Molecular Design Based on 3D-Pharmacophore. Application to 5-HT Subtypes Receptors

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A first definition of a pharmacophore for the serotonin reuptake inhibitors was carried out by considering a three-dimensional model which correlates the chemical structures of series of reuptake inhibitors with their biological affinities. A molecular design was described by analyzing two different 3D serotonin pharmacophores. This successful approach enabled us to consider the design of new serotonin ligands by the same method.

INTRODUCTION

The pharmacophores[1−4] classically determine the fundamental characteristics, in terms of nature and disposition of chemical groups (topologic and topographic patterns), required for a biological affinity. 3D-QSAR software like Catalyst[5] was allowed to obtain pharmacophores 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] (see Figure 1), 3D-QSAR data could provide us with 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. In this publication, this hypothesis is studied with two subtypes of serotonin receptors, the 5-HT_3 and the 5-HT transporter. The selective pharmacomodulations will be analyzed on a tricyclic feature linked to an aminoalkyl side chain (summarized in Figure 1).

Initially, our group developed several selective ligands (Figure 2) for the 5-HT_3 receptor such as compounds **1** or **2**.[9,10] From these data, we described a precise 3D pharmacophore for the partial agonists of a 5-HT_3 receptor.[11]

Beside these results, several of these 5-HT_3 ligands were tested toward the 5-HT transporter. The compound **2** is selective toward the 5-HT_3 receptor[12] (9.96 vs 6.06 for 5-HT transporter (-log IC_{50})) contrary to compound **3**, in the same series, which exhibited an interesting equivalent double affinity for the two subtypes (7.48 vs 7.58).

Since for the serotonin reuptake inhibitors no 3D-pharmacophore analysis based on a multiconformational database had been published before, the first step of this study was this definition. Indeed, the first pharmacophore of 5-HT reuptake inhibitors described in the literature (topographic patterns, Figure 3) was a function of the comparison of serotonin and alaproclate.[13] It includes a primary amine (A), an aromatic ring (B), and a system rich in electrons (C). Thereafter three other models appeared with nearly the same characteristics.[14−16] However, Rupp and al.[15] proposed the presence of a second aromatic ring in a plan perpendicular to the first aromatic ring. These models pointed out some important information but were not sufficiently defined for our 3D-pharmacophore comparisons.

MATERIALS AND METHODS

Training Set and Conformational Analysis. For the definition of the 5-HT reuptake inhibitors pharmacophore, 19 compounds previously described in the literature[15] (Figure 4) were analyzed. The biological data for this set cover 3 orders of magnitude.

The geometry of each compound was built with the Catalyst builder and optimized by using the CHARMM-like force field implemented in the program.[17] A stochastic research coupled to a poling method[18] 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).

In this series, a problem of stereochemistry appeared with two derivatives (**8** and **19**) for which the biological data belonged to the racemic form. In the case, we have considered the two configurations. This problem will be discussed in the result part.

Hypothesis Generation. Four functional groups (basic center, acceptor of hydrogen bonds, 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.[13−16]

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

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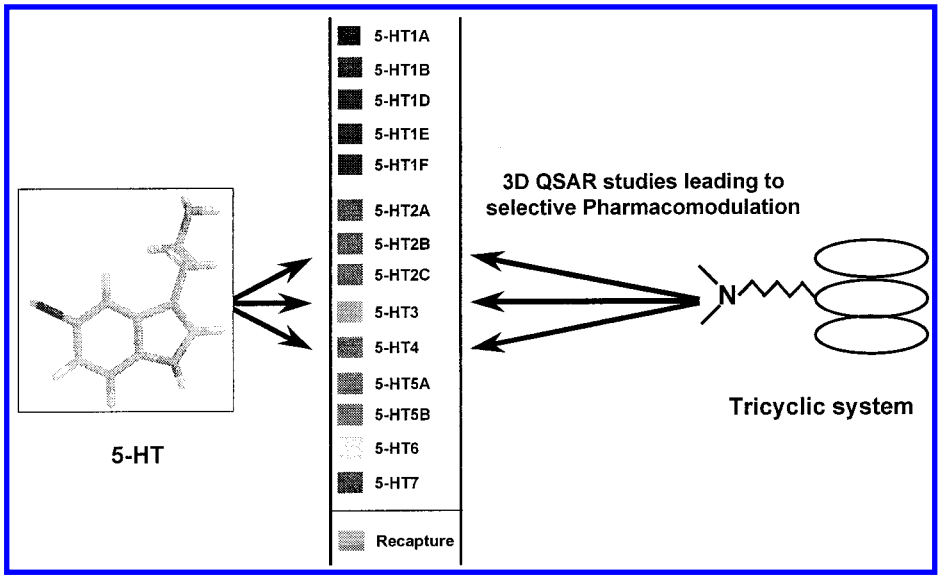

Figure 1. General representation of the pharmacomodulation program.

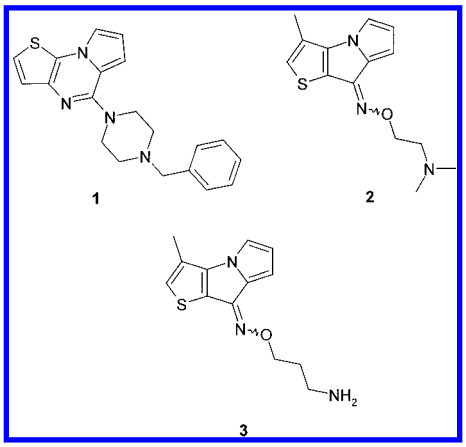

Figure 2. Chemical structures of our 5-HT_3 ligands.

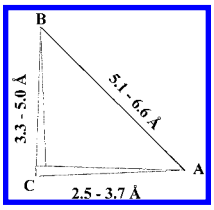

Figure 3. First representation of the serotonin reuptake pharmacophore.

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.[19] 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 hypotheses: 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.[19]

The statistical relevance of the various hypotheses is assessed on the basis of their cost relative to the cost of the null and ideal hypotheses and of their correlation coefficient r.[5,20]

RESULTS

Selection of One Hypothesis. The choice of the hypothesis was based on the following criteria: (1) elimination of the hypotheses with a higher cost of 15 compared to the cost of the best hypothesis and (2) elimination of the hypotheses based only on three chemical characteristics. Following this strategy, we wanted to obtain the most selective possible model.

These two criteria eliminated several generated hypotheses. Among the remaining ones, those for which the whole chemical characteristics was not recognized by the best compounds were dismissed. Finally, the choice was made on the best hypothesis remaining.

The final statistical relation obtained was the following one (Table 1, Figure 5):

$$-\log (IC_{50}) = 0.87(\pm 0.16)*\text{fit value} + 2.59\ (\pm 0.95) \quad (1)$$

$$n = 19,\ r^2 = 0.64,\ s = 0.73$$

The maximum error of prediction for the affinities was 1.64 logarithmic units. In the majority of cases (21 out of 28), the error was lower than one logarithmic unit.

The total cost of the hypothesis selected was equal to 139.5. The costs of the ideal hypothesis and that of the null hypothesis were respectively 105.5 and 190.6. The difference between the total cost of the hypothesis and the cost of the null hypothesis (no correlation) was 51.1. The pharmacophore cost is closer to the fixed cost (ideal hypothesis).

Characteristics of the Pharmacophore. The 3D-pharmacophore (Figure 6) consists of a specific and three-dimensional arrangement of four chemical features corresponding to the following: a hydrophobic group, an amine (basic center on Figure 1), and two aromatic rings.

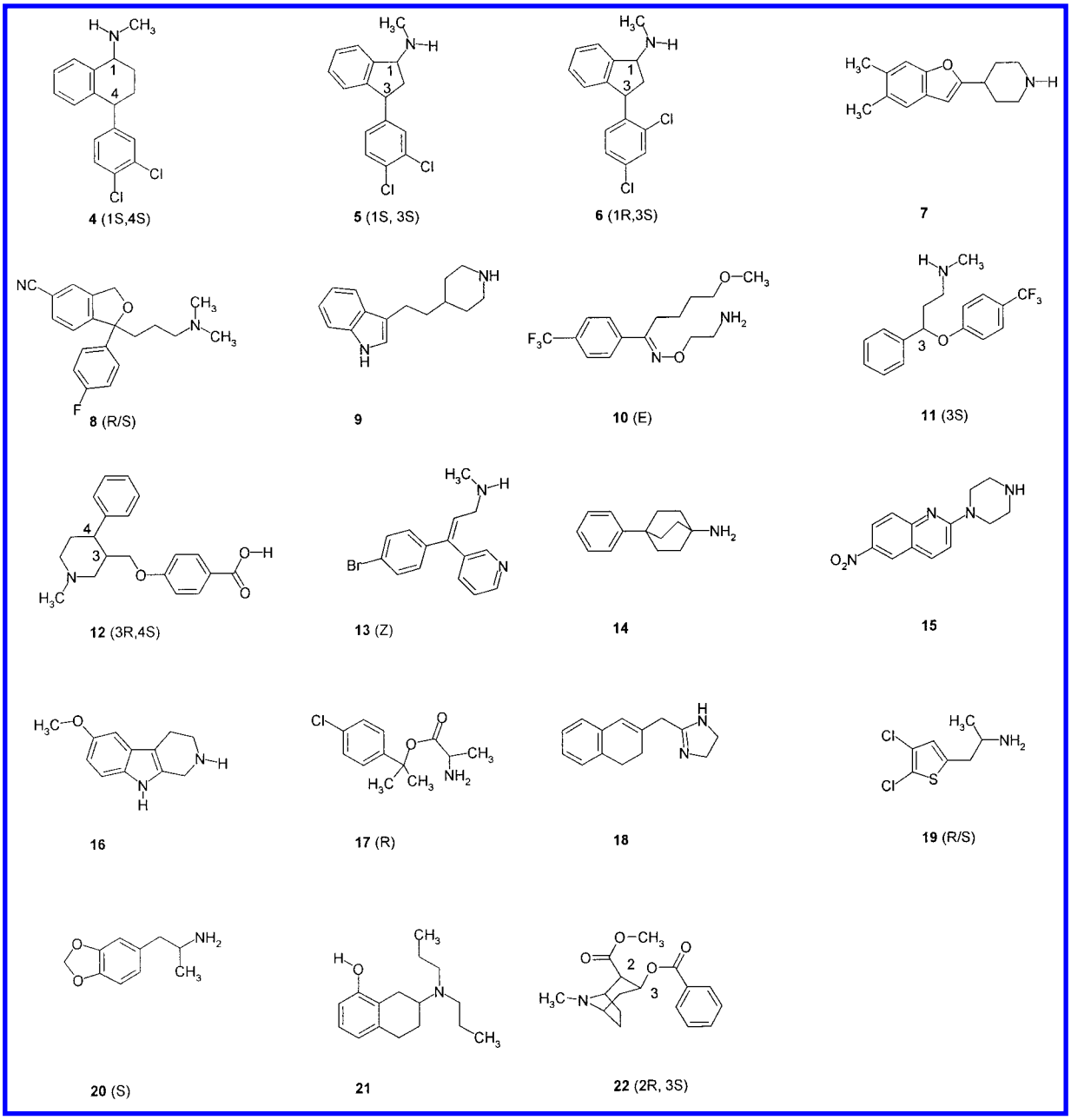

Figure 4. Serotonin reuptake inhibitors considered in the training set.

The distances between the chemical features are recapitulated in Table 2.

The superimposition between **4** (Figure 6), a selective 5-HT reuptake inhibitor, and the pharmacophore led to the following observations: (1) the two aromatic rings are in almost perpendicular plans, (2) the basic center corresponds to the final amine which is distant respectively of 6.6 and 4.1 Å of the aromatic rings 1 and 2, and (3) the hydrophobic group is a substituent of one aromatic ring.

Problem of Stereochemistry. The racemic form of the citalopram (**8**) was considered in this study (this is the form currently marketed by Forest laboratories). The S configuration of citalopram has a fit value, toward the pharmacophore, higher than the R configuration. In fact, (*S*)-citralopram seems to be the active configuration as indicated by the recent developments of this compound.[21] This result shows that this pharmacophore understands the impact of stereochemistry on the affinity of the chemicals.

Comparison with the Models of the Literature. Our pharmacophore is in agreement with the information provided by the former studies. The major difference is the absence of a "system rich in electrons" in the definition of our model. The importance of two aromatic rings is confirmed.[15]

Improvement of the Statistical Equation. We were interested in the possibility of incorporating additional descriptors in order to improve the statistical relation. To this end several descriptors[22] were calculated including physicochemical descriptors (logPow, MR), structural descriptors (number of rotatable bonds, number of H bond acceptor and donor groups, molecular weight), spatial descriptors (Jurs descriptors, shadow indices), and topological descriptors (Wiener index, Hosoya index, Hier & Hall molecular connectivity index, Zagreb index). The genetic function approximation (GFA) algorithm[22] was used to emphasize the significant descriptors. An initial population of 100 randomly constructed equations was first generated.

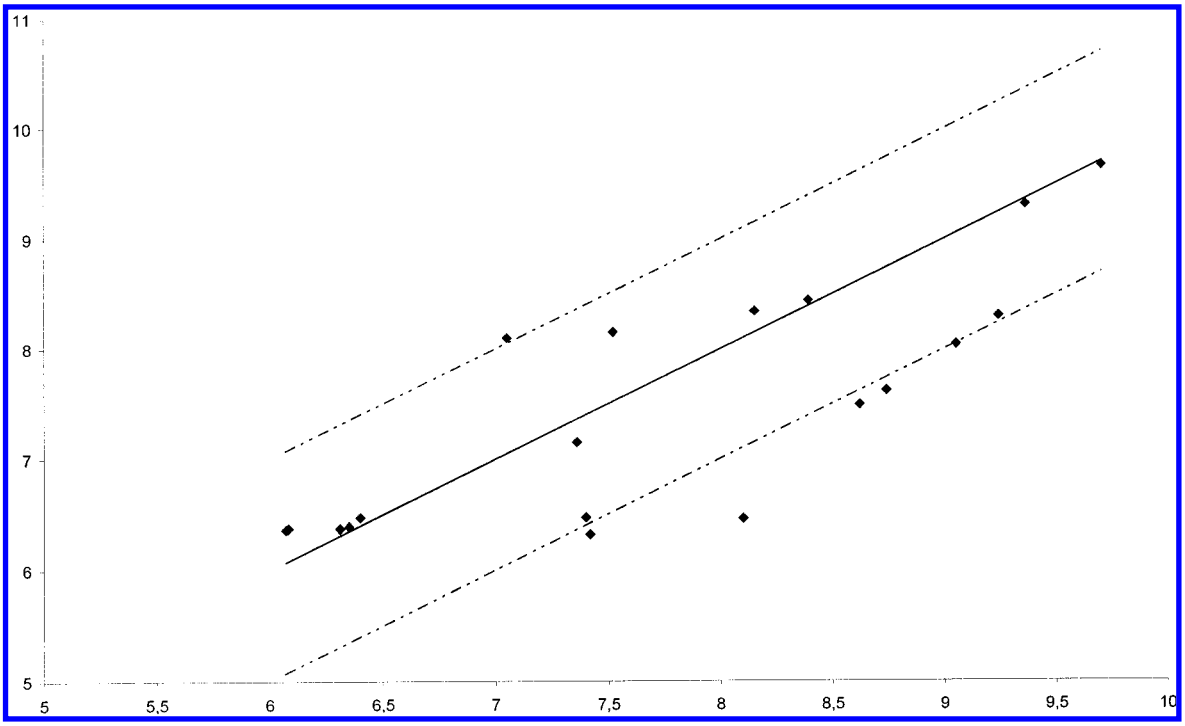

Figure 5. Experimental affinities versus predicted affinities for the 28 compounds ($r^2 = 0.64$).

Table 1. Fit Value, Experimental Affinity, and Estimated Affinity, by Considering This Hypothesis

compd	fit value	exptl affinity -log (IC_{50})*	pred affinity (eq 1)	diff with eq 1	pred affinity (eq 2)	diff with eq 2
4	8.09	9.70	9.66	−0.04	9.82	0.12
5	7.74	9.36	9.31	−0.05	9.35	−0.01
6	6.73	9.24	8.30	−0.94	8.95	−0.29
7	6.47	9.05	8.04	−1.01	8.96	−0.09
8	6.06	8.74	7.62	−1.12	8.46	−0.28
9	5.93	8.62	7.49	−1.12	8.28	−0.34
10	6.87	8.39	8.44	+0.06	8.29	−0.10
11	6.78	8.15	8.34	+0.18	8.41	+0.26
12	4.89	8.10	6.46	−1.64	7.52	−0.58
13	6.59	7.52	8.15	+0.63	7.76	0.24
14	4.75	7.42	6.32	−1.10	6.64	−0.78
15	4.90	7.40	6.47	−0.93	8.05	0.65
16	5.59	7.36	7.15	−0.20	7.61	0.26
17	6.54	7.05	8.10	+1.05	7.01	−0.04
18	4.90	6.40	6.47	+0.07	6.62	0.22
19	4.82	6.35	6.39	+0.04	6.12	−0.23
20	4.80	6.31	6.37	+0.06	6.08	−0.23
21	4.80	6.08	6.37	+0.29	6.59	0.50
22	4.79	6.07	6.36	+0.29	6.63	0.56

The initial population was then evolved for 5000 generations. The mutation probabilities was equaled to 0.5, the smoothing parameter d, which controls the scoring bias between equations of different sizes, was equaled to 1, and the initial equation length was equaled to 4.

The importance of spatial descriptors such as the shadow indices was underlined during this study. Indeed, the best relations included each time this type of descriptors. This set of geometric descriptors helps to characterize the shape of the molecules. The shadow descriptors[23] are calculated by projecting the molecular surface on three mutually perpendicular planes, XY, YZ, and XZ. These descriptors depend not only on conformation but also on the orientation of the molecule. To calculate them, the molecules are first rotated to align the principal moments of inertia with the X, Y, and Z axes. A total of 10 descriptors are calculated in this set including the area of the molecular shadow in the XY plane (Shadow-XY), the area of the molecular shadow in the YZ plane (Shadow-YZ), the area of the molecular shadow in the XZ plane (Shadow-XZ), the fraction of area of molecular shadow in the XY plane over area of enclosing rectangle (Shadow-XYfrac), the fraction of area of molecular

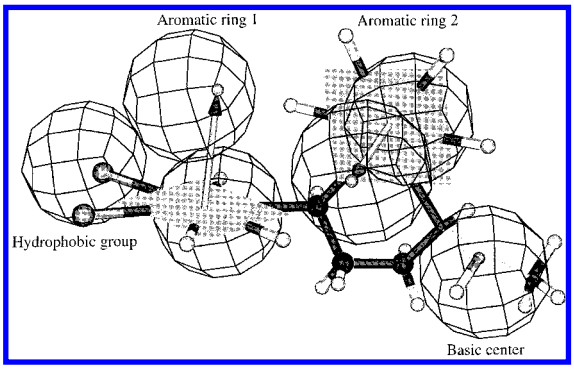

Figure 6. Representation of the 5-HT reuptake inhibitors pharmacophore (alignment of **4** with this pharmacophore).

Table 2. Matrix Distances (in Å) for the Characteristics of the Hypothesis Selected[a]

	hydrophobic group	basic center	aromatic ring			
			PI1	PP2	PI1	PP2
hydrophobic group						
basic center	10.6					
aromatic ring PI1	4.1	6.6				
aromatic ring PP1	4.2	8.3	3.0			
aromatic ring PI2	8.5	4.1	5.1	5.3		
aromatic ring PP2	8.6	4.8	4.9	5.1	3.4	

[a] PI: initial point for the ligand. PP: projected point on the receptor.

Table 3. Shadow Indice Values and Number of Rotation Bonds

	Shadow-Zlength	Shadow-Ylength	Shadow-Xlength	Shadow-nu	Shadow-YZfrac	Shadow-XZfrac	Shadow-XYfrac	Shadow-YZ	Shadow-XZ	Shadow-XY	Rotlbonds
4	8.33	9.98	12.69	1.52	0.66	0.66	0.57	54.63	69.34	72.40	2
5	8.50	8.74	12.53	1.47	0.67	0.62	0.65	49.50	66.52	70.85	2
6	8.39	9.18	12.63	1.50	0.64	0.62	0.63	49.03	66.03	72.86	2
7	6.55	8.11	13.59	2.08	0.63	0.62	0.66	33.59	55.07	72.32	1
8	8.11	11.58	14.44	1.78	0.61	0.63	0.51	57.39	73.42	84.41	6
9	5.82	8.83	13.26	2.28	0.64	0.67	0.66	32.95	51.85	77.53	3
10	6.50	14.63	12.88	2.25	0.64	0.68	0.48	61.14	57.04	90.90	10
11	6.93	13.03	11.84	1.88	0.66	0.64	0.53	59.28	52.53	81.59	6
12	6.76	10.52	16.23	2.40	0.68	0.61	0.58	48.02	67.11	98.17	6
13	6.02	10.74	12.99	2.16	0.71	0.70	0.54	45.88	54.96	75.42	4
14	6.74	6.80	12.34	1.83	0.64	0.60	0.73	29.50	49.88	61.11	2
15	6.40	7.93	14.31	2.24	0.61	0.56	0.70	31.03	51.47	79.11	2
16	4.92	8.31	12.34	2.51	0.67	0.65	0.66	27.21	39.67	67.25	1
17	6.54	9.41	12.31	1.88	0.71	0.68	0.61	44.02	54.77	70.69	6
18	6.94	7.51	13.43	1.93	0.68	0.58	0.66	35.59	53.94	66.55	2
19	6.22	6.96	11.07	1.78	0.64	0.60	0.70	27.92	41.11	54.19	3
20	5.49	7.28	11.74	2.14	0.66	0.67	0.68	26.52	42.86	57.79	3
21	7.23	9.46	12.64	1.75	0.66	0.61	0.65	44.99	55.54	77.90	6
22	7.88	9.99	14.02	1.78	0.63	0.69	0.49	49.50	76.17	68.93	5

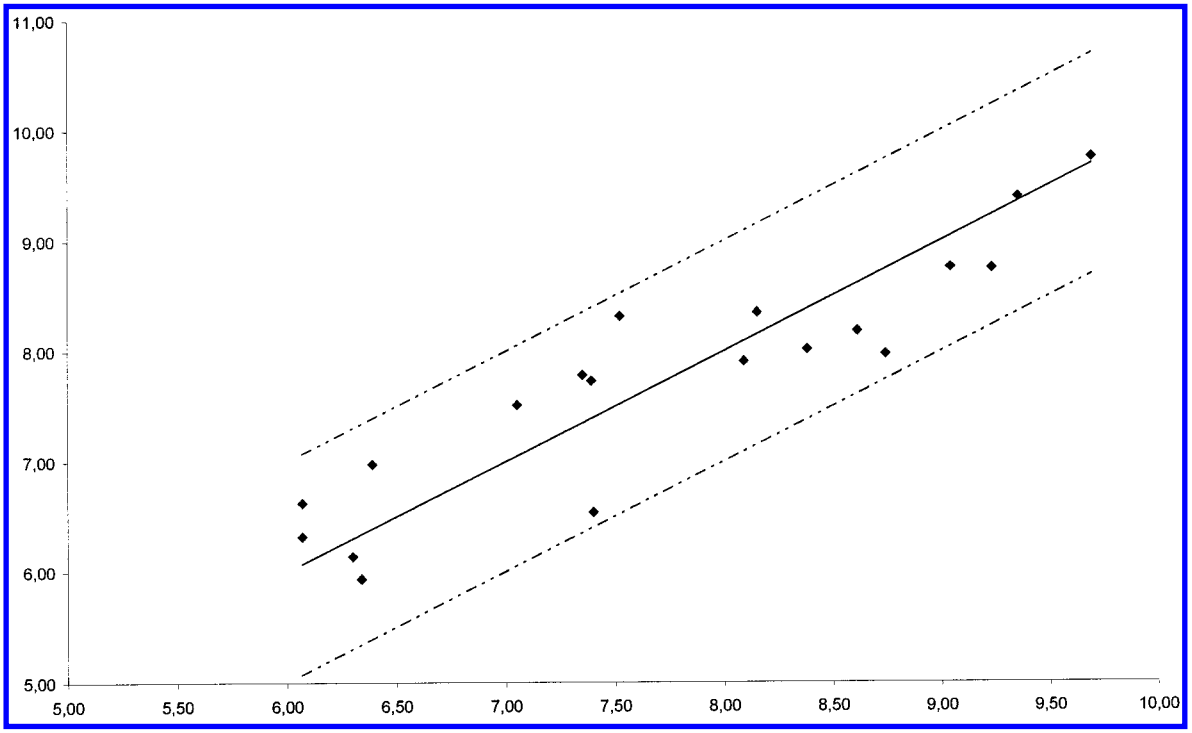

Figure 7. Experimental affinities versus predicted affinities for the 28 compounds ($r^2 = 0.84$).

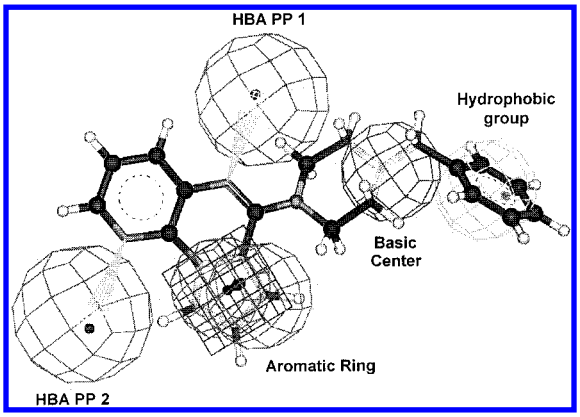

Figure 8. Representation of the partial agonist 5-HT_3 pharmacophore (alignment of **1** with this pharmacophore).

shadow in the YZ plane over area of enclosing rectangle (Shadow-YZfrac), the fraction of area of molecular shadow in the XZ plane over area of enclosing rectangle (Shadow-XZfrac), the length of molecule in the X dimension (Shadow-Xlength), the length of molecule in the Y dimension (Shadow-Ylength), and the length of molecule in the Z dimension (Shadow-Zlength), the ratio of largest to smallest dimension (shadow-nu).

Another point underlined in this study is the flexibility of the compounds by considering as descriptor the number of rotatable bonds (Rotlbonds).

The final statistical relation retained with three descriptors was the following one (Tables 1 and 3 and Figure 7).

$$-\log(IC_{50}) = 0.76(\pm 0.12)*\text{fit value} + 0.06(\pm 0.02)*\text{Shadow-XY} - 0.24(\pm 0.07)*\text{Rotlbonds} - 0.26(\pm 1.04) \quad (2)$$

$$n = 19,\ r^2 = 0.84,\ s = 0.52$$

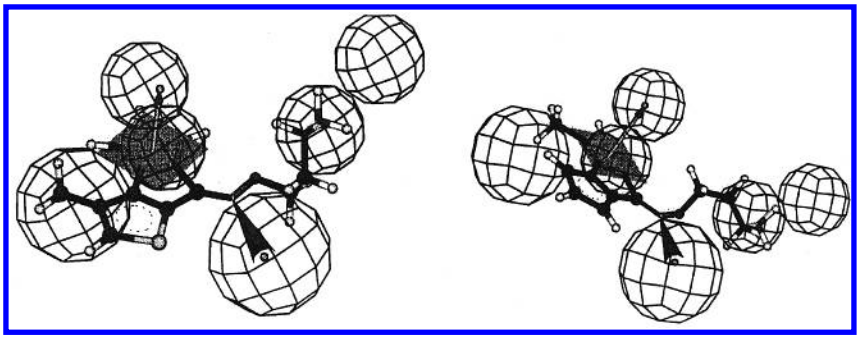

Figure 9. Alignment between **3** (two configurations) and the 5-HT_3 partial agonist pharmacophore.

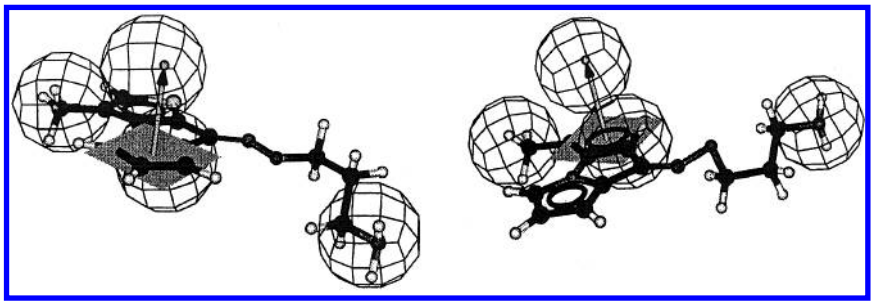

Figure 10. Alignment between **3** (two configurations) and the 5-HT reuptake inhibitors pharmacophore.

With four descriptors, we improved slightly the statistical equation:

$$-\log(IC_{50}) = 0.82(\pm 0.10)*\text{fit value} + 0.05(\pm 0.01)*\text{Shadow-XY} - 0.21(\pm 0.06)*\text{Rotlbonds} - 10.88(\pm 3.76)*\text{Shadow-YZfrac} + 6.98(\pm 2.64) \quad (3)$$

$$n = 19,\ r^2 = 0.90,\ s = 0.43$$

The importance of the two perpendicular hydrophobic area (already pointed out in the pharmacophore) are accentuated by considering the spatial descriptor. This result is interesting because Catalyst theoretically gives the same weight for all features of the pharmacophore (the feature weight of the model must be close to an idealized value of two). The new factor corresponds to the conformational flexibility of the compounds. This last information supplement in an interesting way the preceding 3D-QSAR analysis because no penalty function of the conformational flexibility of a compound (probability to obtain the conformer which fits the pharmacophore) is taken into account in the eq 1.

PHARMACOMODULATION

Comparison between the Partial Agonist 5-HT_3 and the 5-HT Reuptake Inhibitors Pharmacophores. Two chemical features are common to these two pharmacophores (Figures 6 and 8). The first common element is the basic center, and the second is the aromatic ring named 1. The distances between these two features (6.5 vs 6.6 Å) and the heights of the basic center compared to the plane defined by the aromatic ring are very close (0.56 vs 0.52 Å). These data explain the double affinity of **2**.

Relation between the Compound 3 and the Pharmacophores. The compound **3** could theoretically fit three characteristics of the partial agonist 5-HT_3 pharmacophore out of five (aromatic ring, hydrogen bond acceptor, positive ionizable group (amine)). This fit could be obtained, with the same quality, for the two configurations (Figure 9).

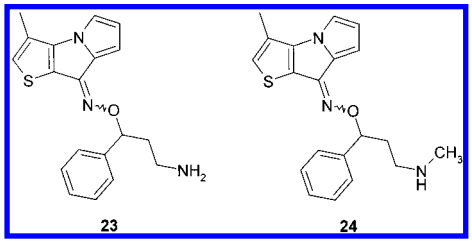

Figure 11. Chemical structures proposed for the pharmacomodulations of **3**.

However, in one case, the thiophene ring fitted the aromatic ring and in the other case it was the pyrrole ring. This pointed out the importance of the tricyclic ring toward the biological results. The predicted affinity is in agreement with the experimental affinity (7.3 vs 7.58 (-log IC_{50})).

The compound **3** could also fit three characteristics of the 5-HT reuptake inhibitors pharmacophore out of four. Like for the previous pharmacophore, the fit could be obtained for the two configurations with the same quality (Figure 10). The predicted affinity is in agreement with the experimental affinity (7.79 vs 7.48 (-log IC_{50})).

Orientation toward a Selective 5-HT Reuptake Inhibitor. To direct the pharmacomodulations, we chose to exploit the differences between the two pharmacophores. The choice was quite naturally orientated (aromatic ring 2 of the pharmacophore and shadow-XY descriptor) toward the addition of a second aromatic cycle on compound **3** (Figure 11). This chemical modification led to the possibility of four diastereoisomers. We analyzed the fit between each diastereoisomer and the pharmacophore. The four diastereoisomers fitted the characteristics of the pharmacophore with different quality (Figure 12, Table 4). The best fit was obtained for the E configuration and the overlap of the two hydrophobic perpendical regions (shadow-XY) was better for the R configurations (Table 4). From these results, the synthesis of this compound (the racemic was obtained) was decided. The experimental results showed three aspects:

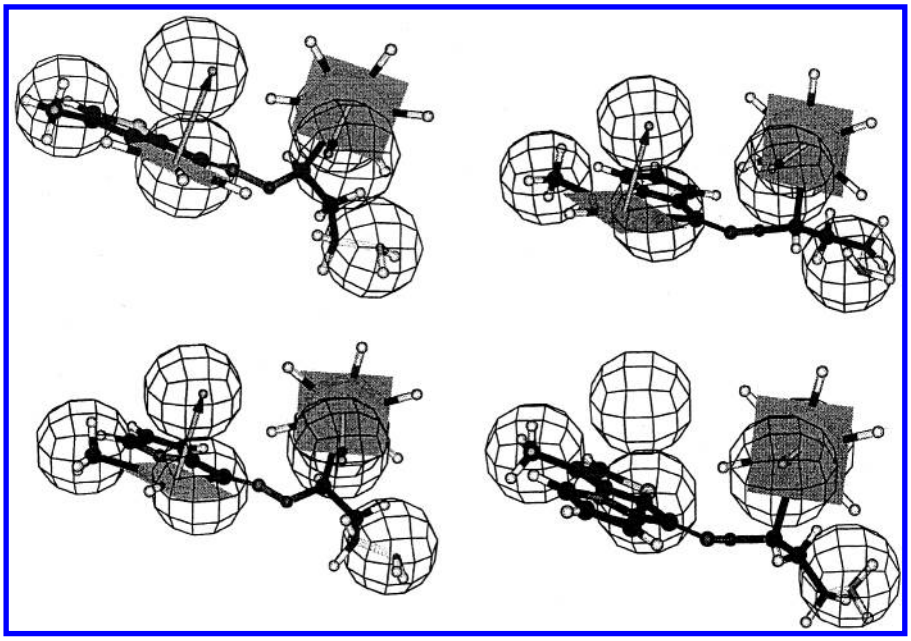

Figure 12. Alignment between **23** (four configurations, ER, ES, ZR, ZS) and the 5-HT reuptake inhibitors pharmacophore.

Table 4. Binding Properties of the New Derivatives

	5-HT_3[a]	config-uration	fit value[b]	Shadow-XY	Rotl-bonds	pred affinity	exptl affinity[c]
23	12%	ER	8.18	93.73	6	10.14	8.32
		ES	7.76	88.49	6	9.50	
		ZR	7.18	90.49	6	9.18	
		ZS	6.98	85.47	6	8.73	
24	11%			same as for **23**			9.7

[a] Experimental affinity toward the 5-HT_3 receptor (percentage of inhibition at 10^{-7} M) for the mixture. [b] Fit value toward the 5-HT transporter. [c] Experimental affinity toward the 5-HT transporter (-log IC_{50}) for the mixture.

1. The affinity of compound **23** is good for 5-HT transporter (Table 4).

2. The prediction from eq 2 was weak (2 logarithmic units for the difference between predicted affinity (average predicted affinity) and the experimental affinity).

3. The selectivity toward the 5-HT transporter increased seriously (Table 4).

In front of this problem of a poor predicted affinity, we noticed that the basic center for several 5-HT reuptake inhibitors corresponds to a secondary amine instead of a primary amine. The synthesis of compound **24** (racemic) was decided, and this modification led to a compound with an experimental affinity closed to the predictive affinity (same predictive affinity for **23** and **24**). With this last observation, the pharmacophore must be completed by the precision of the characteristics of the amine.

CONCLUSION

This study initially defined a new pharmacophore for 5-HT reuptake inhibitors based on a 3D-QSAR. The incorporation of spatial and structural descriptors improves the predictive quality of the equation. The comparison with the 5-HT_3 model enabled us to understand the double affinity of ligands (5-HT_3 vs 5-HT transporter). The analysis of the differences between these two pharmacophores directed the synthesis toward a new selective ligand. This successful molecular design based on 3D-pharmacophore shows the efficiency of this approach to design new serotonin ligands.

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REFERENCES AND NOTES

(1) Korolkovas, A. *Essentials of Molecular Pharmacology, Background for drug design;* New York: Interscience: 1970.

(2) Gund, P. *Three-dimensional pharmacophore pattern searching.* In *progress in molecular and Subcellular Biology*; Hahn, F. E., Ed.; Springer-Verlag: Berlin, 1977; Vol. 5, pp 117−143.

(3) Gund, P. Pharmacophoric pattern searching and receptor mapping. *Ann. Reports Med. Chem.* **1979**, *14*, 299−308.

(4) Humblet, C.; Marshall, G. R. Pharmacophore identification of receptor mapping. *Ann. Reports. Med. Chem.* **1980**, *15*, 267−276.

(5) CATALYST Version 4.5 software; ACCELRYS, 2001.

(6) Hoyer, D.; Clarke, D. E.; Fozard, J. R.; Harting, P. R.; Martin, G. R.; Mylecharane, E. J.; Saxena, P. R.; Humphrey, P. P. International Union of Pharmacology Classification of Receptors for 5-Hydroxytryptamine (Serotonin). *Pharmacol. Rev.* **1994**, *46*, 157−203.

(7) Zifa, E.; Fillion, G. 5-Hydroxytryptamine Receptors. *Pharmacol. Rev.* **1992**, *44*, 401−458.

(8) Peroutka, S. J. 5-Hydroxytryptamine receptor subtypes. *In Serotonin receptors and their ligands;* Olivier, B., Van Wijngaarden, I., Soudijn, W., Eds.; Elsevier (pharmacochemistry library): Amsterdam, 1997; Vol. 27, pp 3−13.

(9) Rault, S.; Lancelot, J. C.; Prunier, H.; Robba, M.; Renard, P.; Delagrange, P.; Pfeiffer, B.; Caignard, D. H.; Guardiola-Lemaitre, B.; Hamon, M. Novel selective and Partial Agonists of 5-HT_3 Receptors. Part 1. Synthesis and Biological Evaluation of Piperazinopyrrolothienopyrazines. *J. Med. Chem.* **1996**, *39*, 2068−2080.

(10) Prunier, H.; Rault, S.; Lancelot, J. C.; Robba, M.; Renard, P.; Delagrange, P.; Pfeiffer, B.; Caignard, D. H.; Misslin, R.; Guardiola-Lemaitre, B.; Hamon, M. Novel selective and Partial Agonists of 5-HT_3 Receptors. Part 2. Synthesis and Biological Evaluation of Piperazinopyridopyrrolopyrazines, Piperazinopyrroloquinoxalines, and piperazinopyridopyrroloquinoxalines. *J. Med. Chem.* **1997**, *40*, 1808−1819.

(11) Daveu, C.; Bureau, R.; Baglin, I.; Prunier, H.; Lancelot, J. C.; Rault, S. Definition of a pharmacophore for partial agonists of serotonin 5-HT3 receptors. *J. Chem. Inf. Comput. Sci.* **1999**, *39*, 362−369.

(12) Baglin, I.; Daveu, C.; Lancelot, J. C.; Bureau, R.; Dauphin, F.; Pfeiffer, B.; Renard, P.; Delagrange, P.; Rault, S. First Tricyclo Oximino Derivatives as 5-HT3 ligands. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 453−457.
(13) Lindberg, U. H.; Thorberg, S. O.; Bengtsson, S.; Renyi, A. L.; Ross, S. B.; Ogren S. O. Inhibitors of neuronal monoamine uptake. 2. Selective inhibition of 5-hydroxytryptamine uptake by alpha-amino acid esters of phenethyl alcohols. *J. Med. Chem.* **1978**, *21*, 448−456.
(14) Humber, L. G.; Lee, D.; Rakhit, S.; Treasurywala, A. M. Computer-assisted investigation of structure−activity relationships in serotonin-uptake blockers. *J. Molecular Graphics* **1985**, *3*, 84−89.
(15) Rupp, A.; Kovar, K. A.; Beuerle, G.; Ruf, C.; Folkers G. A new pharmophoric model for 5-HT reuptake-inhibitors: differentiation of amphetamine analogues. *Pharm. Acta Helv.* **1994**, *68*, 235−244.
(16) Hoepping, A.; Johnson, K. M.; George, C.; Flippen-Anderson, J.; Kozikowski, A. P. Novel conformationally constrained tropane analogues by 6-endo-trig radical cyclization and stille coupling − switch of activity toward the serotonin and/or norepinephrine transporter. *J. Med. Chem.* **2000**, *43*, 2064−2071.
(17) Brooks, B. R.; Bruccoleri, R. E.; Olafson, B. D.; Sates, D. J.; Swaninathan, S.; Karplus, M. CHAMM: A program for macromolecular energy, minimization, and dynamics calculations. *J. Comput. Chem.* **1983**, *4*, 187−217.
(18) Smellie, A.; Teig, S. L.; Towbin, P. Poling: Promoting Conformational Variation. *J. Comput. Chem.* **1995**, *16*, 171−187.
(19) Güner, O. F. *Pharmacophore, Perception, Development, and Use in Drug Design*; IUL Biotechnology Series, 2000; p 504.
(20) Fisher, R. The design of Experiments; Hafner Publishing: New York, 1966; Chapter 2.
(21) Stinson, S. C. Counting on Chiral Drugs. *Special report* 1998; Vol. 76, pp 1−136. http://pubs.acs.org/hotartcl/cenear/980921/drugs.html.
(22) CERIUS2, Version 4.5 software; ACCELRYS, 2001.
(23) Rohrbaugh, R. H.; Jurs, P. C. Descriptions of molecular shape applied in studies of structure/activity and structure/property relationships. *Anal. Chim. Acta* **1987**, *199*, 99−109.

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