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3-D-QSAR/CoMFA and Recognition Models of Benzimidazole Derivatives at the 5-HT$_4$ Receptor

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Abstract—3-D-QSAR/CoMFA methodology and computational simulation of ligand recognition have been successfully applied to explain the binding affinities of a series of benzimidazole derivatives **1**–**24** acting at serotonin 5-HT$_4$Rs. Both derived computational models have facilitated the identification of the structural elements of the ligands that are key to high 5-HT$_4$R affinity. The results provide the tools for predicting the affinity of related compounds, and for guiding the design and synthesis of new ligands with predetermined affinities and selectivity.

Introduction

The discovery of new ligands with affinity for the family of serotonin receptors is an area of active research in Medicinal Chemistry. Within this field the 5-HT$_4$ receptor (5-HT$_4$R), which belongs to the G protein-coupled receptor (GPCR) superfamily, is of considerable interest because it is involved in (patho)physiological processes both in peripheral and central nervous systems.[1] In addition to the clinical use of 5-HT$_4$R ligands in the treatment of gastrointestinal motility disorders, their potential use in the treatment of irritable bowel syndrome, arrhythmias, micturition disturbances and cognitive disorders are currently under investigation.[2] In the course of a program aimed at the design of new specific agents for this receptor, we postulated the first steric model for the recognition of 5-HT$_4$R antagonists.[3] This pharmacophore model led us to design a series of new benzimidazole-4-carboxylic acid derivatives, which were characterized as novel potent and selective 5-HT$_4$R antagonists.[4,5] In this work we have synthesized new related analogues and carried out 3-D-QSAR studies using the comparative molecular field analysis (CoMFA) methodology, in order to rationalize the structure–affinity relationships of benzimidazole derivatives **1**–**24** acting at serotonin 5-HT$_4$Rs. In addition, computational simulation of the complex between the recently postulated bioactive conformation of these ligands[6] (Fig. 1) and a rhodopsin (RHO)-based[7] 3-D model of the 5-HT$_4$R transmembrane domain has allowed us to define the molecular details of interaction. The model of the ligand–receptor complex shows high level of compatibility with the steric and electrostatic properties of the 3-D-QSAR/CoMFA model for the 5-HT$_4$R binding site.

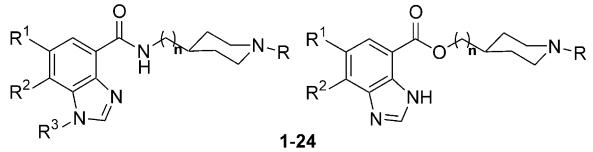

Figure 1. Benzimidazole derivatives acting at the 5-HT$_4$R.

Methods

Chemistry

Compounds **1**–**11** and **20**–**24** were obtained previously in our group.[5] New amides **14**–**16** were synthesized from benzimidazole-4-carboxylic acids **25**–**27**[8] as detailed in Scheme 1. 7-Aminocarboxamides **17**–**19** were prepared from their corresponding 7-nitro derivatives (Scheme 2).

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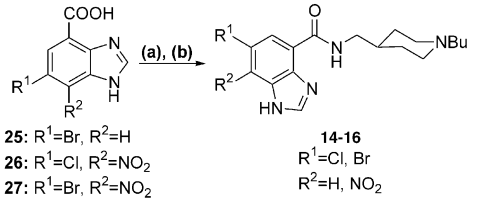
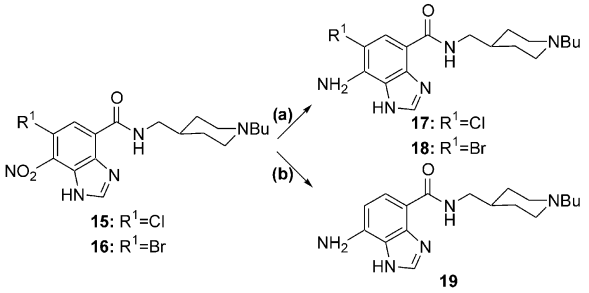
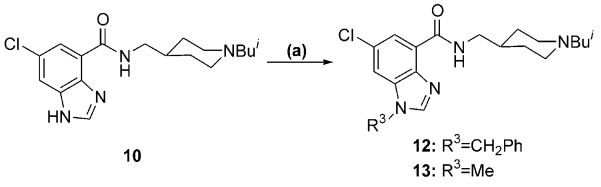


Scheme 1. Reagents: (a) CDI, DMF, 40 °C; (b) (1-butyl-4-piperidyl)methylamine, DBU, DMF, 50 °C.



Scheme 2. Reagents: (a) NH_2NH_2, Ni-Ra, EtOH, rt; (b) (**16**) NH_2NH_2, Pd(C), EtOH, Δ.

1-Alkylated compounds **12** and **13** were obtained by alkylation of **10** as detailed in Scheme 3. The benzimidazole alkylation pattern was assigned on the basis of ^{13}C, DEPT and HMBC NMR spectra of final compounds. All new compounds gave satisfactory combustion analyses (C, H, N). Yields and melting points of the novel derivatives **12**–**19** are shown in Table 1.



Scheme 3. Reagents: (a) NaH, $PhCH_2Cl$, DMF, 30 °C (**12**); BuLi, MeI, THF, −78 °C → rt (**13**).

Biological data

Target compounds were assessed for in vitro affinity at serotoninergic 5-HT_4Rs by radioligand binding assays, using [^{3}H]GR 113808 in rat striatum membranes[9] (Table 2).

3-D-QSAR/CoMFA method

One of the crucial steps in CoMFA is to select a proper alignment rule. In the present study, we have adopted an alignment based on our previous structural analysis of the annular tautomerism in these benzimidazole derivatives in solution, by using NMR and IR techniques and theoretical calculations.[6] Full geometry optimization of the postulated bioactive conformation of compounds **1**–**24** (see Fig. 1), in their piperidine-protonated form, was carried out with the AM1 Hamiltonian model. The QSAR table for the CoMFA study consisted of the pK_i values (dependent variable), the steric and electrostatic fields (independent variables), to mimic the stabilization energy of the ligand–receptor complex, and the solvation energy of the ligand (independent variable). These independent variables were computed as described elsewhere.[10] Partial least squares (PLS) analysis was used to derive linear equations from the resulting matrices. Leave one out (LOO) cross-validation was employed to select the number of principal components and to calculate the cross-validated statistics. All the quantum mechanical calculations were performed with the Gaussian-98 system of programs.[11] The CoMFA study was carried out with the QSAR module of the SYBYL 6.5 program,[12] using default parameters.

Table 1. Yields and melting points of the novel derivatives **12**–**19**

Compd	Yield (%)	Mp
12	90	150–151 °C (ethyl acetate)
13	73	128–130 °C (chloroform/hexane)
14	35	182–184 °C (d) (toluene)
15	84	188–190 °C (d) (ethyl acetate)
16	95	189–190 °C (d) (ethyl acetate)
17	39	232–234 °C (d) (chloroform)
18	51	228–230 °C (d) (chloroform)
19	90	162–164 °C (d)

Model of 5-HT_4R–ligand interaction

The 3-D model of the 5-HT_4R transmembrane domain was constructed by computer-aided model building techniques from the recently reported crystal structure of RHO,[7] in a similar manner to our recent 3-D model of the 5-HT_{1A}R.[13] Thr$^{3.37}$ adopts the g-conformation during the modeling procedure which induces a small bending angle of 4°.[14] Inclusion of this bending in helix 3 facilitates the experimentally derived interactions between the ligand and Asp$^{3.32}$ and Ser$^{5.43}$.[15] The mode of recognition of UCM-21195 (**5**) (the butyl group attached to the piperidine nitrogen was replaced by a methyl group) by Asp$^{3.32}$, Ser$^{5.43}$ and Asn$^{6.55}$, experimentally determined to form the binding pocket,[15] was first obtained by ab-initio geometry optimization (see ref 13 for computational details). This optimized reduced model of the ligand–receptor complex was used to position the complete ligand inside the equilibrated 5-HT_4R transmembrane domain. Subsequently, the complete system was energy minimized (5000 steps).

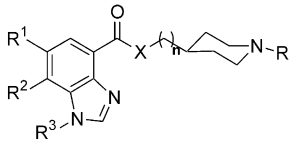
Computational simulations were performed with the Gaussian-98[11] and Amber 5[16] system of programs. Parameters for UCM-21195 were adapted from the Cornell et al. force field[17] using RESP point charges.[18]

Results and Discussion

3-D-QSAR/CoMFA model

5-HT_4R binding data of benzimidazole derivatives **1**–**24** are listed in Table 2. The CoMFA model was developed using a training set of 21 compounds marked with § in Table 2. Randomly chosen compounds **1**, **15** and **23** were not included in this initial set in order to test the derived CoMFA model predictiveness. The 5-HT_4R binding affinities of the training set, expressed as pK_i, were related to the independent variables (steric and

Table 2. Experimental and CoMFA predicted 5-HT$_4$R binding affinities of benzimidazole derivatives **1**–**24**



Compd	R^1	R^2	R^3	X	n	R	$K_i \pm$SEM (nM)[a]	pK_i (M)	pK_i^{pred} (M)
1	H	H	H	NH	0	Me	719±58	6.14	6.38[#]
2	H	H	H	NH	0	Et	>1000	6.00[§]	5.91
3	H	H	H	NH	0	Pr	>1000	6.00[§]	6.02
4	H	H	H	NH	0	Bu	290±54	6.54[§]	6.61
5[b]	H	H	H	NH	1	Bu	13.7±0.9	7.86[§]	7.97
6	H	H	H	NH	1	MSAE[c]	11.4±3.0	7.94[§]	7.94
7	Cl	H	H	NH	0	Me	54.0±2.8	7.27[§]	7.36
8	Cl	H	H	NH	1	Bu	0.32±0.07	9.49[§]	9.32
9	Cl	H	H	NH	1	MSAE[c]	0.11±0.03	9.96[§]	9.99
10	Cl	H	H	NH	1	Bu^i	0.29±0.04	9.54[§]	9.46
11	Cl	H	H	NH	1	Pent	0.54±0.10	9.27[§]	9.26
12	Cl	H	CH_2Ph	NH	1	Bu^i	69.2±9.3	7.16[§]	7.19
13	Cl	H	Me	NH	1	Bu^i	2.2±0.2	8.66[§]	8.66
14	Br	H	H	NH	1	Bu	0.64±0.04	9.19[§]	9.17
15	Cl	NO_2	H	NH	1	Bu	44.6±2.0	7.35	7.59[#]
16	Br	NO_2	H	NH	1	Bu	48.2±6.5	7.32[§]	7.32
17	Cl	NH_2	H	NH	1	Bu	22.4±0.6	7.65[§]	7.75
18	Br	NH_2	H	NH	1	Bu	27.8±3.3	7.56[§]	7.63
19	H	NH_2	H	NH	1	Bu	115±15	6.94[§]	6.85
20	H	H	H	O	0	Me	>10,000	5.00[§]	4.91
21	H	H	H	O	1	Bu	24.6±0.5	7.61[§]	7.59
22	H	H	H	O	1	MSAE[c]	26.1±0.3	7.58[§]	7.59
23	Cl	H	H	O	1	Bu	2.9±0.4	8.54	8.39[#]
24	Cl	H	H	O	1	MSAE[c]	2.3±1.1	8.64[§]	8.67

[§]Compounds of the training set used to develop the CoMFA model. [#]Compounds used to test the CoMFA model.
[a]K_i 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.
[b]**5**: UCM-21195.
[c]MSAE: [(methylsulfonyl)amino]ethyl.

electrostatic fields and solvation energies) by the PLS methodology. The high LOO cross-validated correlation coefficient ($q^2 = 0.79$) reveals that the model is a useful tool for predicting 5-HT$_4$R affinities. In addition, the model yielded conventional r^2 of 0.99 (eight principal components). Steric and electrostatic fields and ΔG_{solv} contribute to the QSAR equation by 43.5:50.3:6.1, respectively. As a further test of robustness, the CoMFA model was successfully applied to the excluded ligands **1**, **15** and **23** (see # symbol in Table 2). The quality of the model is also illustrated by the good agreement between predicted and experimental pK_i for the 21 compounds of the training set (see § symbol in Table 2).

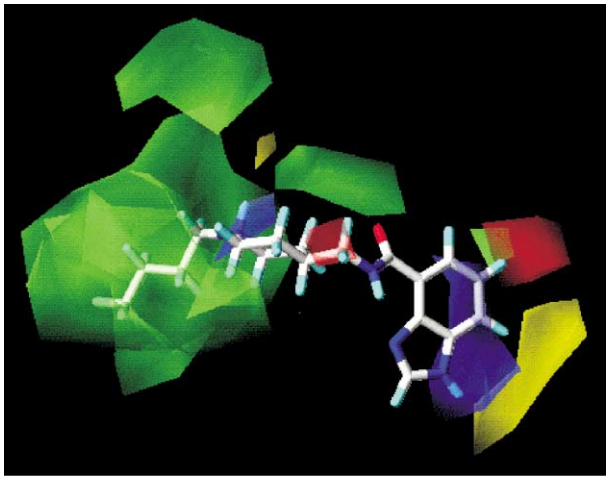


Figure 2. Compound UCM-21195 (**5**) superimposed in CoMFA electrostatic and steric maps, showing contributions to 5-HT$_4$R affinity.

Figure 2 illustrates the CoMFA electrostatic and steric maps using compound UCM-21195 as the reference structure. The color code is as follows: areas where a high electron density provided by the ligand increases (red) or decreases (blue) affinity; areas where occupancy by the ligands increases (green) or decreases (yellow) affinity. The electrostatic map shows a red shaded region at the 6-position of the aromatic ring, indicating the favorable effect of electronegative substituents such as chloro or bromo atom in this position. Steric contours depict also a small favorable (green) area in this position, and an unfavorable (yellow) region near the 7-position, justifying the detrimental effect of the 7-amino or 7-nitro substituents. The volume in the proximity of the basic amino moiety shows a blue region near the nitrogen atom, and a large favorable (green) region near the side chain. In the cases where no methylene unit is linking the acyl group and the piperidine ring, the alkyl group is not accommodated inside this green area. Indeed, these analogues are either inactive or poorly active in the 5-HT$_4$R. Also, analogues with a substituent in the nitrogen atom less voluminous than a butyl group are devoid of 5-HT$_4$R affinity, except

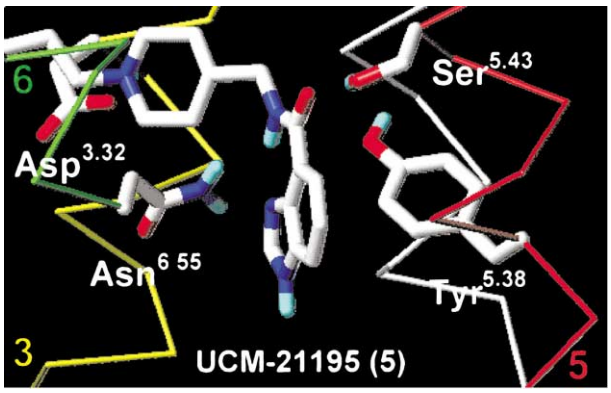


Figure 3. Molecular interactions of ligand UCM-21195 (**5**) with the 5-HT_4R, in a detailed view of the binding site.

compound **7** ($K_i = 54.0$ nM). These results are in good agreement with our pharmacophore model for 5-HT_4R antagonists, where both the presence of a voluminous substituent in the basic nitrogen of the amino moiety and the distance from this nitrogen to the aromatic ring are of great importance for high affinity binding.

Model of 5-HT_4R–ligand interaction

The human 5-HT_4R binding site has been structurally explored by site-directed mutagenesis experiments,[15] revealing that, among others, $Ser^{5.43}$-Ala, $Phe^{6.51}$-Ala and $Phe^{6.52}$-Val/$Asn^{6.55}$-Leu mutants do not bind the antagonist GR 113808. It has been suggested, from these results, that GR 113808 anchors the 5-HT_4R mainly throughout the ionic interaction with $Asp^{3.32}$ and the hydrogen bond (H-bond) with $Ser^{5.43}$.

The computed antagonist–receptor complex for ligand UCM-21195 (see Methods for computational details) depicted in Figure 3 is formed by: (i) the ionic interaction between the NH group in the protonated piperidine of the ligand and the O_δ atom of $Asp^{3.32}$; (ii) the H-bond between the carbonylic oxygen of the amide and the hydroxyl group of $Ser^{5.43}$; (iii) the H-bond between the electron-poor hydrogens in the NH group of $Asn^{6.55}$ and the π electron-rich clouds in the aromatic ring of the ligand, in a similar manner to the proposed H-bond between benzene and water;[19] (iv) a π–σ aromatic–aromatic interaction between the benzimidazole ring of UCM-21195 and $Tyr^{5.38}$ side chain, positioned in the face-to-edge orientation (T-shaped).

The superimposition of the CoMFA electrostatic and steric plots in the modeled 5-HT_4R binding site shows that the green (small) and red areas at the 6-position of the benzimidazole ring, where the Cl/Br atom accommodates, are located between TMHs 5 and 6. The H-bond of the carbonyl group to $Ser^{5.43}$ positions the halogen atom near $Ala^{5.39}$, located in the same face of the helix. An analysis of the conservation pattern at the 5.39 position of all GPCR sequences denoted as Serotonin (67 entries) in GPCRDB[20] (November 2000) shows that Ala is only present in the 5-HT_4R, with the exception of the 5-HT_5R. The other serotonin subtypes possess bulkier residues at this position: 5-HT_1 (Thr), 5-HT_2 (Val, Met), 5-HT_5 (Ala, Thr), 5-HT_6 (Val), and 5-HT_7 (Thr). Thus, the higher stabilization of the 6-halogen substituted ligands in the 5-HT_4R binding site can be attributed to the additional electrostatic and van der Waals interaction of the halogen atom in this small cavity between TMHs 5 and 6.

Conclusions

3-D-QSAR/CoMFA methodology and computational simulation of ligand recognition have been successfully applied to explain the 5-HT_4R binding affinities of a series of benzimidazole-4-carboxylic acid derivatives of piperidines **1**–**24**. Both derived computational models have facilitated the identification of the structural elements of the ligands that are key to high 5-HT_4R affinity. The results provide the tools for predicting the affinity of related compounds, and for guiding the design and synthesis of new ligands with predetermined affinities and selectivity. These studies are now in progress and the results will be reported in due course.

Acknowledgements

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