Molecular Determinants for Binding of Methylenedioxytryptamines at 5-HT/LSD *Receptors*

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Abstract

In investigations of the molecular determinants for the recognition of drugs by a serotonin (5-HT) receptor in the brain, the commonality in the reactivity patterns of 5-HT congeners has been identified. On this basis a mechanistic hypothesis is proposed to explain the rank order of the affinity of drugs that bind to the receptor shared by 5-HT and LSD (the 5-HT/LSD receptor). This hypothesis describes the relation between affinity and the intramolecular rearrangement needed to make a 5-HT congener recognizable at the 5-HT/LSD receptor. The rearrangement of the congener aligns the electrostatic orientation vector of its indole portion in the direction defined by the vector in 5-HT. To further probe this mechanistic hypothesis the molecular determinants for the action of a new series of tryptamine derivatives was studied in which a methylenedioxy substituent is placed at the 5,6 or the 4,5 positions. The reactivity characteristics of these molecules are presented. Theoretical predictions for their activity on the 5-HT/LSD receptor are discussed. These predictions are based on the comparison of the electrostatic potentials of these molecules with the requirements for 5-HT-like recognition by the 5-HT/LSD receptor, and on simulations of molecular interactions with a molecular probe (imidazolium cation) which represents a matching receptor site. The electrostatic nature of the complexes that these molecules form with imidazolium cation is revealed by the decomposition of the stabilization energy. It is shown that the affinity of these new compounds for 5-HT/LSD receptors can be predicted and explained on the basis of the working hypothesis obtained previously. Preliminary experimental data on the binding of 5,6- and 4,5-methylenedioxy derivatives of typtamine to the 5-HT/LSD receptors in brain confirm these findings.

Introduction

As part of continuing studies of the molecular determinants for the recognition of drugs at receptors shared by LSD and serotonin (5-hydroxytryptamine; 5-HT), we have studied the molecular determinants for binding of 5,6-methylenedioxytryptamine (5,6-MDOXT) and 4,5-methylenedioxytryptamine (4,5-MDOXT) at 5-HT/LSD receptors. The structures of 5,6-MDOXT and 4,5-MDOXT as compared to those of the monohydroxy substituted tryptamines 5-HT and 6-HT are shown in Figure 1.

The rank order of affinities of monohydroxy substituted tryptamines (TRYP), i.e., 4-, 5-, 6-, and 7-hydroxytryptamine, has been shown to be 5-HT $\gg$ 4-HT

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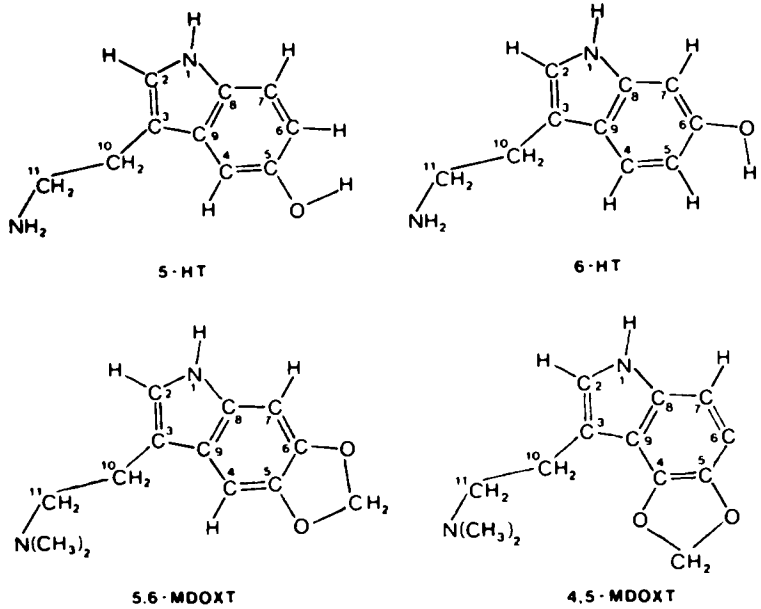

Figure 1. Structures of 5-hydroxytryptamine (5-HT), 6-hydroxytryptamine (6-HT), 5,6-methylenedioxytryptamine (5,6-MDOXT), and 4,5-methylenedioxytryptamine (4,5-MDOXT).

> TRYP ≫ 7-HT [1, 2]. The lowest measured affinity for this receptor site is nearly three orders of magnitude smaller than that of 5-HT. On the basis of the known affinities of the monohydroxy substituted tryptamines for the 5-HT/LSD receptor (Table I), and judging from the structural similarities of the methylene dioxytryptamines to the monohydroxy substituted tryptamines (Fig. 1), it would seem reasonable to expect that 4,5-MDOXT will have a higher binding affinity for 5-HT/LSD receptor sites than will 5,6-MDOXT. However, according to our previous findings, such predictions cannot be made from strictly structural considerations but, rather, from the comparison of the reactivity characteristics of the drugs with the molecular determinants for 5-HT-like recognition on the 5-HT/LSD receptors. Such a comparison is presented and discussed here.

Methods

The Calculations

Ab-initio calculations were carried out for the isolated molecules and for models of their complexes with imidazolium cation. The calculations were performed with the GAUSSIAN 70 program [3] with STO-3*G* basis sets [4]. All tryptamine derivatives were calculated in their neutral form since previous work has shown that the interaction with a negative receptor site can be simulated by a neutralization of the side chain [5]. The reactivity characteristics were analyzed from molecular electrostatic potential maps and electrostatic orien-

TABLE I. Rank order of affinities or substituted indolealkylamines for 5-HT/LSD binding sites.[1]

COMPOUND	K_0(M) (rounded off)	RANK[2] (compared to 5-HT)	$\Delta(\Delta G)$[3]
5-HT	6×10^{-9}	1	—
5-MeO-T	2×10^{-8}	3	0.7
Bufotenine (N,N-dimethyl 5-HT)	4×10^{-8}	7	1.1
Tryptamine	1×10^{-7}	17	1.7
Psilocin (N,N-dimethyl 4-HT)	3×10^{-7}	50	2.3
5-FT	8×10^{-7}	133	2.9
6-HT	7×10^{-6}	1,167	4.2

[1] Obtained from binding experiments with (^3H)5-HT. The competition of the various compounds for the binding sites of 5-HT is calculated from the difference between binding in the presence and absence of $10^{-6}M$ unlabeled LSD and various concentrations of the unlabeled drug.

[2] The rank order is the ratio between the dissociation constant for the compound and that of 5-HT.

[3] Calculated in kcal/mol from the rank number representing the ratio of dissociation constants. It is an estimate of the difference in the free energy of binding of 5-HT and that of another compound.

tation vectors (see [5] for details). A decomposition of the interaction energies in the complex was performed according to the scheme proposed by Morokuma and co-workers [6]. In this scheme the total stabilization energy of the complex is broken down into contributions from the electrostatic interaction of the unperturbed molecules (ES), a term related to the mutual polarization of the molecules (PL), the exchange repulsion (EX), and a composite term representing "charge-transfer" and additional energy contributions (CT + mix).

The Molecular Geometries

All calculations were performed with the tryptamine derivatives as primary amines, in the Falkenberg averaged geometry [7] with the side chain in the least interfering, fully extended conformation. This also corresponds to the geometry in solution (for a review, see [8]). The geometry of the methylenedioxy substituents was constructed from standard bond lengths and angles [9]. The imidazolium cation was calculated in the averaged geometry of the imidazole rings in the N3-H and N1-H tautomers of histamine, obtained from crystallographic data (see [10] and [11], respectively). The model compound of 5,6-MDOXT (see below, Fig. 7) was constructed from the corresponding atoms in 5,6-MDOXT. Hydrogen atoms were placed at standard bond lengths wherever needed. Similarly, formamidinium (FAM) was constructed as described before [12] from imidazolium cation (see below, Fig. 7).

Analysis of Reactivity Criteria

The molecular electrostatic potential maps were studied in planes parallel to the indole ring system in the tryptamines at distances of 2.98, 4.72, and 6.238 bohr. The fixed interplanar separation used in the calculation of the complex was 6.238 bohr. This distance was based on the average "close contact" separation in the crystal structure of stacking complexes of 5-HT [13], and was shown to be essentially identical to the optimal interplanar distance calculated for complexes of tryptamine derivatives with imidazolium [12, 14].

Recently we have also shown that in studies of small molecular complexes such as those investigated here the description of the nature of the interaction obtained at the SCF level with STO-3*G* basis sets does not change when the basis set is extended to the 4-31*G* level [12]. Correlation effects included to second order in a Møller–Plesset formulation do not affect qualitative conclusions from the calculations of these complexes [12]. The interplanar separation in these stacking complexes appears to be a less sensitive parameter for the stabilization energy than does the proper alignment of the model molecules [12].

The electrostatic orientation vectors described elsewhere [5, 15] are obtained here in a simplified manner by connecting the points of minima in the molecular electrostatic potential maps through the regions of most negative values in the potential. This simplified procedure (compared to that described in [5]) is validated by the physical meaning of the potential and by the fact that the electrostatic potentials for the molecules studied here are everywhere negative. The strongest force between the unperturbed molecule and a linear collection of charges will orient the molecule in the direction shown by the alignment of these charges with the electrostatic orientation vector [15].

Results and Discussion

Reactivity Criteria

Fluorescence spectroscopy [16], proton nuclear magnetic resonance [16, 17], and microcalorimetry [18] studies have revealed that 5-HT forms stacking complexes of the electron donor–acceptor type with such molecules as DNA, poly(A), and ATP. These studies have indicated that the indole portion of 5-HT is involved in the stacking, while its ethylamine side chain is engaged in an electrostatic interaction. The reactivity characteristics of 5-HT and its congeners were therefore studied in planes parallel to the indole portion, and included the molecular electrostatic potentials and the electrostatic orientation vectors calculated from the isopotential contour maps [5, 14, 15]. On the basis of these calculated reactivity characteristics of 5-HT and its congeners, and from experimental structure activity relationships it became possible to suggest a set of recognition requirements for interaction at 5-HT/LSD receptors [14, 15, 19]. These are defined in Figure 2.

As described elsewhere [5, 15], 6-HT, which has a much lower binding affinity than 5-HT at 5-HT/LSD sites, possesses an electrostatic orientation vector

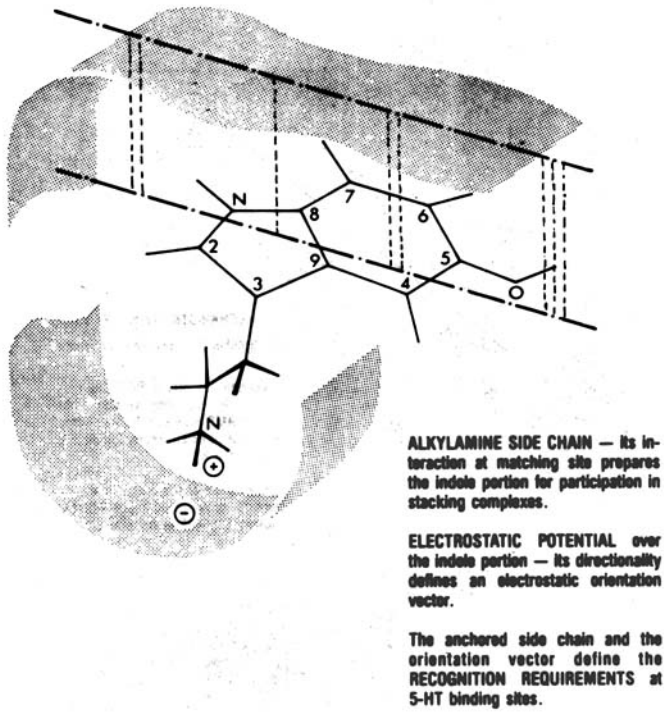

Figure 2. Elements of recognition at 5-HT sites.

nearly perpendicular to that of 5-HT. Other congeners exhibit orientations of these vectors that are less different from that in 5-HT [5]. Such results lead us to the mechanistic hypothesis that a congener of 5-HT achieves 5-HT-like recognition by anchoring its side chain at the same site as that of 5-HT and by orienting its electrostatic vector parallel to that of 5-HT. Reorientation of the vector in a congener can be achieved by a conformation change in the side chain.

As a corollary to this mechanistic hypothesis it was proposed [5, 19] that the energy needed for vector reorientation in any congener reduces the probability for binding at 5-HT sites, thus lowering the affinity of the congener for the binding site. Figure 3 illustrates a conformational change that can rearrange the electrostatic orientation vector of 6-HT to mimic that of 5-HT. *Ab-initio* calculations of the conformation of 5-HT congeners [20] indicate that the energies required to achieve such rearrangements are of the order of 0–4 kcal/mol, well within the range of differences in the $\Delta(\Delta G)$ values calculated from the binding affinities of the congeners (see Tables I and II).

Figures 4 and 5 show the electrostatic potentials of 5,6-MDOXT and 4,5-MDOXT, respectively. These potential maps were calculated in a plane 3.3 Å above and parallel to the indole portion of each molecule. Figure 6 shows a comparison of the relative directions of the electrostatic orientation vectors of

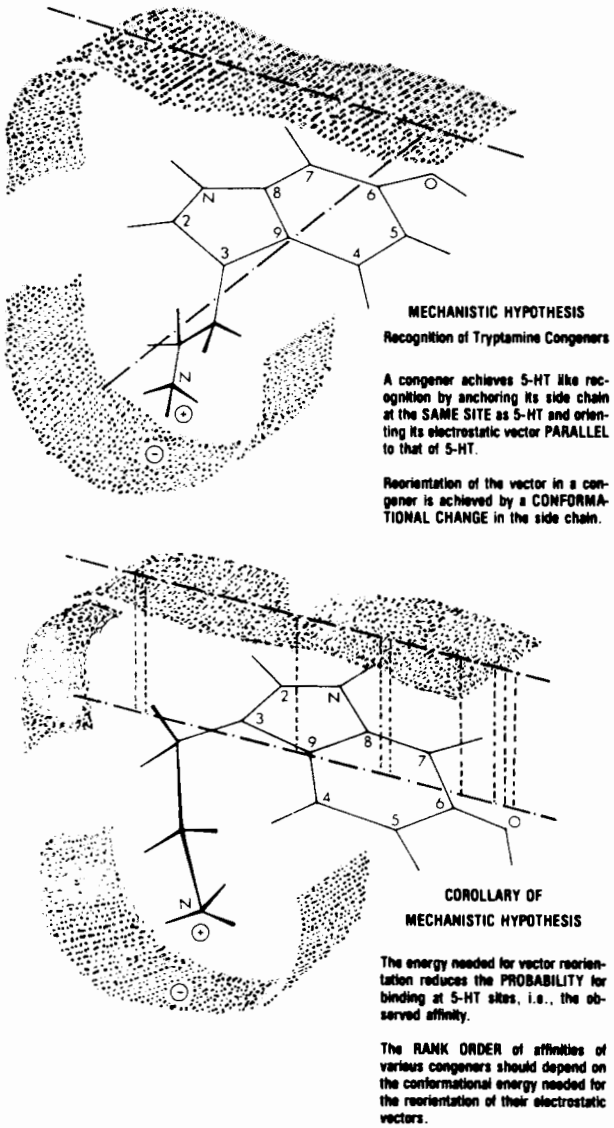

Figure 3. Illustration of the mechanistic hypothesis for 5-HT-like recognition of tryptamine congeners.

5-HT, 6-HT, 5,6-MDOXT, and 4,5-MDOXT. It should be noted that 4,5-MDOXT exhibits an electrostatic orientation vector that is parallel to that of 6-HT. 5,6-MDOXT exhibits two vectors, one nearly parallel to that of 5-HT and one parallel to that of 6-HT. On the basis of the criteria established previously for recognition at 5-HT sites (see above) and because the potential of 5,6-MDOXT contains at least one orientation in which it mimics 5-HT, we concluded that 5,6-MDOXT should have a higher affinity than 4,5-MDOXT

TABLE II. Comparison of affinities of methylenedioxytryptamines with those of 5-HT congeners.[a]

COMPOUND	K_D(M)	RANK	$\Delta(\Delta G)$
5-HT	6×10^{9}	1	—
Bufotenine (N,N-dimethyl 5-HT)	4×10^{8}	7	1.1
Psilocin (N,N-dimethyl 4-HT)	3×10^{7}	50	2.3
5,6-MDOXT	2×10^{6}	333	3.4
6-HT	7×10^{6}	1,167	4.2
4,5-MDOXT	1×10^{5}	1,667	4.4

[a] Definitions are in Table I.

for binding at 5-HT sites. This prediction is in direct contrast to what would have been concluded from a strict structural comparison as discussed in the Introduction.

Table II presents a comparison of the affinities of the methylenedioxytryptamines with those of 5-HT congeners. These experimental results (from [1, 2, 21]) support the predictions made on the basis of the mechanistic hypothesis previously arrived at for 5-HT and its congeners. 5,6-MDOXT does indeed exhibit a higher affinity than does 4,5-MDOXT at 5-HT/LSD binding sites [21].

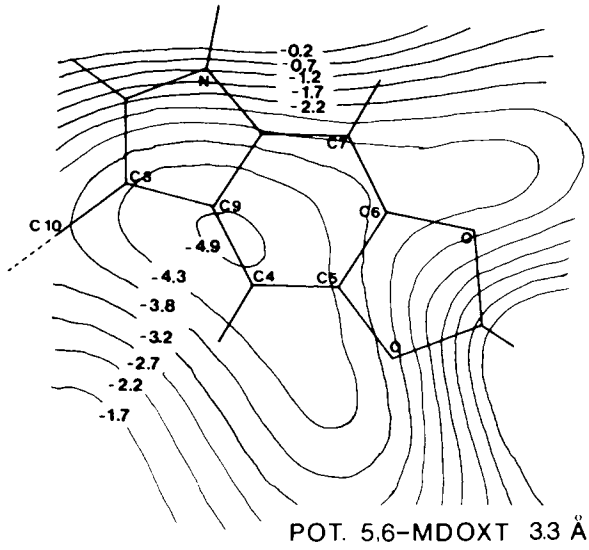

Figure 4. Molecular electrostatic potential of neutral 5,6-MDOXT. Contours represent the electrostatic potential in a plane 3.3 Å above the indole system in the molecule. Values are in kcal/mol.

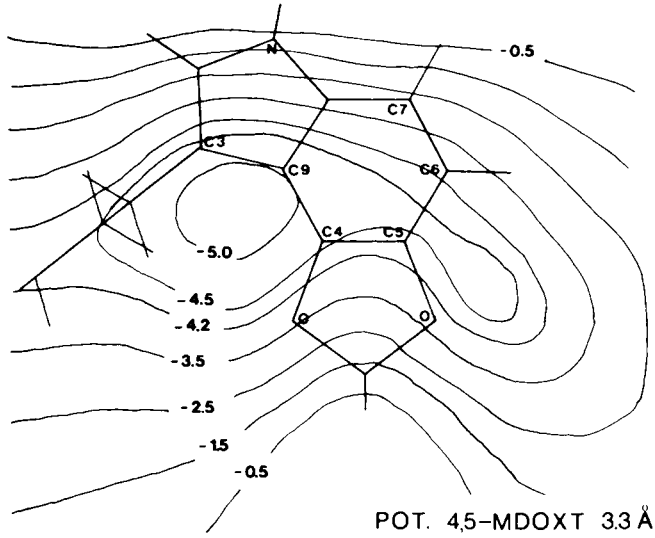

Figure 5. Molecular electrostatic potential of neutral 4,5-MDOXT. Contours represent the electrostatic potential in a plane 3.3 Å above the indole system in the molecule. Values are in kcal/mol.

Examination of the Reactivity Criteria by Calculations on Model Complexes

Calculations on model complexes were undertaken in order to test the validity of the reactivity criteria, i.e., to establish whether the calculated properties of

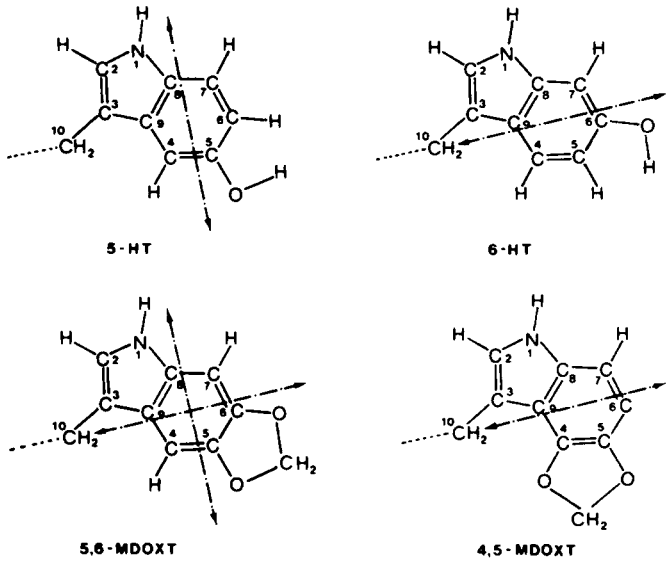

Figure 6. Relative positions of the electrostatic orientation vectors in 5-HT, 6-HT, 5,6-MDOXT, and 4,5-MDOXT.

stacking complexes of methylenedioxytryptamine are consonant with the predictions based on the electrostatic reactivity criteria. Imidazolium cation was chosen for the simulation of interactions with tryptamines on the basis of experimental evidence for complexes between indole derivatives and this molecular ion (for a review, see [22]). Spectroscopic experiments (primarily fluorescence and some nuclear magnetic resonance studies) have described inter- as well as intramolecular complexes that are of particular relevance to the reactivity of indoles in biological systems. These studies have demonstrated the formation of complexes between imidazole, histidine, or histamine and indole, tryptophan, aminotryptophan, or methoxytryptophan. All complexes are formed when the imidazole ring is protonated and "the binding forces between the indole and imidazole moieties are weak and strongly dependent on pH and orientation" [17]. We therefore chose the complexes of neutral tryptamine derivatives with imidazolium cation as models to probe the reactivity criteria and the related mechanistic hypothesis. Because the neutral molecules mimic the properties of the cationic species neutralized by a hypothetical anionic receptor site [5], these complexes simulate the simultaneous interaction of the cationic tryptamine derivatives with an anionic site and with a molecule that can form complexes with the indole portion, as observed in the biological materials [16–18, 22].

Figure 7 shows the model molecules used in the calculation of the complex between 5,6-MDOXT and imidazolium cation. We have shown previously that this type of model construction gives a reliable representation of the reactivity as is indicated by the electrostatic potentials of the components and by the properties of the resulting complexes [12]. In agreement with these earlier findings, Figure 8 illustrates that in the case of 5,6-MDOXT the electrostatic potential of the model 5,6-MDOXT reproduces well the important aspects of the electrostatic potential of the full molecule (Fig. 4). The relative positions of the two molecules in the complex are illustrated in Figure 9. The molecules

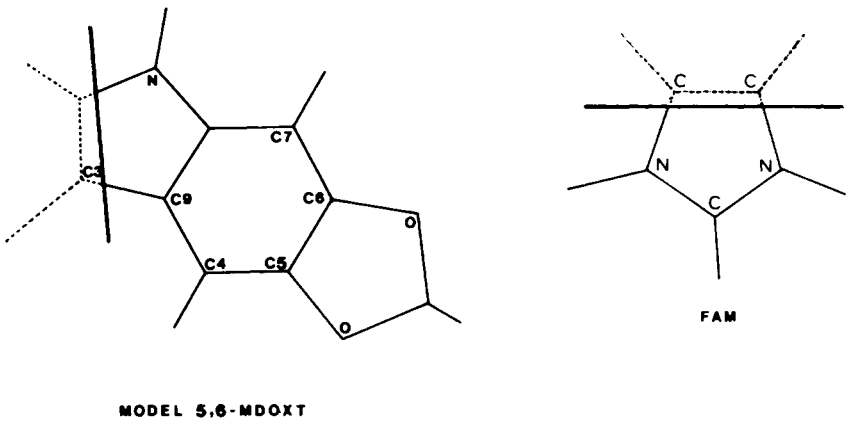

Figure 7. Construction of model 5,6-MDOXT and formamidinium (FAM) from parent compounds.

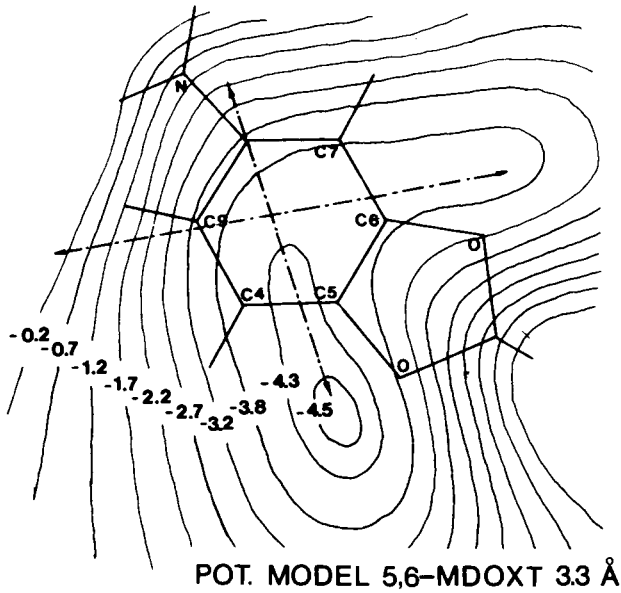

Figure 8. Molecular electrostatic potential of model 5,6-MDOXT. Contours represent the electrostatic potential in a plane 3.3 Å above the molecule. Values are in kcal/mol.

here are shown in the M5 (or MIN-5) geometry which is the optimal alignment found in complexes of 5-HT with imidazolium [12, 15]. This was established by a scan of the electrostatic interaction surface approximated by the monopole–monopole interaction calculated with Mulliken point charges of the unperturbed molecules [15].

A decomposition of the interaction energies in the model complex is given in Table III. The major contributor to the stabilization energy of the complex is shown here to be the electrostatic term (ES). The electrostatic nature of the complex is identical to that found for the complexes of 5-HT and 6-HT with imidazolium cation [12, 14, 23]. This finding explains why the electrostatic orientation vectors can be expected to be good predictors of the properties of

TABLE III. Decomposition of total stabilization energy of model complex.[a]

Type of Contribution	5,6-MDOXT MODEL + FAM (M5)
ES	−3.40
PL	−1.04
EX	0.94
CT + mix	−0.39
TOTAL	−3.89

[a] Energies (kcal/mol) are from an SCF calculation with the STO-3G basis set.

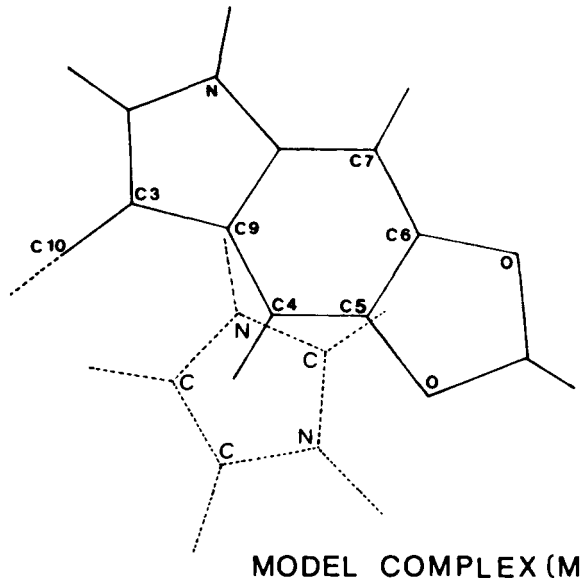

Figure 9. Relative orientations of 5,6-MDOXT and imidazolium cation in the MIN-5 alignment.

stacking complexes observed between these molecules and biological materials [16–18, 22]. This is the reason for the success of the working hyothesis, based on the properties of these oriented complexes, to rationalize and predict the rank order of affinity of the molecules for the 5-HT/LSD receptor [15].

Conclusions

The *directionality* of the electrostatic potential in 5,6-MDOXT and in 4,5-MDOXT is different from what might have been expected on the basis of a structural comparison to 4-HT, 5-HT, and 6-HT. The *elements of recognition* of the MDOXT derivatives at high affinity binding sites of 5-HT/LSD are shown to be compatible with the hypothesis first arrived at for monohydroxy substituted 5-HT congeners. The *rank order of affinity* of the MDOXT derivatives can be predicted on the basis of the *mechanistic hypothesis* previously obtained for 5-HT congeners. Experimenal results confirm the reactivity predictors. Calculations on MDOXT complexes with imidazolium cation show that these complexes, like the complexes of monohydroxy substituted tryptamines with imidazolium, are electrostatic in nature. This fact explains why the orientation vectors for these molecules predict successfully the rank order of affinity of methylenedioxytryptamines at 5-HT/LSD receptor sites, as they predicted the affinities of the monohydroxy derivatives.

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