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First Pharmacophoric Hypothesis for 5-HT$_7$ Antagonism

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Abstract—In order to make the first contribution to the elucidation of essential structural features for 5-HT$_7$ antagonism, a set of thirty 5-HT$_7$ antagonists were selected from the literature. A pharmacophore model was built using Molecular Modeling studies with Catalyst program. The information contained in this model was validated with new synthesized compounds.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT) was discovered over 50 years ago[1] and continues to generate interest as one of the most attractive targets for medicinal chemists. Molecular biological data have revealed the existence of fourteen serotonin receptor subtypes, which can be classified in seven families (5-HT$_{1-7}$).[2] The 5-HT$_7$ subtype is the most recent addition to the burgeoning family of 5-HT receptors.[3] Although the biological functions of the 5-HT$_7$ receptor are poorly understood, preliminary evidences suggest that it may be involved in depression,[4] control of circadian rhythms,[5] and relaxation in a variety of vascular smooth muscles.[6] Nevertheless, the therapeutic utility of 5-HT$_7$ receptor ligands awaits the development of selective agonists and antagonists. During our work only two selective 5-HT$_7$ receptor antagonists (SB-258719[7] and DR4004[8]) were discovered, both from a high-throughput screening of compound libraries. In the meantime of editorial revision, Lovell et al.[9] have reported SB-269970 as a new selective antagonist structurally related to SB-258719. Information on the structural requirements of 5-HT$_7$ ligands is still unknown and its determination is the major aim for developing specific compounds. In a rational drug design, identification of the pharmacophore is one of the most important steps, especially when the structure and properties of the bioreceptor remain unknown. Therefore, our aim in this communication is to report the essential structural features for 5-HT$_7$ antagonism. The validation of the pharmacophore using data of new synthesized compounds suggests consistencies in structural requirements.

Pharmacophore Generation

The study was performed using the software package Catalyst[10] installed on a Silicon Graphics O2 workstation. A set of thirty 5-HT$_7$ antagonists[7,8,11–17] structurally different from a chemical feature standpoint was selected from the reported data as the target training set for Catalyst analysis (Tables 1–4). In cases where the chirality of a stereogenic center was not specified, Catalyst generated and considered alternative stereoisomers. All structures were built de novo using 2D/3-D editor sketcher in Catalyst. Conformational models were calculated using a 15 Kcal energy cutoff (minimization convergence criteria during conformational analysis: energy convergence = 0.1 Kcal/mol/Å, gradient convergence = 0.01 Kcal/mol/Å). The number of conformers generated for each substrate was limited to a maximum of 250. All molecules with their associated conformations were regrouped including the biological data (pK_i). Hypothesis generation was performed and twelve hypotheses were obtained using low energy conformers of the molecules in the training set. After assessing all generated hypotheses, the most plausible one was considered the best. The goodness of the structure–activity correlation was estimated by means of r^2.

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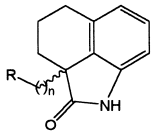
Table 1. Training set used in the generation of the 5-HT$_7$ antagonist pharmacophore

Number	Compound	pK_i (5-HT$_7$)	References
1	Metergoline	8.2	12, 13, 14
2	Mesulergine	8.1	12
3	2-Br-LSD	8.0	11a
4	Methysergide	7.9	12
5	Clozapine	7.9	12
6	(*S*)-Methiothepin	9.0	12
7	Cyproheptadine	7.3	12
8	Mianserin	7.2	15
9	(+)-Butaclamol	7.2[a]	11a, 15
10	Ritanserin	7.8	12
11	Spiperone	7.7	15, 16

[a]This value represents the mean of different pK_i values reported in refs 11a and 15.

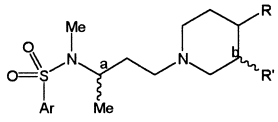
Table 2. Training set used in the generation of the 5-HT$_7$ antagonist pharmacophore



Number	*n*	R	pK_i (5-HT$_7$)[a]
12	2	4-phenylpiperazin-1-yl	7.0
13	3	4-phenylpiperazin-1-yl	8.3
14	4	4-phenylpiperazin-1-yl	8.5
15	4	4-(2-methoxyphenyl)piperazin-1-yl	8.3
16	4	4-(2-cyanophenyl)piperazin-1-yl	8.4
17	4	4-(2-pyridyl)piperazin-1-yl	8.7
18 (DR4004)	4	4-phenyl-1,2,3,6-tetrahydropyridyl	8.7
19	4	4-cyclohexylpiperazin-1-yl	5

[a]Values reported in ref 8.

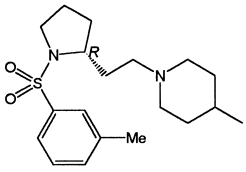
Table 3. Training set used in the generation of the 5-HT$_7$ antagonist pharmacophore



Number	Ar	R	R′	Stereochemistry a	Stereochemistry b	pK_i (5-HT$_7$)[a]
20	1-naphthyl	H	Me	*R,S*	*R,S*	7.2
21	1-naphthyl	H	Me	*R*	*R*	6.9
22	1-naphthyl	H	Me	*R*	*S*	6.2
23	1-naphthyl	H	Me	*S*	*R*	5.8
24	1-naphthyl	H	Me	*S*	*S*	5
25 (SB-258719)	3-methylphenyl	Me	H	*R*	—	7.5
26	1-naphthyl	Me	H	*R*	—	7.5
27	3,4-dichlorophenyl	Me	H	*R*	—	7.5
28	3,4-dibromophenyl	Me	H	*R*	—	7.7
29	4,5-dibromo-2-thienyl	Me	H	*R*	—	7.8

[a]Values reported in ref 7.

Table 4. Training set used in the generation of the 5-HT$_7$ antagonist pharmacophore

Number	Compound	pK_i (5-HT$_7$)[a]
30		8.5

[a]Value reported in ref 17.

Results

According to the hypothesis generated by catalyst, the minimal structural requirements for 5-HT$_7$ antagonism consist of an aromatic ring, a basic nitrogen atom (positive ionizable center), a H-bonding acceptor group and a hydrophobic region at 4.9–5.9 Å apart from the basic center (Fig. 1). For all the molecules in the training set, reasonable low-energy conformers that align on the hypothesis were found. The overall ability of this hypothesis to estimate properly the affinities of all molecules within the training set is shown by the good r^2 value between predicted and estimated affinities ($r^2 = 0.921$). This pharmacophoric assumption was then validated using new naphtholactam and naphthosultam derivatives (Fig. 2). Affinity data (Table 5) suggest consistencies in required structural features.

Compounds **31**–**45**[18] were obtained by treatment of intermediates **46** with the corresponding piperazines and piperidines **47** in the presence of triethylamine and ace-

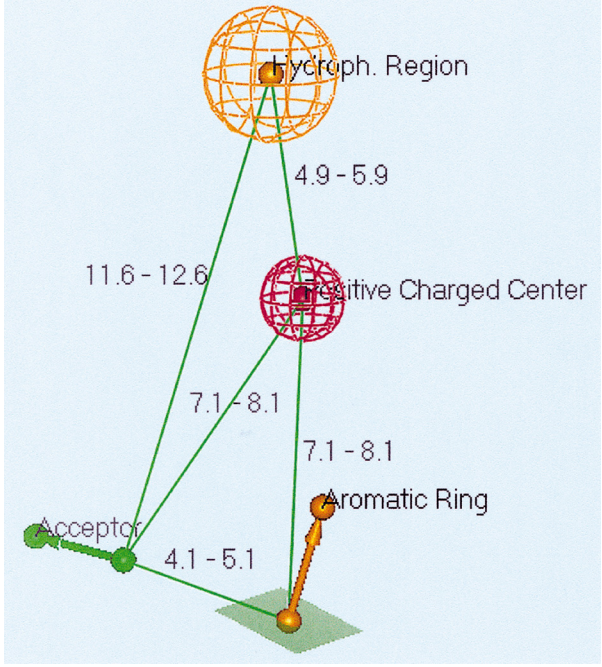


Figure 1. Proposed pharmacophore for 5-HT$_7$ antagonism.

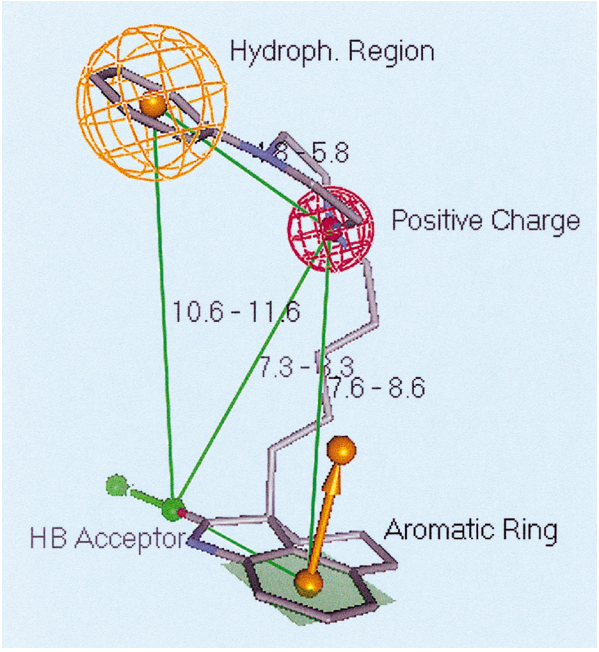
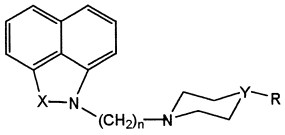


Figure 2. Compound **42** mapped on the hypothesis generated for 5-HT_7 receptor antagonists.

Table 5. Binding affinity of synthesized compounds at 5-HT_7 receptors.[a]



Compd	X	*n*	Y	R	pK_i[a]
31	CO	4	CH	H	< 5
32	CO	4	CH	Me	< 6
33	CO	4	N	Me	< 5
34	SO_2	4	CH	H	< 5
35	SO_2	4	CH	Me	< 5
36	SO_2	4	N	Me	< 5
37	SO_2	5	CH	Me	< 5
38	CO	4	CH	isopropyl	6.7
39	CO	4	N	*o*-methoxyphenyl	7.2
40	SO_2	4	N	*o*-methoxyphenyl	6.7
41	CO	5	N	*o*-methoxyphenyl	7.0
42	CO	5	N	phenyl	7.1
43	SO_2	5	N	*o*-methoxyphenyl	6.6
44	SO_2	5	N	phenyl	6.7
45	SO_2	6	N	*o*-methoxyphenyl	6.7

[a]pK_i = −log K_i. K_i (nM) values are means of two to four assays, performed in triplicate. Inhibition curves were analyzed by a computer-assisted-curve-fitting program (Prism GraphPad) and K_i values were determined from the Cheng–Prusoff equation.

tonitrile (Scheme 1). The reaction of compounds **48** with the appropriate dibromoderivative rendered key intermediates **46**. Respective hydrochloride salts of the synthesized compounds were prepared as samples for biological assays.

Target compounds were assessed for in vitro affinity at the 5-HT_7 receptor by radioligand binding assays, using [^{3}H]-5-CT in rat hypothalamus membranes[19] (Table 5). All the compounds that fit our pharmacophore model (**38**–**45**) show 5-HT_7 affinity (pK_i >6.5), whereas all inactive derivatives (**31**–**37**, pK_i < 6.5) lack the hydrophobic moiety situated at a distance of 4.9–5.9 Å from the nitrogen atom, required for 5-HT_7 binding. These results support our pharmacophoric hypothesis. In particular, compound **42** (Fig. 2) has been selected as a new lead compound for the search for 5-HT_7 receptor ligands.

Conclusions

The pharmacophore model for 5-HT_7 antagonism described herein represents the first contribution to the rational design of agents acting at this recently identified serotonin receptor. The postulated hypothesis was validated with a series of new naphtholactam and naphthosultam derivatives that exhibit affinity for the 5-HT_7 receptor. This study offers structural insight to aid the development of novel 5-HT_7 ligands, which are essential for the knowledge of the (patho)physiological role of this serotonin receptor subtype.

Acknowledgements

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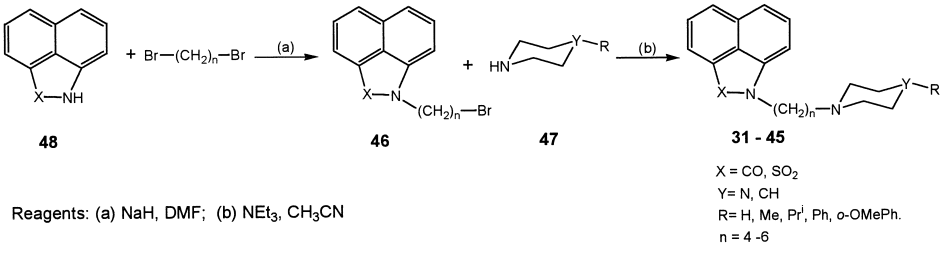

Scheme 1.

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18. New compounds were characterized by IR, ^{1}H, and ^{13}C NMR spectroscopy and gave satisfactory combustion analysis (C, H, N). Spectral data of selected compound **42**: IR ($CHCl_3$, cm^{-1}) 1705 (CON), 1600, 1560, 1496, 1456 (Ar); ^{1}H NMR ($CDCl_3$) δ 1.43 (qt, $J=7.2$ Hz, 2H, CH_2), 1.58 (qt, $J=7.5$ Hz, 2H, CH_2), 1.82 (qt, $J=7.5$ Hz, 2H, CH_2), 2.35 (t, $J=7.5$ Hz, 2H, CH_2N–pip), 2.55 (t, $J=5.1$ Hz, 4H, 2CH_2-pip), 3.15 (t, $J=5.1$ Hz, 4H, 2CH_2-pip), 3.92 (t, $J=7.5$ Hz, 2H, CH_2–NCO), 6.83 (t, $J=7.2$ Hz, 1H, H_4-phenyl), 6.90 (d, $J=7.8$ Hz, 3H, H_2-, H_6-phenyl, H_8), 7.24 (t, $J=6.9$ Hz, 2H, H_3-, H_5-phenyl), 7.45 (t, $J=8.4$ Hz, 1H, H_7), 7.52 (d, $J=8.4$ Hz, 1H, H_6), 7.69 (t, $J=8.1$ Hz, 1H, H_4), 7.99 (d, $J=8.1$ Hz, 1H, H_5), 8.04 (d, $J=7.2$ Hz, 1H, H_3); ^{13}C NMR ($CDCl_3$) δ 24.8, 26.5, 28.6 (CH_2–CH_2–CH_2), 40.1 ($\underline{C}H_2$–NCO), 49.0 (2CH_2-pip), 53.2 (2CH_2-pip), 58.4 (CH_2N-pip), 104.8 (C_8), 115.9 (C_2-, C_6-phenyl), 119.5 (C_4-phenyl), 120.1 (C_6), 124.1 (C_3), 125.1 (C_{8b}), 126.7 (C_{2a}), 128.4, 129.0 (C_4, C_7, C_3-, C_5-phenyl), 128.6 (C_{5a}), 130.6 (C_5), 139.5 (C_{8a}), 151.2 (C_1-phenyl), 168.0 (CO); mp 211–213 °C (CH_2Cl_2:hexane)..
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