. for the design of **7**.[17] In this respect, the structural similarities among **7**, **3** ((−)-norcocaine), **4** (tropine benzoate), and **5** seem worth being considered as an alternative rationale.

It would be reasonable to make a separate receptor mapping study of the *agonist* 5-HT_3 recognition site. Unfortunately, this is not possible at present since the number of selective 5-HT_3 receptor agonists studied is still very limited.[6]

Figure 5 illustrates the putative recognition site topography: the surface corresponds to the envelope of the 5-HT_3 receptor antagonists superimposed in their putative receptor-bound conformations and thus defines a volume accessible to ligands in the active site.

As discussed above, two compounds (**22** and **23**) incorporating the basic features defined by the pharmacophore into as simple a chemical structure as possible have been

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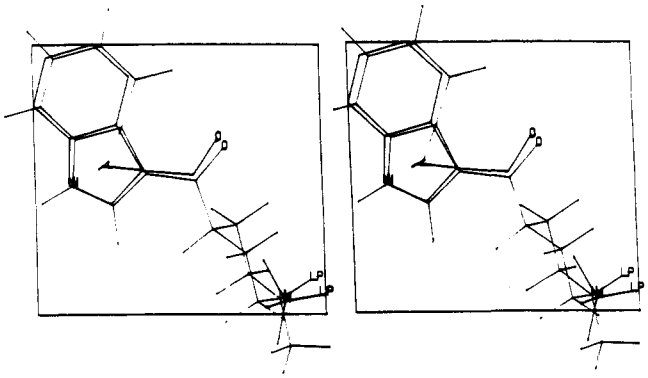

Figure 6. Comparison of compound **22** with the 5-HT_3 receptor pharmacophore.

designed (Figure 6) and synthesized. As was expected from our study, compound **22** displays a relatively high potency (pA_2 = 8.3) in the Langendorff assay (Table I) and is only slightly less potent than previously described antagonists, in spite of its much simpler structure. In addition, its quaternary ammonium derivative (**23**) is even more potent (pA_2 = 9.02).

These results indicate that, as suggested by the conformation–activity study, an aromatic ring, a carbonyl (or equivalent) group, and a correctly oriented nitrogen atom occupying the relative positions defined in Figure 2 are sufficient to provide a compound with 5-HT_3 antagonist properties. However, only the evaluation of totally rigid compounds retaining the molecular determinants described above (Figure 2) would definitively test our model which meanwhile remains a working hypothesis.

The increased affinity of the quaternary ammonium derivative **23** suggests that even the weaker bases recently described, such as **11** or **14**, probably bind in their protonated form to the 5-HT_3 recognition site.

The synthesis of other derivatives is ongoing in order to assess the importance of other structural modifications to the potency and selectivity of 5-HT_3 selective ligands.

Conclusion

A conformation–activity relationship study among most of the known 5-HT_3 receptor antagonists has been performed. A common pharmacophore and receptor map which may account for their activity has been defined. This basic pharmacophore consists essentially of a carbonyl group coplanar to an aromatic ring and a basic center in the relative positions illustrated in Figure 2.

This model has received some support from the synthesis and observed activity of a very simple amino–keto–indole derivative (**22**). In addition, the increased activity of its quaternary ammonium analogue (**23**) indicates that the 5-HT_3 receptor antagonists are more likely to be binding in their protonated form.

More important, the receptor map may represent a versatile working hypothesis which may easily lead to the design of new original putative 5-HT_3 receptor antagonists.

Experimental Section

Synthesis. Melting points were determined with a Büchi apparatus (capillary method) and are uncorrected. ^1H NMR spectra were recorded on a Bruker 90 spectrometer at 90 MHz. Flash chromatography columns were run on silica gel (60 silica gel). Analyses, indicated by elemental symbols, were within ±0.3% of the theoretical values.

***N*-Methylisonipecotyl Chloride (18).** *N*-Methylisonipecotic acid hydrochloride (**16**; 1.677 g, 10 mmol) was dissolved in dry benzene and $SOCl_2$ (5 mL) was slowly added. The mixture was then refluxed for 2 h under argon. Cooling and evaporation to dryness afforded a yellow solid (**18**), which was washed with dry Et_2O and dried under vacuum, mp 150 °C. This solid was used without further purification.

(1*H*-Indol-3-yl)(1-methyl-4-piperidinyl)methanone (20). Dry Et_2O and then EtBr (0.604 mL, 8 mmol) were added to magnesium (243 mg, 10 mmol) under an argon atmosphere. The mixture was progressively warmed from room temperature to reflux. After 30 min the mixture was cooled and indole (947 mg, 8 mmol) in 10 mL of dry Et_2O was added dropwise. After a further 1 h under reflux, the reaction mixture was cooled before addition of **18** (10 mmol) in THF (10 mL) and of Et_3N (1.41 mL, 10 mmol). After refluxing overnight, the mixture was cooled and 10 mL of water was added cautiously followed by EtOAc. The insoluble solid obtained was filtered, washed with H_2O and EtOAc, and dried. On recrystallization from MeOH/EtOAc pure **20** was obtained (16%): mp >250 °C; MS *m/z* 243 MH^+. Anal. ($C_{15}H_{18}N_2O$) C, H, N.

***N*-(Trifluoroacetyl)isonipecotic Acid (17).** Isonipecotic acid (12.04 g, 100 mmol) was dissolved at −10 °C in CH_2Cl_2 under an atmosphere of argon. Et_3N (14.78 mL, 105 mmol) was progressively added, followed by trifluoroacetic anhydride (14.97 mL, 105 mmol). The reaction mixture was stirred for 1 h at room temperature and then poured into H_2O. The organic phase was separated and evaporated to dryness. The crude material thus obtained was dissolved in Et_2O and extracted into a 10% $NaHCO_3$ solution. Acidification to pH = 4 with concentrated HCl and extraction with CH_2Cl_2 yielded, after evaporation, a white solid, which was recrystallized from Et_2O/hexane, affording pure **17** (73%): mp 117 °C; ^1H NMR (90 MHz, $CDCl_3$) δ 10.7 ppm (br s, 1 H), 4.5–3.8 (m, 2 H), 3.55–3.0 (m, 2 H), 2.9–2.5 (m, 1 H), 2.3–1.7 (m, 4 H); ^{19}F NMR (90 MHz, $CDCl_3$, s).

***N*-(Trifluoroacetyl)isonipecotyl Chloride (19).** *N*-(Trifluoroacetyl)isonipecotic acid (2.46 g, 42 mmol) was dissolved in 100 mL of Et_2O at 0 °C under an argon atmosphere. Et_3N (6.33 mL, 45 mmol) was added, followed by $SOCl_2$ (3.32 mL, 45 mmol), and the reaction mixture was stirred at room temperature overnight. The precipitate was filtered and the organic phase was evaporated to dryness under a high vacuum. A mixture of an oil and a solid was obtained. The oil was taken up with dry Et_2O, which after evaporation afforded 5.82 g (23.8 mmol) of the acid chloride as an oil. The remaining solid was the anhydride. Warming of this solid product in dry benzene with 1.5 equiv of $SOCl_2$ at 60 °C for 2 h produced an additional amount of acid chloride (overall quantitative yield): ^1H NMR (90 MHz, $CDCl_3$) δ 4.5–3.85 ppm (m, 2 H), 3.5–2.9 (m, 3 H), 2.3–1.6 (m, 4 H).

(1*H*-Indol-3-yl)[1-(trifluoroacetyl)-4-piperidinyl]methanone (21). Following essentially the same procedure as for **20**, 7.55 g of a crude, yellow solid was obtained from 42 mmol of starting acid chloride. Purification by flash chromatography (silica, CH_2Cl_2/HOAc 93/7) followed by recrystallization from EtOAc/Et_2O/hexane afforded pure **21** as a white solid (3.58 g, 31%), mp 198 °C. Additional product could be recovered from the mother liquors. Anal. ($C_{16}H_{15}N_2O_2F_3$).

(1*H*-Indol-3-yl)(4-piperidinyl)methanone (22). Trifluoroacetamide **21** (3.64 g, 11.2 mmol) was dissolved in warm MeOH (60 mL) and K_2CO_3 (4.66 g, 34 mmol) in 30 mL of H_2O was added. After stirring for 2 h at room temperature, MeOH was evaporated. A white solid crystallized, which was filtered and washed with H_2O and CH_2Cl_2 and then dried in a vacuum (2.54 g, 99%), mp 218 °C. Anal. ($C_{14}H_{16}N_2O$) C, H, N.

1,1-Dimethyl-4-[(1*H*-indol-3-yl)carbonyl]piperidinium Iodide (23). Amine **22** (550 mg, 2.4 mmol) and a large excess of MeI (1.5 mL) in 20 mL of MeOH/CH_2Cl_2 2/1 were stirred at room temperature for 24 h, then Et_2O (50 mL) was added. A solid was filtered and recrystallized from MeOH (450 mg, 48.8 %), mp 275 °C. Anal. ($C_{16}H_{21}N_2OI$) C, H, N.

Biology. The 5-HT_3 antagonist properties of compounds **22** and **23** have been measured in a Langendorff assay as previously described.[35]

Acknowledgment. We would like to thank Dr. M. Gittos for his fruitful comments and André Zimmermann, Martine Host, and Marie Louise Part for their excellent technical assistance.

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Supplementary Material Available: The atomic coordinates of the molecules considered in their minimum energy and receptor-bound conformations can be provided by the author on request.

Registry No. 1, 364-62-5; 3, 18717-72-1; 4, 19145-60-9; 5, 40796-97-2; 6, 85181-40-4; 7, 89565-68-4; 8, 89565-91-3; 9, 126501-69-7; 10, 99614-60-5; 11, 117186-80-8; 12, 109889-09-0; 13, 109872-41-5; 14, 115956-12-2; 15, 4774-24-7; 16, 71985-80-3; 17, 126501-70-0; 18, 41776-24-3; 19, 126501-71-1; 20, 69903-28-2; 21, 126501-72-2; 22, 5275-02-5; 23, 126501-73-3; 24, 99614-58-1; indole, 120-72-9; isonipecotic acid, 498-94-2.

Angiotensin-Converting Enzyme Inhibitors. 9.[1] Novel [[*N*-(1-Carboxy-3-phenylpropyl)amino]acyl]glycine Derivatives with Diuretic Activity

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A series of molecules 1 having sulfonamide diuretic moieties covalently linked to non-sulfhydryl angiotensin-converting enzyme inhibitors (ACEI) were prepared and tested for both activities. IC_{50} values for ACEI as low as 7 nM were observed. Discernable diuretic activity was seen for several hydrochlorothiazide-based molecules. Effects of the ACEI and diuretic structures on the respective potencies are discussed.

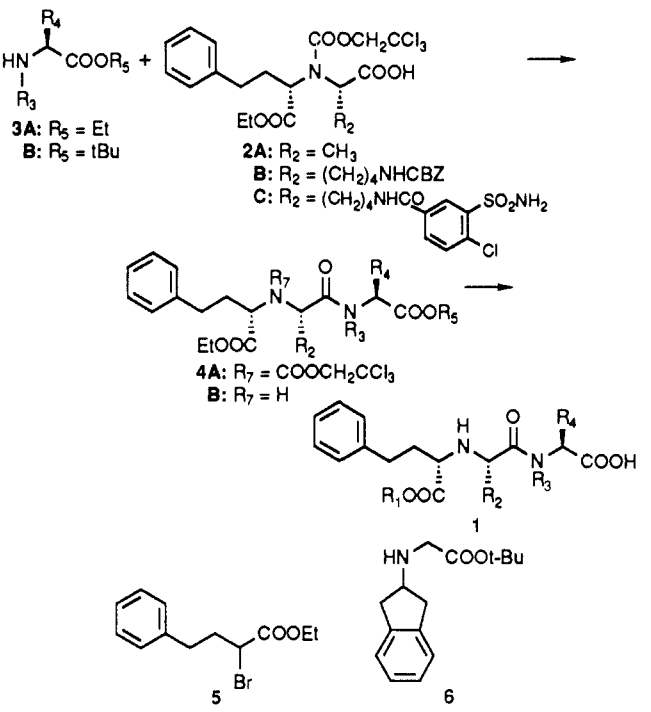
The creation of novel antihypertensive agents remains a principal focus of medical research. In the last decade a significant advance has been made with the introduction of angiotensin-converting enzyme (ACE) inhibitors. Compounds such as captopril[2] and enalapril[3] have captured a significant share of the market, and many similar compounds are currently under clinical investigation.[4]

It has been found that hypertensive patients do not always respond adequately[5] to simple treatment with an ACE inhibitor. In these cases therapeutic success often depends upon adding a diuretic[6] to the treatment regimen. Physiologically this has been explained as an elevation of circulating renin levels due to diuretic-induced sodium depletion.[7] The resulting angiotensin II (AII) production becomes a major contributor to blood pressure elevation. ACE inhibition under these conditions now removes this contribution, leading to a much higher treatment success rate. In general the necessary diuretic dosage has been found to be less than that required for an equivalent response to diuretics alone.

We considered it worthwhile to introduce diuretic activity into known ACE inhibitors from our own[8] and other[3] laboratories. Our goal was an agent with potent ACE activity and only mild diuretic activity. In this way we hoped to retain the beneficial effects of diuretic coadministration while minimizing the hypokalemic[9] and uricosuric[10] side effects frequently associated with diuretic therapy. Such an approach[11] would allow for improvement in initial treatment success rate without the need to titrate two dosage regimens. In addition the simpler dosage would favor patient compliance.

Consideration of the known structure–activity relationships of existing diuretics[12] led us to conclude that the substitution pattern of indapamide,[13] hydrochlorothiazide,[14] furosemide,[15] and bumetanide[16] might readily

Scheme I



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