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A 3D-pharmacophore model for σ_2 receptors based on a series of substituted benzo[d]oxazol-2(3*H*)-one derivatives

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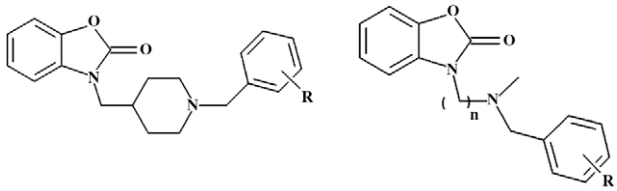
ABSTRACT

In this work we developed a 3D-pharmacophore model for σ_2 receptor based on 19 benzooxazolone derivatives. The best 3D-pharmacophore hypothesis, consisting of five features: a positive ionizable, a hydrogen bond acceptor, a hydrophobic aromatic, a hydrophobic aliphatic, and a generic hydrophobic provided a 3D-QSAR model with a correlation coefficient of 0.97 and a RMSD of 0.48.

Sigma receptors are classified in σ_1 and σ_2 subtypes[1] and are localized in different tissues, including the central and peripheral nervous systems (CNS and PNS, respectively).[2] In the CNS, these receptors are involved in the modulation of neurotransmitter release, in memory and cognitive processes, and in locomotor activity, whereas their role in the PNS and their signal transduction still await a clarification.[3] To date, the σ_1 receptor has been cloned,[4] whereas σ_2 subtype isolation and characterization have not been carried out yet, although a number of studies reported evidences linking σ_2 receptors to potassium channels and intracellular calcium release in NCB-20 cells.[2,5] Also, considering σ_2 receptor overexpression in several tumor tissues,[6] potent and selective σ_2 ligands could be employed as radiotracers to label tumor cells by diagnostic imaging techniques such as positron emission tomography (PET)[7] or single photon emission computed tomography (SPECT).[8] Although this diagnostic potential of σ_2 receptor agents has been suggested, the physiological role of this receptor and the mechanism of overexpression induction in tumor cells needs to be elucidated. Notwithstanding all these limiting issues, σ_2 receptor ligands can be considered a tool for cancer diagnosis[9] and a novel pharmacological perspective in cancer therapy.[10] To date, the endogenous ligand for the σ_2 receptor, if it exists, is unknown, and the signal pathway involved in receptor activation has to be discovered. Furthermore, the best known σ_2 receptor ligands, such as DTG (1,3-di-2-tolylguanidine) and (+)-(1*R*,5*R*)-(*E*)-8-benzilidene-5-(3-hydroxyphenyl)-2azabiciclo[3.3.1]nonan-7-one, display poor selectivity toward the σ_1 subtype,[11] and only a small number of other σ_2 selective compounds have been discovered so far.[12] In this scenario, we recently proposed a simple but effective 3D-pharmacophore model for σ_1 receptor ligands.[13] With reference to our molecular sets and their different selectivity towards σ receptors, one of our reviewers pointed out that the development of a 3D-pharmacophore model for σ_2 ligands was equally important and timely, due to the scarcity of available information currently preventing the derivation of reliable models for the σ_2 receptor subtype. Thus, the purpose of this communication is to report the structural features for σ_2 selective binding. Using Catalyst,[14] we developed a 3D-pharmacophore model from a set of 19 σ_2 ligand previously synthesized (**1–3**, Table 1).[13,15]



1a-f **n = 3 (2a-e); 4 (3a-g)**

The best output hypothesis Hypo1 (Fig. 1), with a positive ionizable atom (PI), a hydrogen bond acceptor group (HBA), a hydrophobic aromatic site (HYAr), a hydrophobic aliphatic site (HYAl), and a generic hydrophobic site (HY), presents good statistical values (see

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Table 1
Experimental and 3D-pharmacophore estimated affinity values of the σ_2 ligands

Compound	*n*	R	$K_i\sigma_2$ (nM)			
			Exptl	Est	Error[a]	Mapping[b]
1a	—	H	246	323	1.3	1 1 1 1 1
1b	—	2-Cl	299	259	−1.2	1 1 1 1 1
1c	—	4-Cl	427	183	−2.3	1 1 1 1 1
1d	—	4-OCH_3	95	139	1.5	1 1 1 1 1
1e	—	4-CH_3	116	221	1.9	1 1 1 1 1
1f	—	2,4-$(CH_3)_2$	187	244	1.3	1 1 1 1 1
1g	—	4-Ph	10,000	6613	−1.5	1 1 0 1 1
2a	3	H	1121	674	−1.7	1 0 1 1 1
2b	3	2-Cl	456	647	1.4	1 0 1 1 1
2c	3	4-Cl	192	566	2.9	1 0 1 1 1
2d	3	4-CH_3	991	1166	1.2	1 0 1 1 1
2e	3	2,4-$(CH_3)_2$	2064	1385	−1.5	1 0 1 1 1
3a	4	H	120	69.9	−1.7	1 1 1 1 1
3b	4	2-Cl	37.4	55.8	1.5	1 1 1 1 1
3c	4	4-Cl	36.2	32.2	−1.1	1 1 1 1 1
3d	4	4-OCH_3	20.8	29.0	1.4	1 1 1 1 1
3e	4	3-CH_3	31.8	31.7	−1.0	1 1 1 1 1
3f	4	4-CH_3	22.9	27.1	1.2	1 1 1 1 1
3g	4	2,4-$(CH_3)_2$	6.94	5.37	−1.3	1 1 1 1 1

[a] Values in the error column represent the ratio of the estimated to experimental affinity, or its negative inverse if the ratio is less than one.
[b] The value 1 means a mapped hypothesis feature while 0 means missing mapping feature. Features are in the following order: HBA, HYAl, HYAr, HY, PI.

Supplementary data). The fixed, null, and total costs are 119.2, 215.7, and 141.4 bits, respectively. The difference between the null and the fixed costs, which should be higher than 70 to guarantee a robust correlation, is 96.5 in our case. This corresponds to a chance of true correlation in the data greater than 90%.[16] Furthermore, the total cost is much closer to the fixed cost than to the null cost, indicating that a meaningful model is obtained. Lastly, there is also a significant correlation between measured and estimated activities, quantified by the high value of the correlation coefficient $\rho = 0.97$ and by the low root-mean-square deviation RMSD = 0.48. Hypo1 was further evaluated for statistical significance using two methods: (i) the Fisher method[17] as implemented in the *CatScramble* module of Catalyst and (ii) the *leave-one-out* test. According to (i), the affinity values were scrambled randomly 19 times, and 19 new hypotheses were generated. None of the outcome hypotheses had a cost lower than the reported hypothesis. Thus, there is at least 95% probability that Hypo1 represents true correlation in the data. Method (ii) consists of re-computing the hypothesis by excluding from the training set one molecule at a time. Basically, this test is performed to verify whether or not the correlation is strongly dependent on one particular compound in the training set. The test is positive if the affinity of each excluded molecule is correctly predicted by the corresponding one-missing hypothesis. The value of ρ, the feature composition of the pharmacophore, and the quality of the predicted affinity of the excluded molecule were used as measures for the assessment of the statistical test. For each of the 19 new hypotheses generated according to this method we did not obtain meaningful differences between Hypo1 and each hypothesis resulting from the exclusion of one compound at a time.

In compound **3g** (Fig. 1), the aromatic ring of the benzooxazolone moiety matches HY feature, while the HYAr feature is nicely over lapped by the additional phenyl ring. The carbonyl group and the basic nitrogen atom of aliphatic linker match the HBA and PI functions, whilst the hydrocarbon portion of the spacer maps the remaining HYAl feature. The estimated affinity for **3g** is 5.37 nM, while the corresponding experimental K_i value is 6.94 nM. Interestingly, compound **2d** maps all pharmacophore features except the HYAl feature, the spacer length being too short to satisfy the 3D-pharmacophore spatial requirements. The calculated K_i value for this compound is 1196 nM, in agreement with the poor experimental affinity value of 991.

As expected, the proposed pharmacophore model for the σ_2 receptor shows remarkable similarities but also notable differences with the validated 3D-QSAR we developed for σ_1 receptors,[13] as evidenced in Figure 2.

In fact, although a basic amino nitrogen atom between two hydrophobic sites are still pharmacophoric elements required for affinity, in the case of σ_2 receptors the positions and distances between these features are different. The primary hydrophobic site corresponds with the hydrophobic aromatic sphere, which is mapped by the phenyl group connected to the aliphatic spacer, at a distance of 3.96 Å from the positive ionizable feature of the amino group, in agreement with our σ_1 receptor model[13] (3.58 Å), Laggner's model (4.1 Å),[18] and the optimum distance suggested by Glennon et al. (2.5–3.9 Å).[19] However, the secondary hydrophobic site is matched by the generic hydrophobic sphere that maps to the aromatic ring of the benzooxazolone group at a shorter distance (4.96 Å) with respect to the σ_1 receptor model (8.50 Å).

The presence of the hydrophobic aliphatic feature (HYAl) constitutes a necessary requisite for binding with high affinity to σ_2 receptors, while it does not seem to play a major role for selectivity towards this receptor. This notion can be easily proved taking compounds **1d** and **3d** as a proof-of-concept.

Experimentally, neither 3-[[1-(4-methoxybenzyl)piperidine-4-yl]methyl]benzo[*d*]oxazol-2(3*H*)one (**1d**) nor 3-[3-[*N*-(4-methoxybenzyl)-*N*-methylamino]butyl]benzo[*d*]oxazol-2(3*H*)-one (**3d**) are endowed with selectivity towards one of the two receptor types. Indeed, the K_i values for **1d** are equal to 95 nM for σ_2 (Table 1), and 83 nM for σ_1,[13] while the corresponding values for **3d** are 20.8 nM for σ_2 (Table1), and 21 nM for σ_1,[13] respectively. Accordingly, these compounds are able to map both pharmacophore models for σ_2 and σ_1, as illustrated in the first four panels of Figure 3.

In both **1d** and **3d**, and in both 3D-pharmacophore models for σ_2 and σ_1 receptors, the basic nitrogen atom is located oven the PI features, and one of the hydrophobic aromatic feature (HYAr) is mapped by the phenyl moiety bound to linker fragment of the molecule. The generic hydrophobic feature (HY) is mapped by the aromatic ring of the benzooxazolone in case of the σ_2 hypothesis in both compounds (Fig. 3(top and middle left)), whilst the 4-OCH_3 substituent serves as the hydrophobic site in the case of the σ_1 model (Fig. 3(top and middle right)).

The hydrophobic aliphatic feature (HYAl) of the σ_2 pharmacophore is located over a fragment of the piperidine ring in **1d**, while it nicely overlaps the butyl spacer in **3d**. Interestingly, for σ_1 the second HYAr feature is overlapped by the benzooxazolone phenyl group in both compounds. Lastly, the HBA feature is still represented by the carbonyl oxygen of the benzooxazolone moiety in both molecules. The non-perfect overlaps between some of the features of these 3D-QSARs and compound **1d** (see, for instance, the HBA feature in Fig. 3(top)) account for the intermediate values of the predicted affinity for this molecule for both receptors: 83 nM for σ_2 (Table 1) and 102 nM for σ_1,[13] respectively. On the other hand, as supported by the last panel in Figure 3 showing the superposition of the two conformations of **1d** and **3d** as extracted from their mapping onto the σ_2-pharmacophore model, the enhanced flexibility of the aliphatic linker with respect to the more constrained heterocyclic piperidine spacer allows compound **3d** to adopt the necessary configuration for a nice overlap on all features of both 3D-pharmacophore models. Accordingly, the estimated affinity of **3d** for σ_2 and σ_1 receptors are 29 nM (Table 1) and 42 nM, thus corroborate the hypothesis that a bridging scaffold endowed with higher conformational freedom is important for enhancing receptor binding affinity and not for improving receptor ligand selectivity.

In order to validate our model by finding novel ligands for our target protein, a 3D search database was performed for our

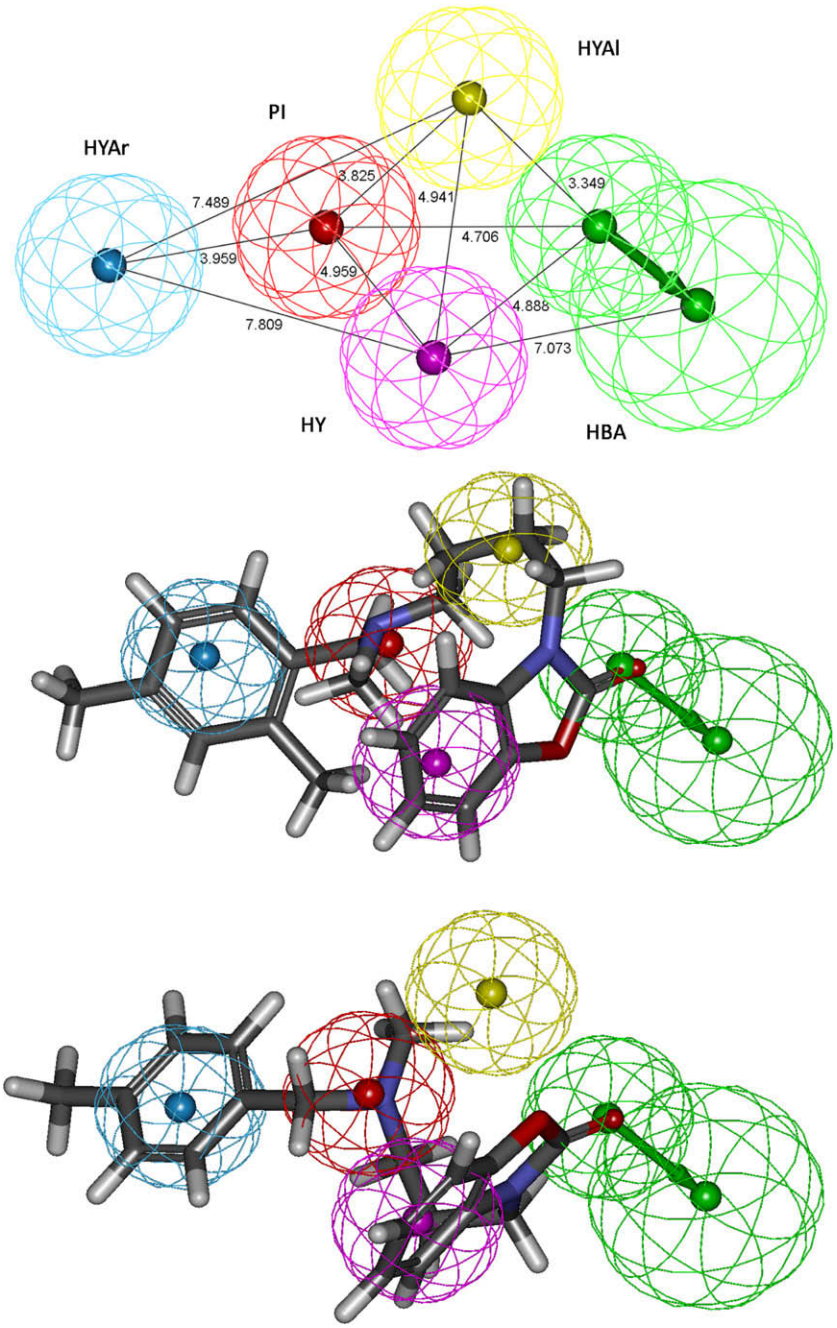

Figure 1. Top-scoring pharmacophore model Hypo1 for compounds of series **1**–**3** (top), and mapping of compounds **3g** (middle) and **2d** (bottom) onto Hypo1. The hypothesis features are portrayed as mashed spheres, color-coded as follows: red, PI; light blue, HYAr; pink, HY; light green, HBA; yellow, HYAl. HBA is actually represented as a pair of spheres (the smaller sphere represents the location of the HBA atom on the ligand and the larger one the location of an HB donor on the receptor). Selected distances (Å) are labeled. Compounds are portrayed as atom-colored sticks (red, O; gray, C; blue, N; white, H).

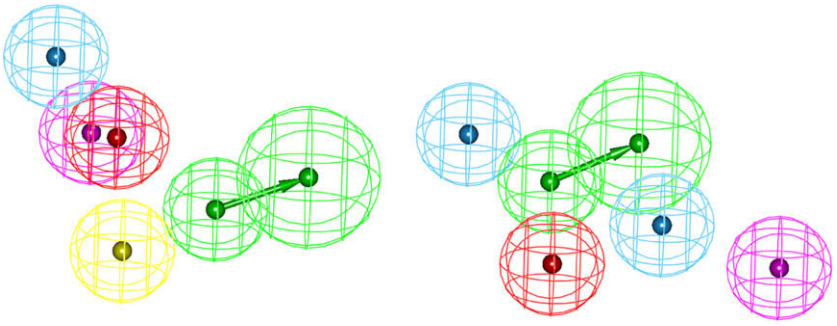

Figure 2. Comparison of the 3D-pharmacophore model derived in this work for σ_2 receptor s (left) and for σ_1 receptors (right).[13] Colors as in Figure 1.

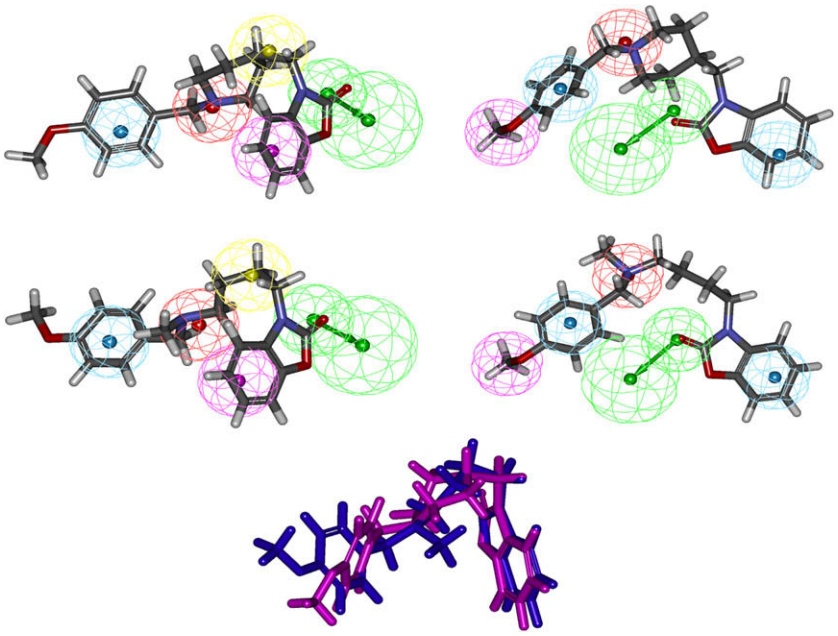

Figure 3. Mapping of compound **1d** onto the 3D-pharmacophore model for σ_2 (top, left) and σ_1 (top, right) receptors. Mapping of compound **3d** onto the 3D-pharmacophore model for σ_2 (middle, left) and σ_1 (middle, right) receptors. (Bottom) Superposition of the molecular conformations of **1d** (hot pink) and **3d** (blue) as extracted from the corresponding mapping onto the σ_2-pharmacophore Hypo1.

Table 2
3D-Pharmacophore estimated affinity values of the best compounds identified in the Maybridge database

Compound	Maybridge Code[a]	$K_i\sigma_{2(est)}$	$K_i\sigma_{1(est)}$	$K_i\sigma_{1(est)}/K_i\sigma_{2(est)}$
4	JFD 02217	7.54	16.4	2.2
5	RF 00799	9.54	62.6	6.6
6	JFD 02226	11.2	235	21
7	DSHS 00452	11.3	1.46e+11	10e+10

[a] For details about structure, IUPAC nomenclature and pharmacophore mapping onto σ_2 and σ_1 hypotheses of these compounds see Supplementary data.

hypothesis. The search with our σ_2-pharmacophore model for new ligands within the Maybridge database produced 59 initial hits. These were filtered for compounds **4**, **5**, **7** and **8** with the best predicted K_i value <20 nM (Table 2). We also compared these values with the estimated K_i of these molecules toward our σ_1 pharmacophore model mentioned previously.[13] As expected, all retrieved compounds are endowed with selectivity towards σ_2 model, with $K_i\sigma_1/K_i\sigma_2$ of at least 2.

Coupled to the recently σ_1 model developed by our group, the 3D-pharmacophore model presented in this work will undoubtedly aid the rational design of new agents acting at this still relatively unknown sigma receptors. Design and synthesis of new derivatives that fulfill these models are currently in progress in our laboratories, and the results will be reported in due course.

Acknowledgment

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Supplementary data

Supplementary data (parameter Table for the 10 top hypotheses generated, *CatScramble* Table, and entire molecular modeling procedure material) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.009.

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