Molecular Design Based on 3D-Pharmacophore. Application to 5-HT$_4$ Receptor

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A definition of a pharmacophore for the 5-HT$_4$ antagonist was carried out by considering a three-dimensional model which correlates the chemical structures of series of antagonists with their biological affinities. A molecular design is described by analyzing the differences between two 3D serotonin pharmacophores. This successful structural modification demonstrates the efficiency of this approach to design new serotonin ligands.

INTRODUCTION

The pharmacophore[1−4] classically determines the fundamental characteristics, in term of nature and disposition of chemical groups (topologic and topographic patterns), required for a biological affinity. 3D-QSAR software like Catalyst[5] allowed to obtain pharmacophore among active compounds in a multiconformation structure database. Within the framework of the cationic neurotransmitters, such as 5-HT, which can interact with a series of subtypes receptors[6−8] (Figure 1), 3D-QSAR data could provide us the elements explaining the existing relations linking these subtype receptors (analogies, differences between the pharmacophores). Consequently, molecular design based on 3D pharmacophore could be carried out to obtain new ligands with a unique or a multiple controlled affinity for one or more subtype receptors. In a recent publication,[9] this hypothesis was studied with two subtypes of serotonin receptors, the 5-HT$_3$ and the 5-HT transporter. The analysis of the differences between these two pharmacophores directed the synthesis toward a new selective 5-HT transporter ligand by a structural modification of a 5-HT$_3$ ligand.

In this publication, we studied the transition from 5-HT$_3$ to 5-HT$_4$. The bibliography shows clearly that some 5-HT$_3$ ligands are not denied of affinity for 5-HT$_4$ receptors.[10,11] Indeed, some compounds such as metoclopramide have a double affinity and act as 5-HT$_3$ antagonists and 5-HT$_4$ agonists. Moreover, structural modifications of 5-HT$_3$ ligands, based on conformational restriction, led to selective ligands toward 5-HT$_4$ receptor[11,12] (conformational restriction of derivatives such as metoclopramide for instance).

Our general project aims at studying the structural modification of a basic skeleton, formed by a tricyclic system bearing various substituents and particularly an aminoalkyl chain, to obtain new 5-HT ligands (Figure 1). The design of new 5-HT$_4$ antagonists is nowadays a challenge for treating many physiological pathology like atrial arrythmia[13] or irritable bowel syndrome.[14]

In the recent past, our group developed several selective partial agonists of the 5-HT$_3$ receptors.[15,16] From these data, we described a first precise 3D pharmacophore for these compounds.[17] Initially, the biological tests showed that these ligands did not exhibit any affinity for 5-HT$_4$ receptor in the micromolar range (Table 1). This behavior was evidenced by the structural difference between our compounds and some well-known antagonists 5-HT$_3$ in term of nature and distance between the functions of the respective pharmacophores.[17,18]

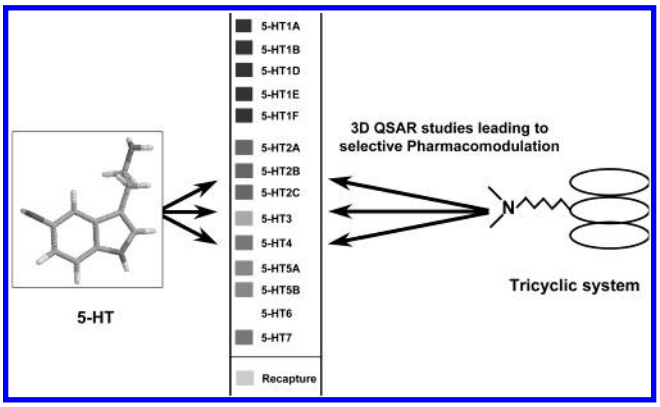
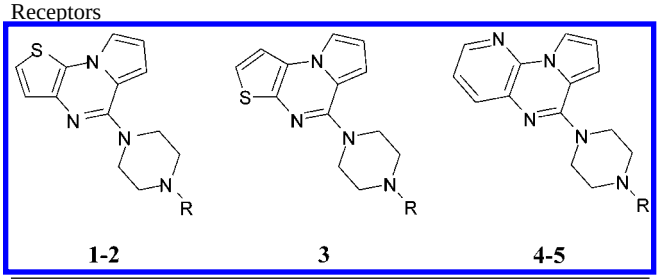


Figure 1. General representation of the structural modification program.

Table 1. Binding Properties of Some Partial Agonists of 5-HT$_3$ Receptors



			5-HT4 (% of inhibition)	
compound	R	5-HT3 (-logIC$_{50}$)	10^{-6} M	10^{-8} M
1	H	7.92	18%	10%
2	CH_2Ph	8.85	21%	16%
3	Allyl	9.04	11%	0%
4	Allyl	12.09	11%	0%
5	CH_2Ph	11.4	26%	6%

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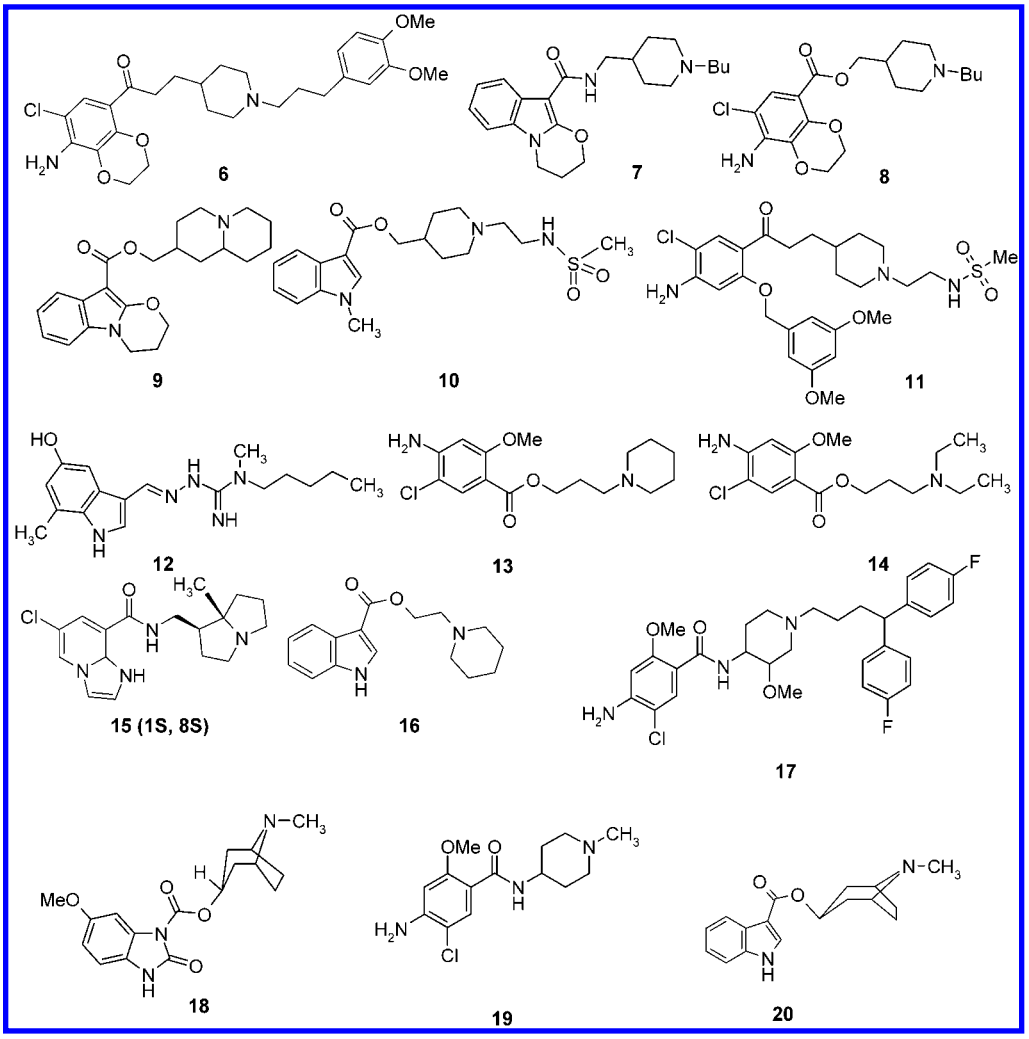


Figure 2. 5-HT_4 ligands considered in the training set.

Table 2. Matrix Distances (in Å) for the Characteristics of the Two Antagonists 5-HT_4 Pharmacophores

	aromatic ring	basic center	HBA
aromatic ring			
basic center	8.0/8.2[a]		
HBA	3.4/3.6[a]	6.8/5.4[a]	

[a] Gaster et al. vs Lôpez-Rodriguez et al.[19,20]

From these considerations and results, our approach was as follows: (1) to define a pharmacophore for 5-HT_4 receptor by a 3D QSAR approach, (2) to compare the two models (partial agonist 5-HT_3 vs antagonists 5-HT_4), (3) to propose some structural modifications, and (4) to analyze the results.

Today, two pharmacophores for the antagonists 5-HT_4 are described in the literature. They both include the same three components:[19,20] an aromatic ring, a hydrogen bond acceptor, and a basic center. In the two cases the basic center is clearly apart from the aromatic plan (1.6 Å vs 3.6 Å for the height). Table 2 summarizes the characteristics of these models in term of distances between the components.

MATERIALS AND METHODS

Training Set and Conformational Analysis. For the definition of the 5-HT_4 pharmacophore, 15 compounds previously described in the literature[21−24] (Figure 2) were analyzed. This training set was made up according to criteria's suitable for the active analogue approach and those suitable for the 3D-QSAR study.

The geometry of each compound was built with the Catalyst builder and optimized by using the CHARMM-like force field implemented in the program.[25] A stochastic research coupled to a poling method[26] was applied to generate conformers for each compound of the training set (20 kcal/mol maximum compared to the energy of the most stable conformer).

Hypothesis Generation. Four functional groups (basic center, hydrogen bond acceptor, aromatic cycle, and hydrophobic group) were selected for the generation of the hypothesis. The choice of these functional groups was based on their presence or their absence in the structure of the best compounds (highest affinities) of the training set and on the characteristics of previous models described in the literature.[19,20]

The default parameters of Catalyst were kept to the following values: weight variation, 0.302; mapping coefficient, 0; spacing, 2.97 Å; and activity uncertainty, 3. The weight variation controls how large a range of feature weights the hypothesis generator will explore during the hypothesis generation. The mapping coefficient controls the importance of having compounds with similar structures map to a hypothesis in a similar way. The spacing specifies the

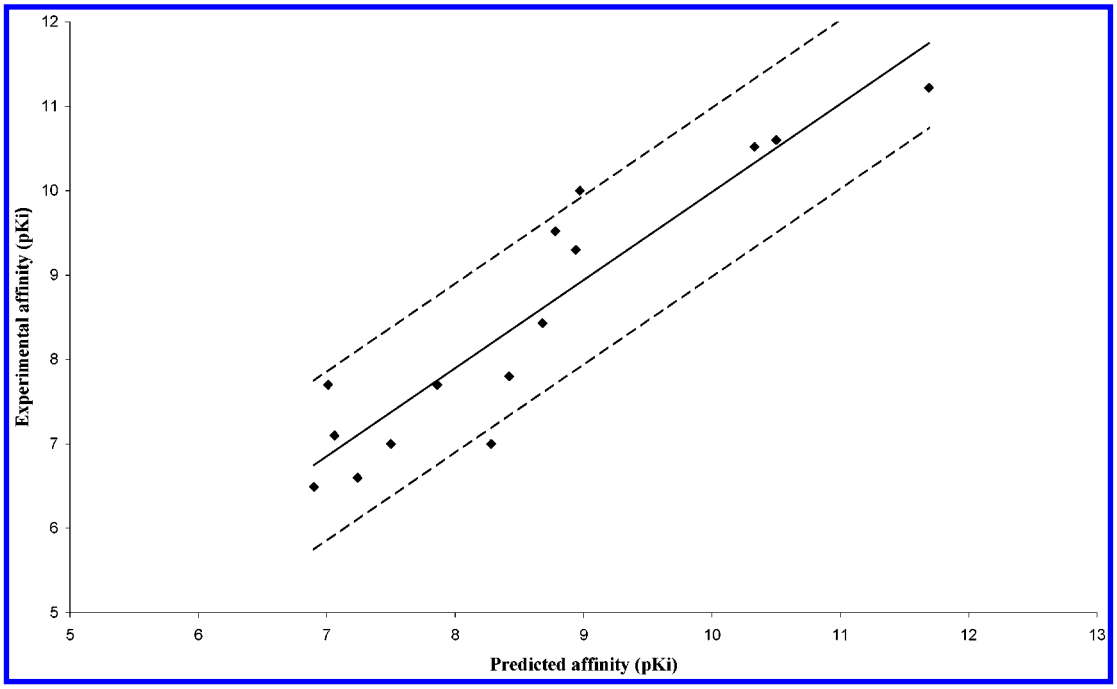


Figure 3. Predicted affinities versus experimental affinities for the 16 compounds ($r = 0.92$; $s = 0.63$).

minimum distance between features of generated hypothesis (the size of our compounds led to keep this value). An uncertainty of 3 in the biological activity means that the activity is located somewhere in the interval "activity/3" to "activity*3".

When generating hypotheses, Catalyst tries to minimize a cost function consisting in three terms.[27] One term, the weight cost, increases in a Gaussian form as the feature weight in a model deviates from an idealized value of two. The second term, the error cost, penalizes the deviation between the estimated activities of the training set and their experimentally determined values. The third term, the configuration cost, penalizes the complexity of the hypothesis. During hypothesis generation, Catalyst calculates the cost of two theoretical hypothesis: the ideal hypothesis, in which the error cost is minimal and the slope of the activity correlation line is one and the null hypothesis, where the error cost is high and the slope of the activity correlation line is zero.[27]

The statistical relevance of the various hypotheses is assessed on the basis of their cost relative to the cost of the null and ideal hypothesis and of their correlation coefficient r.[5,28]

RESULTS.

Selection of One Hypothesis. The choice of the hypothesis was based on the following criteria: (1) elimination of the hypotheses for which the whole chemical characteristics was not recognized by the best compounds of the training set and (2) conservation of the hypotheses with the smallest cost values if these hypotheses had the same chemical characteristics and nearly the same distances between these functions.

These two criteria eliminated eight out of 10 hypotheses. For the remaining ones, the statistical parameters are very close (Table 3). The final choice was made on the pharmacophore comprising an additional chemical characteristic (an aromatic ring). The costs of the ideal hypothesis and that of the null hypothesis were respectively 75 and 150.4.

Table 3. Statistical Parameters of the Two Best Hypotheses

	hypothesis 1	hypothesis 2
total cost	89.3	91.1
r	0.92	0.92
s	0.63	0.63

Table 4. Fit Value, Experimental Affinity, Estimated Affinity, by Considering This Hypothesis

compound	fit value	experimental affinity* pK_i	predicted affinity pK_i	difference
6	11.69	11.22	11.59	+0.36
7	10.5	10.60	10.40	−0.20
8	10.33	10.52	10.21	−0.31
9	8.97	10.00	8.85	−1.15
10	8.78	9.52	8.68	−0.85
11	8.94	9.30	8.82	−0.48
12	8.68	8.43	8.57	+0.14
13	8.42	7.80	8.31	+0.51
14	7.86	7.70	7.74	+0.05
15	7.01	7.70	6.92	−0.78
16	7.06	7.10	6.96	−0.14
17	8.28	7.00	8.12	+1.12
18	7.5	7.00	7.39	+0.39
19	7.24	6.60	7.13	+0.53
20	6.9	6.49	6.80	+0.30

The maximum error of prediction for the affinities was 1.1 logarithmic units (Table 4, Figure 3).

Characteristics of the Pharmacophore. The 3D-pharmacophore (Figure 4) consists of a specific and three-dimensional arrangement of five chemical features corresponding to two hydrophobic groups, an amine (basic center on Figure 4), one hydrogen bond acceptor, and one aromatic ring.

The distances between the chemical features are recapitulated in Table 5.

The superimposition between **8** (Figure 4) and the pharmacophore led to the following observations: one hydrophobic group is a substituent of the aromatic ring and the hydrogen bond acceptor is in the plane of the aromatic ring.

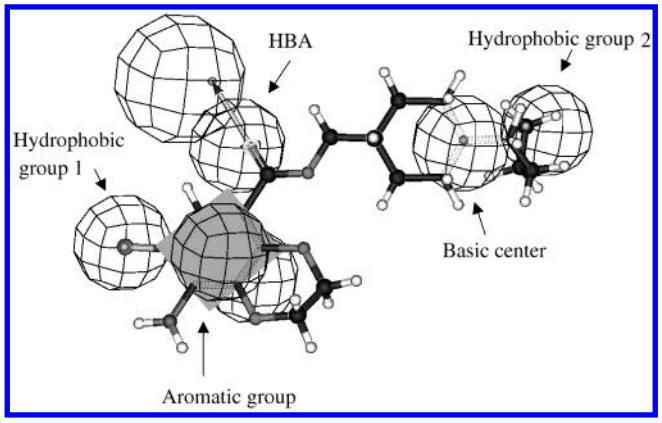


Figure 4. Alignment between **8** and the 5-HT$_4$ antagonist pharmacophore.

Table 5. Matrix Distances (in Å) for the Characteristics of the Hypothesis Selected[a]

			hydrophobe			aromatic	
	HBA IP	HBA PP	group 2	group 1	basic center	group IP	group PP
HBA IP							
HBA PP	3.0						
hydrophobe group 2	10.7	12.7					
hydrophobe group 1	4.9	5.8	15.0				
basic center	7.4	9.6	4.4	11.6			
aromatic group IP	3.9	6.3	12.3	3.6	8.5		
aromatic group PP	4.5	7.0	11.3	4.5	8.4	3.0	

[a] HBA, hydrogen bond acceptor; IP, initial point on the ligand; PP, projected point on the receptor.

Comparison with the Models of the Literature. Compared to the previous pharmacophores, the distances between the chemical groups are slightly longer: 8.5 Å instead of 8.0 Å for the distance aromatic ring−basic center and 7.4 Å instead of 6.8 Å or 5.4 Å for the distance hydrogen bond acceptor−basic center.

This evolution is in relation with the height of the basic center compared to the plane defined by the aromatic ring (0.5 Å vs 1.6 or 3.6 Å[19,20]).

Structural Modification. Three elements (an aromatic ring, a hydrogen bond acceptor and a basic center) are common with our 5-HT$_3$ and 5-HT$_4$ pharmacophores (Figure 4, Figure 5). Yet, the major differences are as follows: The distance aromatic ring−basic center which is longer for the 5-HT$_4$ antagonists (8.5 Å vs 7.2 Å) than for the 5-HT$_3$ partial agonists and the size and the nature of the hydrophobic substituent on the basic center.

The exact correspondence (in term of distances and chemical features) between two elements of the pharmacophores allowed us to modify only the side chain on the basic tricyclic group. Initially, we tested a structural modification of our tricyclic system with the basic chain of compound **8** and **10** (alkyl piperidyl group) which were also present in compounds **7** and **11**. Under these conditions, our compounds were in agreement with the pharmacophore of

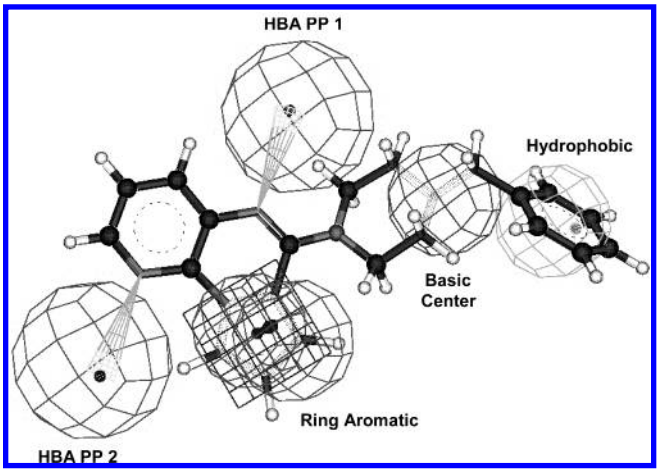


Figure 5. Alignment between **2** and the 5-HT$_3$ partial agonist pharmacophore.

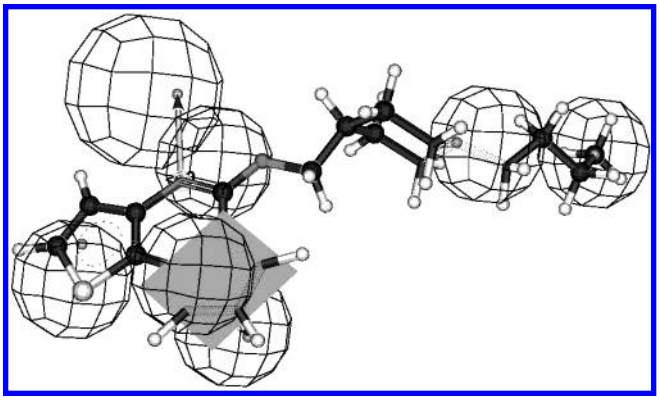
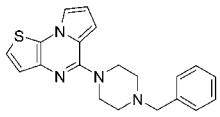
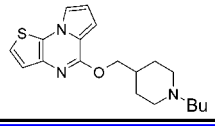


Figure 6. Alignment between **21** and the 5-HT$_4$ antagonist pharmacophore.

Table 6. First Proposed Structural Modification

	Structure	5-HT$_3$ receptor	5-HT$_4$ receptor
2		8.85 (-log IC$_{50}$)	21%[a] 16%[b]
21		20%[c] 0%[d]	7.58 (pKi)

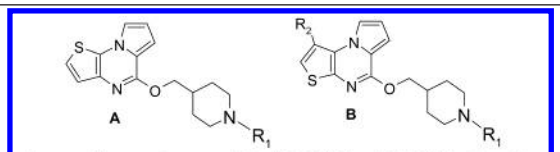
[a] Percentage of inhibition at 10^{-6} M. [b] Percentage of inhibition at 10^{-8} M. [c] Percentage of inhibition at 10^{-5} M. [d] Percentage of inhibition at 10^{-7} M.

the 5-HT$_4$ antagonists (Figure 6), and straightforward, we obtained a remarkable inversion of affinity with the preservation of a great selectivity for 5-HT$_4$ receptor (Table 6).

Thereafter, a series of new derivatives was synthesized including in particular an additional hydrophobic substituent on the tricyclic feature (in agreement with our 5-HT$_4$ pharmacophore), a different orientation for the thiophenic ring and a modification of the hydrophobic group on the final amine (Table 7). The reinforcement of affinity for some compounds of this series, particularly those which bear a methyl group such as **38**−**44** (R_2 = Me) is understood by observing the rise of the quality of the fit between these compounds and the pharmacophore (Table 8, Figure 7).

Some other side chains were also considered such as for the case of compounds **44** and **45**. Their proper binding

Table 7. Evolution of the Affinity in Function of the Structural Modifications



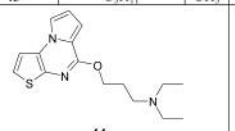
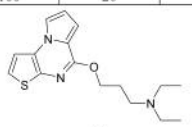
Compound	R_1	R_2	% inhibition at 10^{-6} M	% inhibition at 10^{-8} M	pKi
A					
22	H	-	74	6	
23	CH_3	-	100	0	
24	C_2H_5	-	100	58	7.86
25	$CH_2CH_2CH=CH_2$	-	100	23	
26	$CH(CH_3)CH_2CH_3$	-	56	2	
27	CH_2Ph	-	100	0	
28	C_3H_7	-	100	97	7.74
29	C_4H_9	-	100	62	7.58
30	C_5H_{11}	-	99	55	7.57
B					
31	CH_3	H	100	20	
32	C_2H_5	H	92	0	
33	$CH_2CH_2CH=CH_2$	H	99	49	
34	C_3H_7	H	100	54	7.91
35	C_4H_9	H	100	56	7.60
36	C_5H_{11}	H	100	13	
37	CH_3	CH_3	100	13	
38	C_2H_5	CH_3	100	30	
39	$CH_2CH_2CH=CH_2$	CH_3	100	28	
40	CH_2Ph	CH_3	100	11	
41	C_3H_7	CH_3	100	78	8.53
42	C_4H_9	CH_3	100	75	8.12
43	C_5H_{11}	CH_3	100	20	
 44			 45		
44	-	-	100	28	
45	-	-	100	28	

Table 8. Evolution of the Fit Values

	fit values[a]	predicted affinity (pK_i)
28	8.56	8.45
41	9.64	9.63

[a] Best Fit option for the calculation.

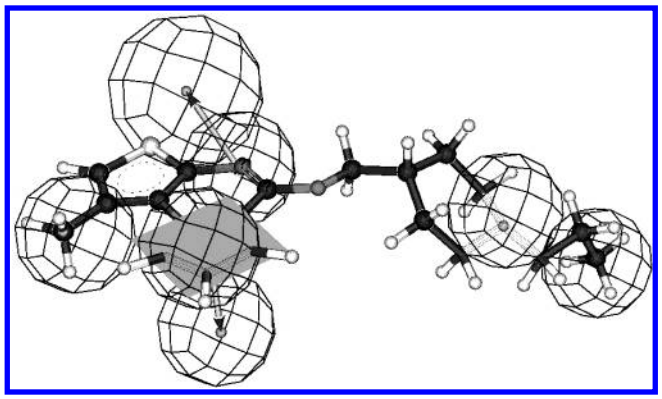


Figure 7. Alignment between **41** and the 5-HT_4 antagonist pharmacophore.

results (Table 7) are in agreement with the results observed for their direct analogues, the metoclopramide-like compounds **13** and **14**.

CONCLUSION

This study defined a pharmacophore for 5-HT_4 receptors based on a 3D-QSAR. The comparison with the 5-HT_3 model enabled us to understand the relation between our first 5-HT_3 ligands and the 5-HT_4 ligands. This analysis directed the synthesis toward new selective 5-HT_4 ligands. This successful molecular design based on 3D-pharmacophore shows the efficiency of this approach to design new serotonin ligands. We have now designed ligands for 5-HT transporter, 5-HT_3, and 5-HT_4 receptor. Today, we worked[29] on other subtypes and particularly 5-HT_5, 5-HT_6, and 5-HT_7.

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