Molecular Modeling Studies Focused on 5-HT$_7$ versus 5-HT$_{1A}$ Selectivity. Discovery of Novel Phenylpyrrole Derivatives with High Affinity for 5-HT$_7$ Receptors

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The present study discusses the well-known 5-HT$_7$/5-HT$_{1A}$ selectivity issue through a new series of phenylpyrrole derivatives. The first hits emerged from a virtual screening performed on a chemolibrary. Further study led to an optimization of a preliminary 5-HT$_7$ pharmacophore model. The importance of each pharmacophoric feature is confirmed, but these characteristics have to be coupled to geometric constraints in order to achieve a 5-HT$_7$ selectivity. Indeed, 5-HT$_{1A}$ affinity probably arises from extended conformations, whereas a bent one appears to be best suited for 5-HT$_7$ selectivity.

INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter that exerts its effects on the central and peripheral nervous system by interacting with a large variety of receptors. In 1986, Bradley et al.[1] classified 5-HT receptors into three main types, namely "5-HT$_1$-like", 5-HT$_2$, and 5-HT$_3$. The term "5-HT$_1$-like" encompassed a broad range of receptors with certain common features: (1) a more potent stimulation by 5-carboxamidotryptamine (5-CT) than by 5-HT, (2) a blockade by methiothepin and methysergide, and (3) a resistance to blockade by selective 5-HT$_2$ or 5-HT$_3$ receptor antagonists. Subsequently, the application of molecular biology techniques has allowed the discovery of numerous additional 5-HT receptors, and a classification of serotonin receptors based on binding properties alone has been superseded by a more comprehensive approach, which includes structural as well as transductional properties. In 1994, the 5-HT receptors were reclassified into seven receptor subfamilies (5-HT$_1$−5-HT$_7$) on the basis of their sequence homology, pharmacology, and signal transduction cascade.[2] The 5-HT$_7$ receptor (5-HT$_7$R) is the most recent addition to this burgeoning family of receptors. The 5-HT$_7$R has been identified in mice,[3] rats,[4−6] guinea pigs,[7] pigs,[8] and humans[9] by the application of molecular cloning. Despite growing efforts to understand the physiological role of this receptor, the function of 5-HT$_7$ receptors has not been clearly established, although its implication in the regulation of circadian rhythms,[5] depression,[10] epilepsy,[11] relaxation of vascular smooth muscles,[12] and migraine pathogenesis[13] is known. Its high affinity for 5-HT and 5-CT suggests that it might have been included in previous characterizations of "multiple" 5-HT$_1$ receptor binding sites using [^{3}H]5-HT or [^{3}H]5-CT as radioligands.[14] Moreover, in view of the evidence that 5-HT$_7$ and 5-HT$_{1A}$ receptors show some similarities in their pharmacological profiles,[15] a selectivity issue is encountered for these two receptor subtypes. For instance, 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT), a compound that was previously considered to be a selective 5-HT$_{1A}$ receptor agonist, is also a partial agonist at the 5-HT$_7$ receptor with moderate affinity. This implies that the 5-HT$_7$R might have contributed to 8-OH-DPAT responses, previously believed to be 5-HT$_{1A}$-mediated responses.

Because no crystallographic structure of 5-HT receptors is yet available, the concept of pharmacophores[16−19] is useful to understand the existing relationships linking these receptor subtypes. 3D-QSAR software such as Catalyst[20] allows a pharmacophore to be obtained among active compounds in a multiconformational structure database. Previous studies were carried out on ligands for the 5-HT$_3$ receptors,[21] 5-HT$_4$ receptors,[22] 5-HT$_7$R,[23] and 5-HT reuptake inhibitors[24] with these approaches.

In this study, we discuss the problem of 5-HT$_7$ versus 5-HT$_{1A}$ selectivity through a new series of phenylpyrrole derivatives discovered by virtual screening (Figure 1). Our recently described 5-HT$_7$ pharmacophore (Figure 2)[23] is then used to understand the 5-HT$_7$ affinity and selectivity of these new discovered ligands and of ligands derived from the literature (Figure 3).[23,25−29] The importance of each characteristic of the pharmacophore is particularly investigated.

MATERIALS AND METHODS

Virtual Screening (2D-Database Searching). The virtual screening was performed on our chemolibrary (http://www.cermn.unicaen.fr/) using the MOE program.[30] The basis for comparison during the screen was a 2D pharmacophore defined from a molecular graph and included in a fingerprint. The typed graph distance method was employed. In this case, the fingerprint is a set of all the triples of the form (u, v, d), where u and v are atom types and d is

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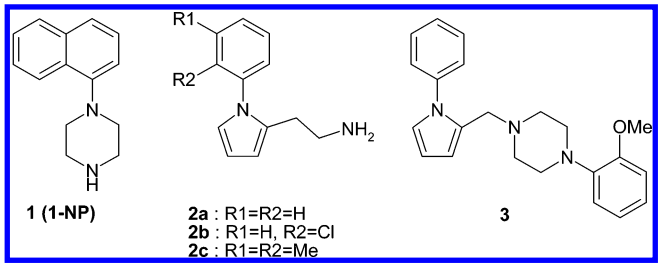


Figure 1. Query molecule (**1**) for similarity searching and results of the virtual screening (**2a**−**c**). Compound **3** represents the lead for the pharmacomodulations.

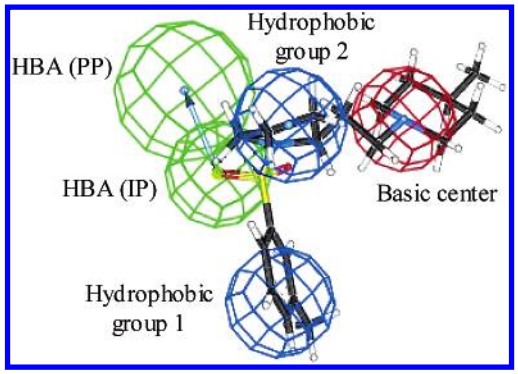


Figure 2. Alignment between **11** and the 5-HT$_7$ pharmacophore (IP: initial point for the ligand; PP: projected point on the receptor).

the graph distance between the two atoms (the number of bonds in the shortest path between the atoms in the chemical graph). Each atom is assigned one of the following types: acid group, basic group, hydrogen bond acceptor, hydrogen bond donor, both hydrogen bond acceptor and donor, hydrophobic group, or none of the above. The Tanimoto similarity coefficient[31] was used to quantify the degree of structural resemblance between pairs of molecules.

5-HT$_7$ Pharmacophore Model. The definition of the pharmacophore for the 5-HT$_7$ antagonists was carried out with Catalyst[20] by searching the common chemical features of selective antagonists from the literature (see ref 23 for methodology). This 3D pharmacophore (Figure 2) consists of one basic center, two hydrophobic groups, and one hydrogen bond acceptor (HBA).

Conformational Analysis. A torsional scan along the N/C−C−S−C(Me) dihedral angle was performed for three model compounds: 1-(methylthio)*H*-pyrrolo[1,2-a]pyrazine (representing **7**), 2-(methylthio)pyridine (for **8**), and 4-(methylthio)pyridine (for **13**). Density functional theory (DFT) calculations consisted of a 0−180° geometry optimization with the specified coordinate freezing and a 5° increment, using the hybrid density functional B3LYP[32] and a 3-21+G* basis set with the Jaguar program.[33]

Binding Assays. Binding assays on human and rat 5-HT$_7$R were performed according to Shen et al.[4] and Ruat et al.[34] The methods described by Hall et al.[35] were used for the binding assays on rat 5-HT$_{1A}$ receptors.

RESULTS AND DISCUSSION

Virtual Screening (2D-Database Searching). The first step of this study was to perform a 2D similarity search on a chemolibrary in order to discover novel 5-HT$_7$R ligands. Compound **1** (1-NP, Figure 1) has been chosen for its relatively simple and rigid structure. It is able to bind to 5-HT$_7$R (K_i = 83 nM)[9] as well as to other serotonin receptors. It is also claimed to be used for the treatment of urinary incontinence and urinary retention for its 5-HT$_7$R action.[36]

The virtual screening was performed on our chemolibrary (6663 compounds) using the MOE program.[30] This similarity searching led to 132 structures with a Tanimoto similarity coefficient[31] greater than 0.75. Three compounds displayed a similarity value greater than 0.90, corresponding to the **2a**−**c** derivatives (Figure 1; Tanimoto similarity coefficient = 0.94). The structural resemblances between **1** and **2a**−**c** consist of one basic center and two hydrophobic groups, at comparable distances. The pharmacological results showed a primary 5-HT$_7$R affinity for **2c** (72% inhibition at 10^{-6} M). Besides, these data showed that the higher the phenyl was substituted, the more the 5-HT$_7$R affinity increased. This observation led to the following hypotheses. Either a bulky hydrophobic group is favorable for 5-HT$_7$R affinity or the phenyl substitution leads to favorable interactions with the 5-HT$_7$R by forcing the aryl group out of the plane of the pyrrole ring. Six chemolibrary-stored compounds of the same phenylpyrrole family were then selected and tested. Compound **3** (Figure 1) emerged from the analysis of the pharmacological results, with 91% inhibition of the 5-HT$_7$R binding at 10^{-6} M. From **3**, structural modifications were performed in order to improve the 5-HT$_7$R affinity of this new series.

Molecular Design of the Phenylpyrrole Derivatives and Selectivity Studies. In particular, two pharmacomodulation procedures were carried out. First, phenyl-substituted compounds were synthesized according to the **2a**−**c** results discussed above. This led to compounds **4a**,**b** (Table 1). Second, because derivatives with the pyrrole substituted on position 3 could be obtained, their isomeric counterparts **5a**,**b** were studied. At that time, a patent stopped us from performing the binding assays on human 5-HT$_7$R. Thus, remaining compounds were evaluated only on rat 5-HT$_7$R. Affinity values are listed in Table 1. It is worth noticing that binding values of this novel series of phenylpyrrole derivatives toward the rat 5-HT$_7$R appear to be slightly lower than those toward the human 5-HT$_7$R. Nevertheless, these data showed that compounds with the pyrrole substituted on position 3 have higher 5-HT$_7$R affinities than compounds with the pyrrole substituted on position 2.

Ortho-methoxyphenylpiperazine (*o*-MPP) being one of the most commonly used pharmacophoric moieties in high affinity ligands for 5-HT$_{1A}$ receptors,[37] **4a**,**b** and **5a**,**b** were also tested toward these receptors. As expected, the results for the most active 5-HT$_7$R compounds, **5a** and **5b**, showed a comparable affinity for the 5-HT$_{1A}$ receptors (Table 1). To improve the 5-HT$_7$R affinity, other pharmacomodulations on the basis of our 5-HT$_7$ pharmacophore (Figure 2) were also carried out. Because the first synthesized phenylpyrrole derivatives lack a HBA group, we decided to introduce such a function. In accordance with the phenylaporphine derivative **9** (Figure 3), a potent and selective 5-HT$_7$R antagonist[26] that correctly fits the pharmacophore (Figure 4), we decided to incorporate a cyano group. This led to compounds **4c** and **5c** (Table 1), with a high 5-HT$_7$R affinity for **5c** (K_i = 18.7 nM). Despite a satisfying accordance with the 5-HT$_7$ pharmacophore (Figure 4), an absence of selectivity over 5-HT$_{1A}$ receptors was still observed. These results can be explained by the fact that such compounds can adopt a

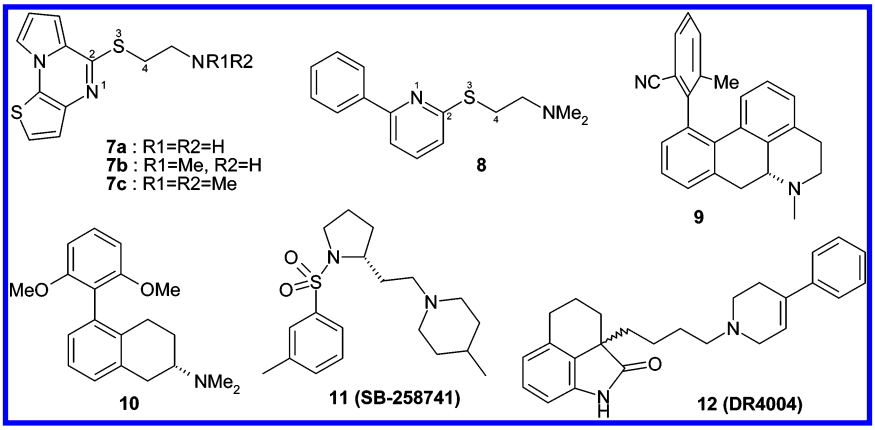
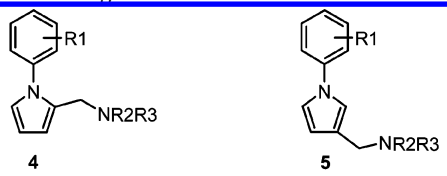
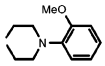
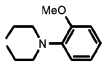
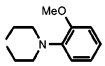


Figure 3. 5-HT$_7$ receptor ligands.

Table 1. Experimental 5-HT$_7$ and 5-HT$_{1A}$ Receptor Binding Affinities



Compd	R_1	R_2R_3	5-HT$_7$ (% Inhibition)[a] 10^{-6} M	5-HT$_7$ (% Inhibition)[a] 10^{-8} M	K_i (nM)[a] 5-HT$_7$	5-HT$_{1A}$ (% Inhibition) 10^{-6} M	5-HT$_{1A}$ (% Inhibition) 10^{-8} M	K_i (nM) 5-HT$_{1A}$
4a	2-CH_3		63	0		34	3	
5a			95	22	21.1	97	22	41.3
4b	2,3-CH_3		68	2	111	59	0	
5b			91	0	16.6	97	7	
4c	2-CN		24	0		59	0	
5c			96	14	18.7	96	16	

[a] Data from binding assays on rat 5-HT$_7$ receptors.

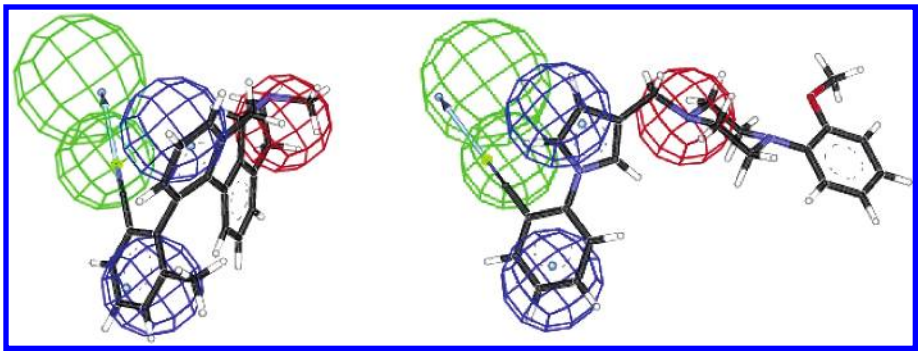


Figure 4. Mapping of compounds **9** (left) and **5c** (right) onto the 5-HT$_7$ pharmacophore.

relatively linear conformation, leading to a geometry similar to the extended conformation of the long-chain arylpiperazine derivatives, accepted as bioactive at the 5-HT$_{1A}$ receptors.[38] For instance, compound **6** (NAN-190, Figure 5) is a well-known 5-HT$_{1A}$ receptor ligand that should bind to this receptor in a linear conformation.[39,40] To compare compounds **5c** and **6**, we designed a 5-HT$_{1A}$ pharmacophore. Several 5-HT$_{1A}$ pharmacophores have been published,[41−44] and it is generally accepted that there are at least two essential chemical features for ligand recognition. These characteristics consist of one aromatic ring separated from a basic nitrogen by 5.2−5.7 Å. Both structural units are present in compound **6** and correspond to the *o*-MPP moiety. Taking this information into account, we constructed a 5-HT$_{1A}$ pharmacophore with Catalyst[20] by fixing the positions of the corresponding aromatic ring and basic center on the extended crystallographic structure of **6**, obtained from the Cambridge Structural Database.[45] To complete this pharmacophore, we fixed the positions of one additional hydrophobic group and one additional HBA group (Figure 5). The distances between the chemical features are recapitulated in Table 2. As shown in Figure 5, compound **5c** can adopt a linear geometry, which

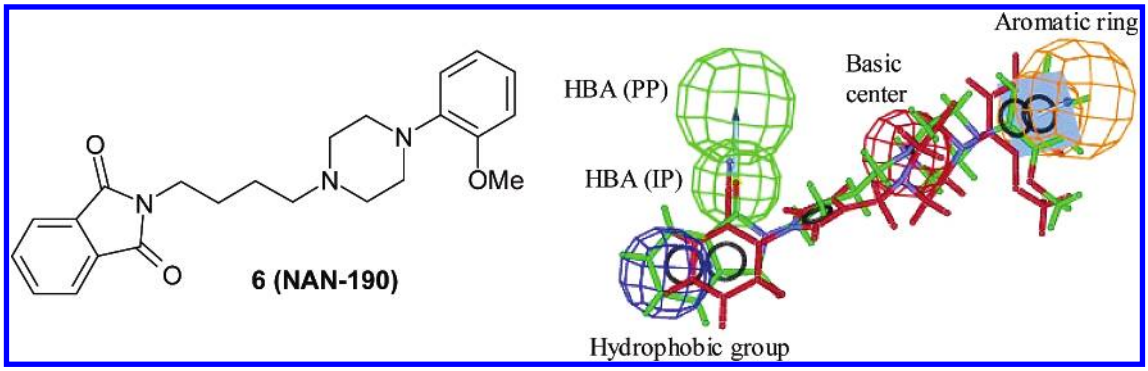


Figure 5. Compound **6** (left) and alignments between **5c** (red) and **6** (green) with the constructed 5-HT$_{1A}$ pharmacophore (right).

Table 2. Matrix Distances (Å) for the Characteristics of the 5-HT$_{1A}$ Pharmacophore[a] (in Parentheses: Corresponding 5-HT$_7$ Elements)

	HBA (IP)	HBA (PP)	hydrophobic group	aromatic ring (IP)	basic center
HBA (IP)					
HBA (PP)	3 (3)				
hydrophobic group	4.4 (4.7)	7 (7.8)			
aromatic ring (IP)	12.2	11.9	15.8		
basic center	6.6 (6.3)	6.8 (7.2)	10.2 (7.3)	5.7	

[a] IP = initial point for the ligand; PP = projected point on the receptor.

Table 3. Binding Properties of Compounds **7a**, **7b**, and **7c**

	5-HT$_7$ (% Inhibition)[a]		5-HT$_{1A}$ (% Inhibition)	
compd	10^{-6} M	10^{-8} M	10^{-6} M	10^{-8} M
7a	90	88	83	1
7b	93	80	82	3
7c	100	72	94	0

[a] Data from binding assays on human 5-HT$_7$ receptors.

gives a correct fit with the 5-HT$_{1A}$ pharmacophore. In that case, the 3-methylene pyrrole moiety of **5c** acts as a spacer equivalent to the butylene linker of **6**. Thus, two pharmacophoric elements (HBA and basic center) are common and geometrically very close for the 5-HT$_{1A}$ and 5-HT$_7$ pharmacophores (6.6 vs 6.3 Å; Table 2). The tendency to act as a 5-HT$_7$R or 5-HT$_{1A}$ receptor ligand seems to depend on the flexibility of the molecule, as shown previously.[40]

Comparison between Our 5-HT$_7$ Pharmacophore and Ligands with High 5-HT$_7$R Affinity. In a previous publication,[23] we described the new series of compounds **7** (Figure 3) with high 5-HT$_7$R affinity (Table 3) and selectivity over the 5-HT$_3$ receptors, the 5-HT$_4$ receptors, and the 5-HT transporter. An alignment between the lead compound, **7a**, and all of the characteristics of our 5-HT$_7$ pharmacophore was then proposed. The resulting conformation, which was obtained with the empirical CHARMM-like force field[46] implemented in the Catalyst program, displayed a value of 97° for the N1−C2−S3−C4 torsion angle (Figure 3). To further investigate the conformational space of such ligands, we ran a search with Mogul,[47] a knowledge base of molecular geometry using data derived from the Cambridge Structural Database. The resulting distribution for such a moiety showed the preferred torsion angle to be close to 0°. For compound **7a**, a value close to 0° was confirmed by DFT calculations (triangle curve, Figure 6). Indeed, the 0−180° scan on a

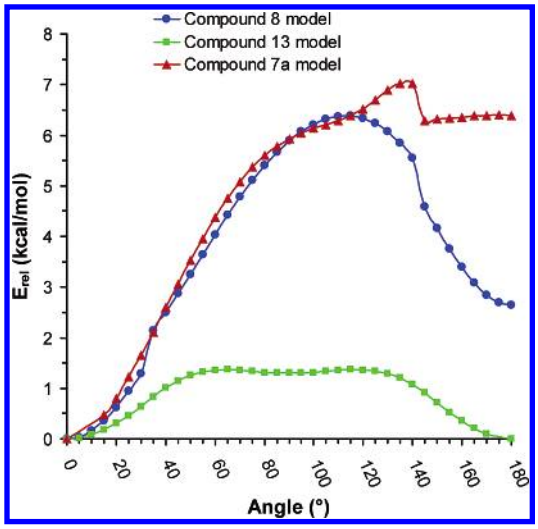
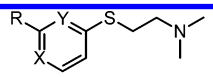


Figure 6. Torsional scan along the N/C−C−S−C(Me) dihedral angle. B3LYP/3-21+G* (in kcal/mol) energies are relative to the lowest energy conformation of each molecule.

model structure yields a local minimum at a 0° torsion angle 6.1 kcal/mol lower in energy than at 97° (not a minimum) and 6.4 kcal/mol lower than at 180°. The higher energy value observed for the 180° torsion angle is due to steric hindrance with the fused-pyrrole ring. For compound **8**, a recently described 5-HT$_7$R partial agonist[25] that presents the same N1−C2−S3−C4 moiety (Figure 3), the same tendency was observed (circle curve, Figure 6). However, a local minimum at 180° and 2.6 kcal/mol higher in energy was also calculated, most likely due to less steric hindrance between the methylene and the 3-hydrogen on the pyridine ring. Finally, an analysis of a model of compound **13** yielded two global minima at 0° and 180°, with a small (1.4 kcal/mol) barrier of rotation and a shallow local minimum located at 90° and 1.3 kcal/mol higher in energy (square curve, Figure 6). This higher conformational freedom led to a decrease in 5-HT$_7$ affinity (Table 4). Taking into account such 0° conformational minima, the resulting bent geometries led to the most likely alignments, represented in Figure 7. Only a partial fit between the pharmacophore and compounds **7a** and **8** was observed, in which the zone corresponding to the HBA cannot be reached. Thus, one basic center and two hydrophobic groups must be sufficient for providing a high 5-HT$_7$R affinity. It can also be noted that a bulky hydrophobic group is not harmful, as shown by compound **14** (Table 4).

Besides, when comparing Figure 6 with Tables 3 and 4, a direct relationship between the conformational flexibility of the N1/C1−C2−S3−C4 moiety and the 5-HT$_7$/5-HT$_{1A}$

Table 4. Thiopyridine-Based 5-HT$_7$ Ligands[25]



Compd	R	X	Y	K_i (nM)[25] 5-HT$_7$	K_i (nM)[25] 5-HT$_{1A}$
8		CH	N	0.6	16
13		N	CH	100	1200
14		CH	N	1.3	17

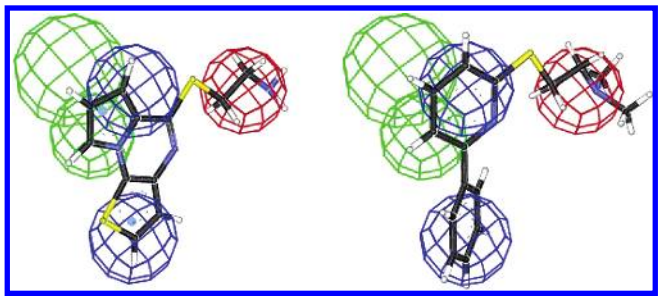


Figure 7. Proposed alignments of **7a** (left) and **8** (right) with the 5-HT$_7$ pharmacophore.

selectivity is highlighted. Indeed, a 27-fold selectivity is observed for compound **8**, with a 6.4 kcal/mol rotational barrier (Figure 6, circle curve), whereas the more flexible compound **13**, with a 1.4 kcal/mol rotational barrier (Figure 6, square curve), only leads to a 12-fold selectivity. For our compound **7a**, with a 7.1 kcal/mol rotational barrier (Figure 6, triangle curve), one can expect a selectivity above 20- or 30-fold. This hypothesis can be inferred from the binding affinities reported in Table 3.

As a result, three characteristics (one basic center and two hydrophobic groups) coupled with a constrained bent geometry provide a very high 5-HT$_7$R affinity and a significant selectivity over the 5-HT$_{1A}$ receptors in this series of compounds.

Comparison between Our 5-HT$_7$ Pharmacophore and Selective 5-HT$_7$R Ligands. Taking into account the results observed for our series of compounds, the importance of the HBA group was reconsidered. This characteristic arises from the 5-HT$_7$ pharmacophore generated by searching the common chemical features of selective antagonists from the literature,[23] including **9**, **11**, and **12** (Figure 3). These compounds display a 37-, 316-, and 81-fold selectivity over the 5-HT$_{1A}$ receptors, respectively.[26,28,29]

The alignment between the highly constrained compound **9** and the pharmacophore (Figure 4) shows an unambiguous orientation for the HBA group. For this series of compounds, the HBA seems to play a crucial role in the 5-HT$_7$R selectivity because the nonsubstituted analogue (R)-11-phenylaporphine is nonselective.[26] By considering the methoxy group as a potential HBA, the closely related compound **10** (Figure 3) supports such a hypothesis (Figure 8). Holmberg et al.[27] mentioned another hypothesis for the 5-HT$_7$R selectivity of **10**: the ortho substituents could force the aryl group out of the plane of the tetralin and thus reduce favorable interactions with the 5-HT$_{1A}$ receptors. Concerning the more selective compound **11**, highly conformational constraints are observed too. This led to the perfect alignment presented in Figure 2. The bent geometry and the ideal orientation of the HBA are found in this mapping. Other 3D-QSAR studies showed the importance of the sulfonamide moiety in the interaction with the 5-HT$_7$R.[48,49] Indeed, this HBA group seems to form a hydrogen bond with the hydroxyl side chain of Ser$^{5.42}$ or Thr$^{5.43}$ (according to the receptor numbering scheme of Ballesteros and Weinstein[50]). Concerning compound **12**, the bent geometry results from its stereocenter and allows a correct positioning and orientation of the amide group as a HBA (Figure 8).

Because these potent selective 5-HT$_7$R ligands also exhibit a constrained bent geometry, such a conformational restriction could play a crucial role in the selectivity toward 5-HT$_7$R compared to 5-HT$_{1A}$ receptors. Considering the phenylaporphine derivatives, the HBA group could participate in a higher selectivity in some cases.

CONCLUSION

This study initially discusses the well-known 5-HT$_7$/5-HT$_{1A}$ receptor problem of selectivity through new phenylpyrrole derivatives. This new series of compounds was

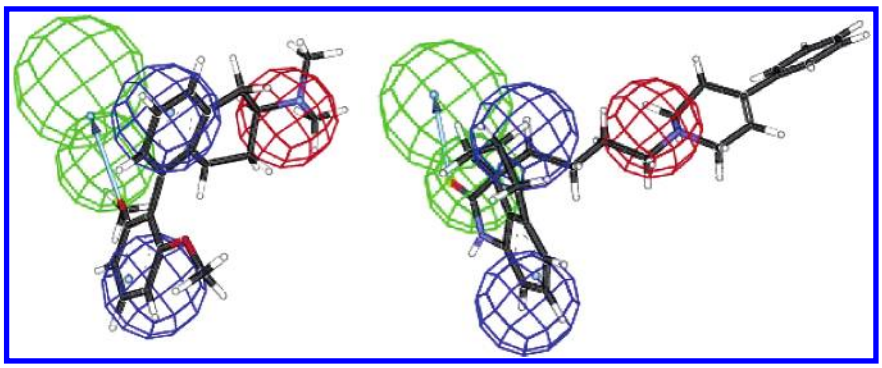


Figure 8. Mapping of compounds **10** (left) and **12** (right) onto the 5-HT$_7$ pharmacophore.

discovered by a virtual screening performed on our 6663 structure chemolibrary. Initially, a 2D similarity searching based on **1** led to the first hit, compound **2c**. Afterward, the phenylpyrrole moiety was the basis for a substructure search of the same chemolibrary. Compound **3** emerged from the analysis of the pharmacological results, with a moderate 5-HT_7R affinity. Starting from **3**, pharmacomodulations were carried out and led to new high affinity 5-HT_7R derivatives. Particularly, the introduction of a hydrogen bond acceptor group was suggested from a 5-HT_7 pharmacophore recently published. Though a correct mapping of **5c** could be obtained for all of the pharmacophoric features, selectivity toward 5-HT_{1A} receptors was not achieved. This result probably arises from the fact that these phenylpyrrole derivatives can adopt an extended conformation best suited for 5-HT_{1A} receptor affinity, whereas a bent one appears to be necessary for 5-HT_7R selectivity. The study of selective 5-HT_7R ligands described by our group or issued from the literature seems to support such a hypothesis.

This work provides an optimization of our preliminary pharmacophore model for 5-HT_7R ligands. In light of this study, the four essential pharmacophoric features coupled with a constrained bent geometry could be the real characteristics required for 5-HT_7R selectivity versus 5-HT_{1A} receptors.

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