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Conformationally constrained butyrophenones as new pharmacological tools to study 5-HT_{2A} and 5-HT_{2C} receptor behaviours

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Abstract

This study presents new pharmacological and molecular modelling studies on a recently described series of conformationally constrained butyrophenones. Alignment-free three-dimensional quantitative structure-activity relationship models developed on the basis of GRid Independent descriptors and partial least squares regression analysis, allow feasible predictions of activity of new compounds and reveal structural requirements for optimal affinity, particularly in the case of the 5-HT_{2A} receptor. The requirements for the 5-HT_{2A} affinity consist in a precise distance between hydrogen bond donor (protonated amino group) and hydrogen bond acceptor groups, as well as an optimal distance between the protonated amino group and the farthest extreme of the compounds. Another significant result has been the characterisation of two structurally similar compounds as interesting pharmacological tools (1-[(4-Oxo-4,5,6,7-tetrahydrobenzo[b]furan-5-yl)ethyl]-4-(6-fluorobenzisoxazol-3-yl)piperidine and 1-[(4-Oxo-4,5,6,7-tetrahydrobenzo[b]furan-6-yl)methyl]-4-(6-fluorobenzisoxazol-3-yl)piperidine). In spite of their structural similarity, the first compound shows clearly higher affinity for the 5-HT_{2C} receptor (about 100 fold) and higher Meltzer ratio (1.17 vs. 0.99) than the second. Moreover, the first compound inhibits arachidonic acid release in a biphasic concentration-dependent way in functional experiments at the 5-HT_{2A} receptor and it acts as inverse agonist at the 5-HT_{2C} receptor, behaviours that are not shown by the second compound.

Keywords: 3D-QSAR models; 5-HT_{2A}; 5-HT_{2C}; Butyrophenone; GRIND; Inverse agonism; Receptor conformations

1. Introduction

Clozapine and other 'atypical antipsychotics' have been shown to be effective against the negative symptoms of schizophrenia. Blockade of 5-HT_2 receptors has been postulated as the origin of the distinctive pharmacological profile of these drugs, causing an incidence of tardive diskinaesia and other extrapyramidal side effects lower than those found with classical antipsychotics [1].

The involvement of 5-HT_2 receptors in the pharmacological profile of atypical antipsychotics is supported by many biological, pharmacological and clinical studies [2,3]. Initially, many studies pointed to the 5-HT_{2A}

Abbreviations: 3D-QSAR, three-dimensional quantitative structure-activity relationship; 5-HT, serotonin; AA, arachidonic acid; CHO, Chinese hamster ovary; D, dopamine; FFD, fractional factorial design; GPCR, G protein-coupled receptor; GRIND, GRID independent descriptors; HBA, hydrogen bond acceptor; HBD, hydrogen bond donor; IP, inositol phosphates; LOO, leave-one-out; LV, latent variable; PLA_2, phospholipase A_2; PLC, phospholipase C; PLS, partial least squares.

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subtype as the most involved in schizophrenia [4–11]. Meltzer and coworkers [12,13] suggested that the ratio of the pK_is of antipsychotic agents at the 5-HT_{2A} and D_2 receptors, reflects the atypical profile; this ratio appears to be >1.12 for atypical antipsychotics, and <1.09 for classical antipsychotics.

However, it seems now possible that some of the effects of atypical antipsychotics that have been attributed to their blockade of the 5-HT_{2A} receptor may instead be due to the blockade of 5-HT_{2C}. In particular, affinity for 5-HT_{2C} is another important feature for discriminating classical from atypical antipsychotics [14]: clozapine, olanzapine, seroquel and other atypical antipsychotics have indeed greater affinity for 5-HT_{2C} (and in addition for 5-HT_{2A}) than for D_2 receptors [15]. Blockade of 5-HT_{2C} has been indicated as responsible for the relatively mild extrapyramidal side effects observed for atypical antipsychotics (since 5-HT_{2C} rather than 5-HT_{2A} blockade can prevent the extrapyramidal side effects induced by haloperidol [16]). On the other hand, 5-HT_{2C} antagonists, by enhancing dopamine release in the cortex, would efficiently counteract the hypofrontality, which contributes to the negative symptoms of schizophrenia [17,18]. Furthermore, a 5-HT_{2C} receptor polymorphism (Cys23Ser) has been associated to psychotic symptoms in Alzheimer [19].

On the other hand, since G protein-coupled receptors (GPCRs), as any other biomolecule, exist as collections of conformations in equilibrium [20,21], the affinity and efficacy of the drugs will be related with their absolute and relative affinity for each conformation of each receptor, as well as with the changes that each drug is able to induce in such conformations. A current challenge in receptor pharmacology is the development of experimental and computational methods, as well as pharmacological tools, able to detect different conformational states related with different physiological and pathological mechanisms.

One of the consequences of the existence of pools of conformations is that GPCRs may adopt different states/conformations promoted by agonists that could differentially activate diverse biochemical pathways [22]. It has been described that 5-HT_{2A} and 5-HT_{2C} receptors, in a ligand-dependent way, differentially couple to phospholipase C (PLC) –mediating inositol phosphates (IP) accumulation– and phospholipase A_2 (PLA_2) –mediating arachidonic acid (AA) release– pathways.

Another consequence of the conformational variability of the receptors is the existence of constitutive activity, since it is known the ability of some conformations of the receptors to couple with G proteins and to signal a cellular response in the absence of agonists [20,23–25]. This finding led to the reclassification of the antagonists into inverse agonists and neutral antagonists, as a function of their ability to lower or leave unchanged, respectively, the basal activity of the system.

Taking this into account, the present study presents new pharmacological and three-dimensional quantitative structure-activity relationship (3D-QSAR) studies carried out using 5-HT_2 ligands recently published [26], with the aim of better describing particular pharmacological behaviours that make some of such ligands interesting as new pharmacological tools, as well as describing the structural features related with pharmacological properties of the aforementioned compounds.

2. Chemistry

The present study is based in a series of conformationally constrained butyrophenones, the synthesis and standard pharmacological characterisation of which were recently described [26]. A sample of these compounds is shown in Fig. 1 and Table 1.

3. Pharmacology

3.1. Antagonism of serotonin at 5-HT_{2A} receptors from rat aorta

The experiments were performed as previously described, expressing the antagonistic potency as pA_2 [26].

3.2. Human 5-HT_{2C} binding assays

The experiments were performed as previously described [26] by using [^{3}H]mesulergine to label human 5-HT_{2C} receptors and 1 μM mianserin as non-specific masking ligand. Data were fitted by non-linear regression using GRAPH PAD PRISM v2.01 (GRAPH PAD Software). The affinity of compounds were measured as pIC_{50} (−log of concentration that displace the 50% of total binding).

Table 1
Binding affinities of relevant compounds from Ref. [26], measured at rat 5-HT_{2A} and bovine 5-HT_{2C} receptors

Compound	pK_i 5-HT_{2A}	pK_i 5-HT_{2C}
1 (QF0104B)	8.80±0.80	6.63±0.14
2 (QF0108B)	8.57±0.03	6.98±0.10
3 (QF0307B)	8.60±0.80	6.78±0.07
4 (QF0510B)	8.76±0.20	7.06±0.06
5 (QF0603B)	6.84±0.12	7.09±0.17
6 (QF0703B)	8.97±0.09	7.16±0.01
7 (QF0902B)	8.17±0.18	7.16±0.08
8 (QF1004B)	7.97±0.03	⩽5
9 (QF2004B)	8.80±0.88	7.24±0.06

Values represent the mean±S.E.M. of three experiments.

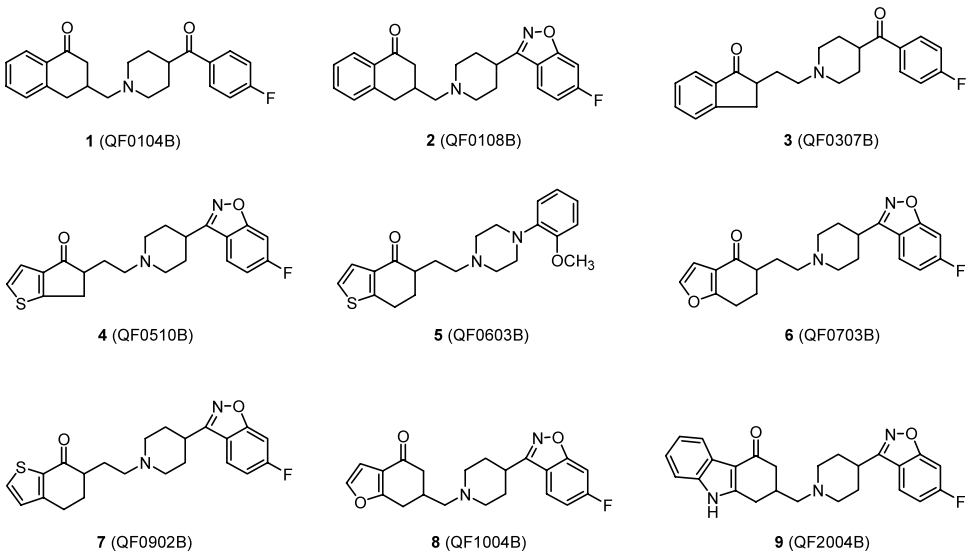

Fig. 1. Structures of relevant compounds.

3.3. IP accumulation and AA release measurement

Both effector pathways were simultaneously evaluated from the same experiment by using the method described by Berg et al. [22]. The experiments were performed at human 5-HT_{2A} receptors transfected in (Chinese Hamster Ovary) CHO cells (200 fmol mg^{-1} protein) and in human 5-HT_{2C} receptors transfected in CHO cells (5–10 pmol mg^{-1} protein). At 5-HT_{2A} receptors, the experiments were performed by inhibiting, with the antagonists/inverse agonists studied, the IP accumulation and AA release induced by 1 μM 5-HT. At 5-HT_{2C} receptors, the experiments were performed by inhibiting, with the antagonists/inverse agonists studied, the basal IP accumulation and AA release. Data were fitted by non-linear regression using GRAPH PAD PRISM v2.01 (GRAPH PAD Software). The potency of the compounds was measured as pIC_{50} (−log of concentration that inhibits the 50% of the maximal stimulation) and their efficacy as the percentage of inhibition of the maximal stimulation.

4. 3D-QSAR analysis

The compounds and pK_i values were extracted from Table 2 of Ref. [26], discarding compounds for which no binding experiment is available or having a pK_i lower than 5. According to these criteria, the 5-HT_{2A} series contained 52 compounds, and the 5-HT_{2C} one 43 molecules. For each compound, 3D structure was obtained starting from its 2D formula and applying the CORINA software [27]. All compounds were considered to have +1 formal charge, which was assigned to the nitrogen atom of the piperidinic ring or to one of the nitrogens of the piperazinic ring. In order to keep the consistency required for the QSAR analyses, in the compounds having a piperazinic ring (which has two protonable positions) the charge was assigned to the nitrogen closest to the cycloalkanone moiety. The series was analysed using a novel 3D-QSAR methodology based in GRid independent descriptors (GRIND) [B], which is implemented in the ALMOND 3.0.3 software [28]. This methodoly has the advantage, in comparison with other 3D-QSAR methods, that the compounds do not require to be superimposed, thus removing one of the most subjective and time-consuming steps of the analysis. Moreover, the molecular description used is rather tolerant with respect to the conformation of the ligands. Details about GRIND can be found elsewhere [29]. Here, the method was applied with the following options: O, N1 and TIP (molecular shape) probes, 100 nodes and 50% of importance given to the field values. The GRID ALM directive was set equal to 1. The method produced 6 correlograms of 67 variables each, thus giving a total of 402 variables.

The regression analysis of the pK_is vs. the GRIND variables was carried out using the partial least squares (PLS) method, also implemented in the ALMOND software. The optimal number of latent variables (LV) was selected on the basis of the cross-validation analysis

Table 2
Potencies of competition for [^{3}H]ketanserin and [^{3}H]mesulergine at human 5-HT_{2A} and 5-HT_{2C} receptors respectively, functional potencies (pA_2) measured at rat 5-HT_{2A} receptors and Meltzer ratios of compounds **6** and **8**

Compound	pK_i 5-HT_{2A}	pK_i 5-HT_{2C}	pA_2	Meltzer ratio
6 (QF0703B)	9.14±0.11	7.46±0.30	9.25±0.05	1.17
8 (QF1004B)	8.26±0.12	5.40±0.52	7.95±0.07	0.99

Data are expressed as mean±S.E.M. of three experiments.

results, but LV producing only small increases on the values of the q^2 were not incorporated into the models. Cross-validation tests were carried out using the standard leave-one-out method. In all the models, a soft variable selection strategy consisting in two sequential fractional factorial design [30] runs was also applied.

5. Results and discussion

In previous works [26,31–35] we studied the antipsychotic profile of different series of compounds at D_2, D_4, 5-HT_{2A} and 5-HT_{2C} receptors, focusing in 5-HT_{2A} and 5-HT_{2C} receptors. Moreover, in order to identify the structural requirements determining the affinity against 5-HT_{2A} and 5-HT_{2C} receptors, 3D-QSAR analyses using the CoMFA and GRID/GOLPE methodologies were carried out. These methodologies required a certain hypothesis on the superposition of the compounds.

The alignment-free 3D-QSAR model for the 5-HT_{2A} affinities presented in the present article has been developed using a series of 52 compounds, which includes the reference compounds haloperidol, ketanserin and risperidone. For this series, the analysis produced a PLS model of rather good quality (LV = 2; $r^2 = 0.85$; $q^2 = 0.74$). However, the observation of the scatterplot of experimental vs. calculated activities permits to identify an important outlier (compound QF1007B). This compound has a structure nearly identical to that of QF1008B but their affinity differs in more than 2.5 log units. The source of this surprising activity difference is being investigated but in the meanwhile the removal of compound QF1007B from the series increased considerably the model quality ($n = 51$; LV = 2; $r^2 = 0.89$; $q^2 = 0.81$). Fig. 2a shows the resulting scatterplot of experimental vs. calculated activities. A detailed interpretation of the model obtained is out of the scope of this article. However, it is noteworthy that the model identifies some structural features important for the affinity for the 5-HT_{2A} receptor, like the presence of groups able to interact at a precise distance with hydrogen bond donor (HBD) (protonated amino group) and hydrogen bond acceptor (HBA) groups (Fig. 3a), and also provides also a precise optimal distance between the favourable interaction region originated by the protonated amino group and the farthest extreme of the compounds (Fig. 3b). Both findings are compatible with the binding mode suggested in a previous work [26] where we postulated the participation of Ser159 in the binding, being such residue located at a distance of Asp155 that is in agreement with the distance now found in the GRIND model and shown in Fig. 3a. On the other hand, the distance shown in Fig. 3b is probably delimiting the depth of the binding pocket or, alternatively, showing the position where interactions with hydrophobic residues could take place.

GPCRs, as any other molecule, exist in many conformations as a consequence of the thermal excitation. Subsets of such conformations ('ensembles') are able to interact with certain G proteins mediating the corresponding physiological functions. Therefore, the manifestation of the receptor functions result from the relative populations of the different conformations. The modification of the relative populations of the functional ensembles by means of formation of drug-receptor complexes is one of the bases of drug effect

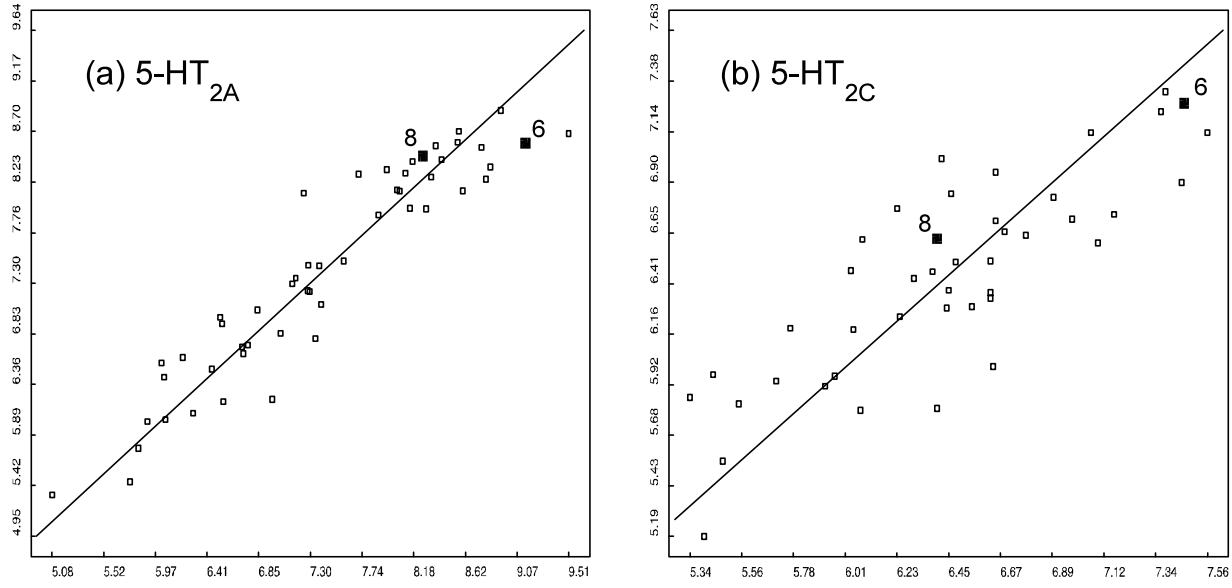

Fig. 2. Scatterplot of experimental vs. calculated binding affinities obtained for the (a) 5-HT_{2A} and (b) 5-HT_{2C} series. Compounds **6** and **8** are highlighted.

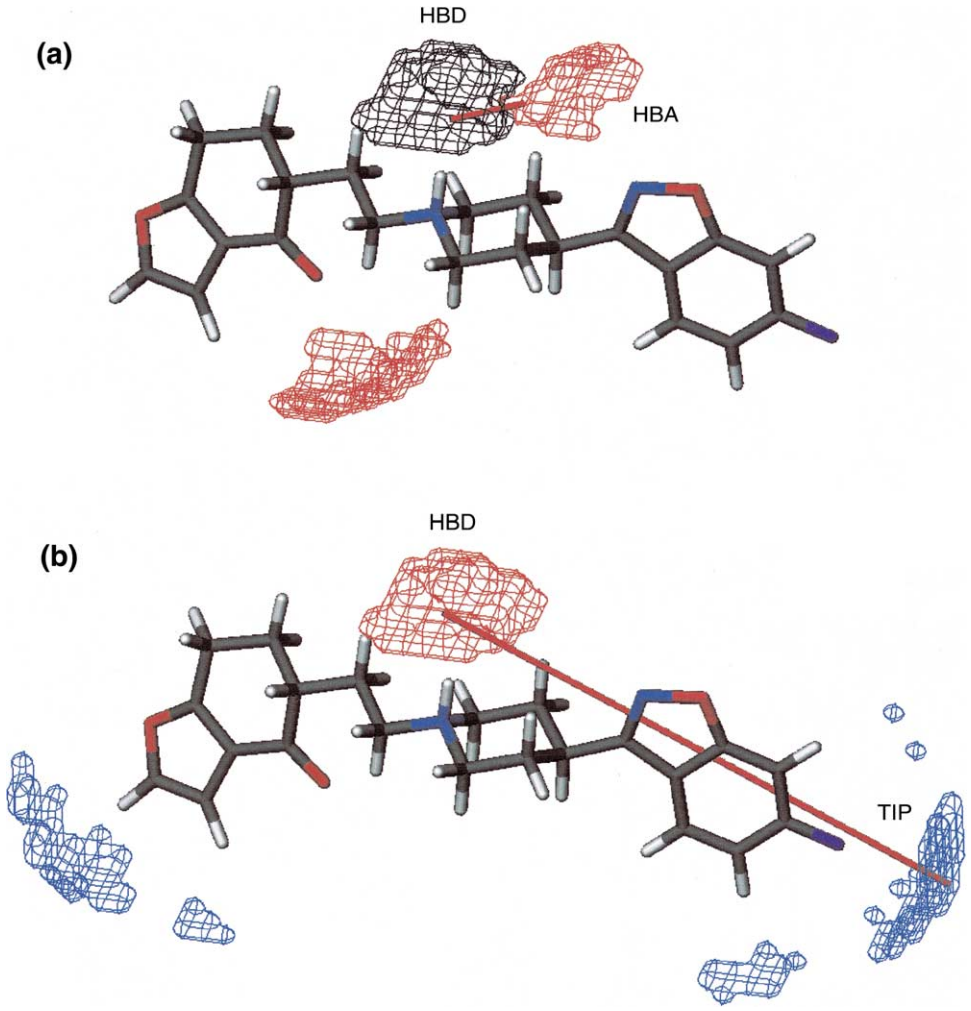

Fig. 3. Regions identified as important for increasing the 5-HT_{2A} binding affinity. Fig. 5a shows the optimal relative location of HBD and HBA regions. Fig. 5b displays the optimal distance between the HBD region generated by the protonated amino group and the farthest extreme of the molecule (TIP) represented by the molecular shape field.

[20]. The present work aims to carry out a sound study of the activation of 5-HT_2 receptors that could unveil conformation-dependent mechanisms.

Since it is difficult to establish structure-activity relationships for series of drugs that have both different pharmacological profiles and strong structural differences, we selected the compounds **6** (QF0703B) (1-[(4-Oxo-4,5,6,7-tetrahydrobenzo[b]furan-5-yl)ethyl]-4-(6-fluorobenzisoxazol-3-yl)piperidine) and **8** (QF1004B) (1-[(4-Oxo-4,5,6,7-tetrahydrobenzo[b]furan-6-yl)methyl]-4-(6-fluorobenzisoxazol-3-yl)piperidine) for the aforementioned pharmacological study, both being closely related from the structural point of view and active at 5-HT_{2A} and 5-HT_{2C} receptors, but showing different pharmacological profiles: the potency of compound **6** at the 5-HT_{2C} receptor is approximately 100 times (2 log units) higher than that of compound **8**, and their Meltzer ratios vary from 1.17 in the case of compound **6** (characteristic of an atypical antipsychotic), to 0.99 in the case of **8** (characteristic of a typical antipsychotic) (see Table 2).

Several conformations for both native and recombinant human 5-HT_{2A} receptors have labelled with agonists and discriminated by antagonists [36]. In order to determine if our compounds **6** and **8**, which are potent 5-HT_{2A} antagonists (Table 2), discriminate different conformations of the 5-HT_{2A} receptor, we have used a functional method of identification of conformational differences. This method consists in assessing the functional effector response at the PLC and PLA_2 pathways activated by the 5-HT_{2A} receptor. In particular, we have studied the way our compounds inhibit the stimulation elicited by 1 μM 5-HT at human 5-HT_{2A} receptors transfected in CHO cells, by measuring simultaneously IP accumulation and AA release (Table 3 and Fig. 4). We have observed that both compounds inhibited completely, but with different potencies, the IP accumulation, being their concentration-dependent curve adjustments most compatible with monophasic sigmoids (Fig. 4a). When we measured AA release inhibition, we observed an analogous behaviour

Table 3
Potency (pIC_{50}) of compounds **6** and **8** at human 5-HT_{2A} receptors transfected in CHO cells, by measuring simultaneously IP accumulation (PLC pathway) and AA release (PLA_2 pathway)

Compound	PLC	PLA_2	
	pIC_{50}	pIC_{50} high	pIC_{50} low
6	9.96±0.26	9.83±0.12	6.76±0.31
8	6.48±0.20		6.64±0.09

Data are expressed as mean±S.E.M. of three experiments.

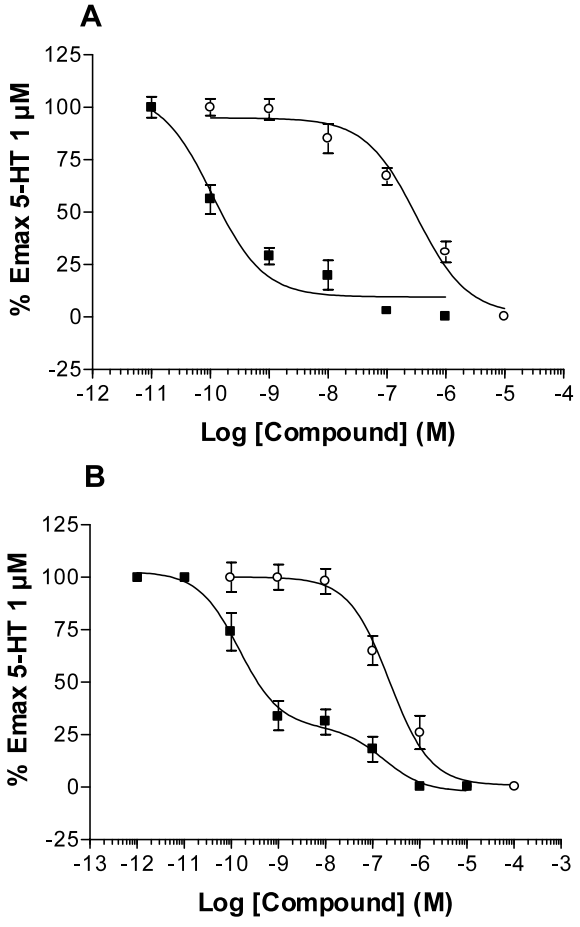

Fig. 4. Inhibition of 1 µM 5-HT -induced stimulation of IP accumulation (A) and AA release (B) by compounds **6** (■) and **8** (○) at human 5-HT_{2A} receptors transfected in CHO cells. Values represent mean± S.E.M. of three experiments.

of compound **8**. However, compound **6** inhibited AA release in a biphasic concentration-dependent way (Fig. 4b). These results suggest that both compounds disallow, but with different potency, 5-HT_{2A} receptors to adopt the serotonin-induced conformations compatible with the activation of the G protein linked to the PLC pathway. On the other hand, whilst compound **8** acts in a similar way in relation to the PLA_2 pathway, compound **6** shows a particular dual behaviour that could indicate its capability to discriminate two conformational ensembles responsible of the activation of the PLA_2 pathway with different efficacies. These results point out the usefulness of this kind of compounds and experiments to detect and discriminate pathway-dependent functional conformations of receptors. Moreover, the results show the dramatic influence that small structural differences between ligands can have in their pharmacological profile.

The alignment-free 3D-QSAR model for the 5-HT_{2C} affinities has been developed using a series of 43 compounds, which includes the reference compounds haloperidol, ketanserin and risperidone. For this series, the analysis produced a much worse PLS model (LV = 3; $r^2 = 0.79$; $q^2 = 0.36$), than that obtained for the 5-HT_{2A} series, probably because the series contains less compounds and the range of binding affinities covered is much smaller (2.22 vs. 4.43 log units). Fig. 2b shows the scatterplot of experimental vs. calculated activities. Even if the quality of the 5-HT_{2C} model is limited, the previously described structural requirement for 5-HT_{2A} receptor are qualitatively similar to those found in the present case, but with small differences that could be determinant for the selectivity.

Multiple conformational ensembles of native 5-HT_{2C} receptors have been described by means of studies made in human brain, which are related to different degrees of constitutive activity. It is known that RNA editing of human 5-HT_{2C} receptors gives rise to different isoforms, which express different degrees of constitutive activity [37]. LSD, a hallucinogenic drug that induce psychotic-like symptoms, changes its efficacy depending on the particular isoform of 5-HT_{2C} receptor, in the same way that antipsychotic drugs change its affinity and efficacy for 5-HT_{2C} receptors depending on the degree of constitutive activity.

Our selected compounds (**6** and **8**) compete for [^{3}H]mesulergine binding at human 5-HT_{2C} receptors (see Fig. 5), showing a concentration-dependent competition curves whose slopes do not significantly differ from unity. The pK_i observed for compounds **6** and **8** were 7.46 and 5.40, respectively. Moreover, we have checked their ability to withdraw constitutively active conformations from the conformational equilibrium (i.e., acting as inverse agonists), by testing their ability to reduce the basal IP accumulation of human 5-HT_{2C} receptors transfected in CHO cells (Fig. 6 and Table 4).

Table 4
Efficacy (Emax) and potency (pIC_{50}) as inverse agonists of compounds **6** and **8** at human 5-HT_{2C} receptors transfected in CHO cells

Compound	Emax (% inhibition)	pIC_{50}
6	72.15±0.67	5.97±0.30
8	0	unable to determinate

Data are expressed as mean±S.E.M. of three experiments.

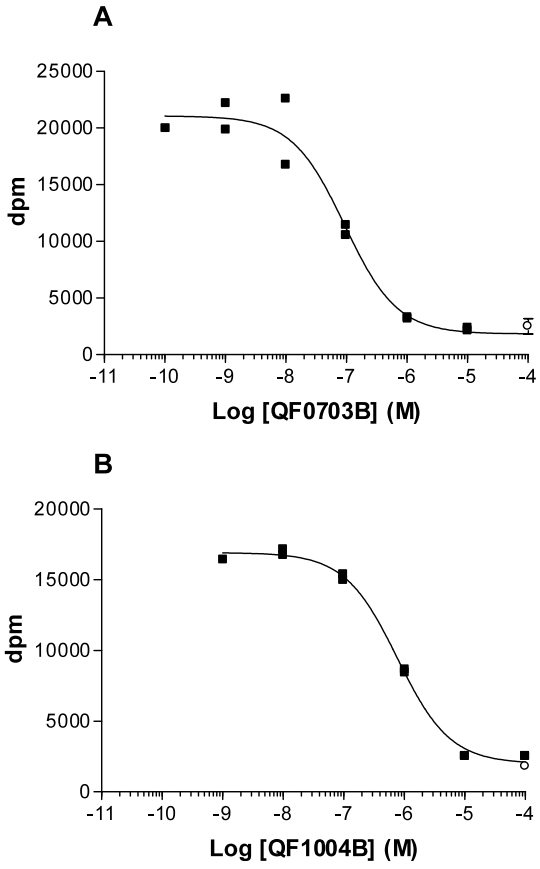

Fig. 5. Binding competition experiments at human 5-HT_{2C} receptors with **6** (QF0703B) (A) and **8** (QF1004B) (B), the radioligand used was [^{3}H]mesulergine (■), non-specific binding was measured with 10^{-6} M mianserine (○). The assay was performed with duplicate points.

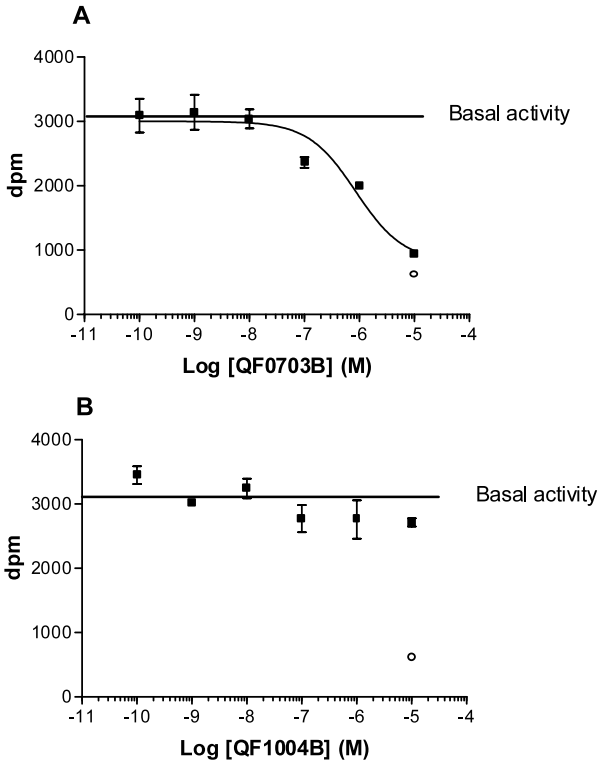

Fig. 6. Concentration–effect curves of compounds **6** (A) and **8** (B) on basal IP accumulation at human 5-HT_{2C} receptors transfected in CHO cells. 1 μM clozapine (○) was used as control. Values represent mean ± S.E.M. of three experiments.

These receptors were transfected at high expression levels (5–10 pmol mg^{-1} protein) consequently showing constitutive activity. The inverse agonist clozapine [38] was used as control in the experiments. Compounds **6** and **8** show again a different behaviour when interacting with constitutively active conformations of the 5-HT_{2C} receptor: compound **6** is able to reduce basal IP accumulation in a concentration-dependent manner revealing its inverse agonist behaviour at the 5-HT_{2C} receptor, while compound **8** is unable to reduce the 5-HT_{2C} constitutive activity showing its neutral antagonist character.

The previously described 3D-QSAR models account for the differences in affinity of compounds **6** and **8** (see Fig. 2), but such models cannot and do not aspire to explain particular pharmacological behaviours of these compounds that are related with effector pathways. 3D-QSAR models, which aim to be relevant for sizeable series of compounds, are focused on identifying major pharmacophoric patterns necessary for a certain biological activity, but such models are not relevant to identify fine-tuning dynamic structural features of individual ligand-receptor complexes that affect pharmacological behaviours related with modifications in the conformational populations of the receptors.

In summary, the present study has developed 3D-QSAR models for 5-HT_2 receptors that allow feasible predictions of activity of new compounds and reveal structural requirements for optimal affinity, particularly in the case of the 5-HT_{2A} receptor. Furthermore, two of the compounds of the series have been characterised as interesting pharmacological tools to study the influence on the antipsychotic profile of: (a) different selectivity for 5-HT_2 receptor subtypes; (b) different 5-HT_{2A}/D_2 ratios (Meltzer ratio); (c) different conformation-dependent functional behaviours at 5-HT_{2A} and 5-HT_{2C} receptors.

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