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Article

Dibenzofuranylethylamines as 5-HT$_{2A/2C}$ Receptor Agonists

Thirumal Yempala, José Brea, María Isabel Loza, Douglas J. Matthies, Gerald Zapata-Torres,* and Bruce K. Cassels*

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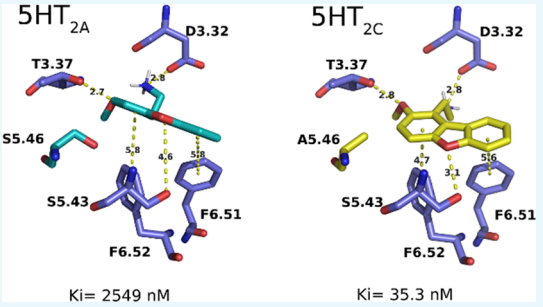
ABSTRACT: The human 5-HT$_2$ receptor subtypes have high sequence identity in their orthosteric ligand-binding domain, and many agonists are poorly selective between the 5-HT$_{2A}$ and 5-HT$_{2C}$ subtypes. Nevertheless, their activation is associated with different pharmacological outcomes. We synthesized five phenethylamine analogs in which the benzene ring is replaced by a bulky dibenzo[*b*,*d*]furan moiety and found a couple with >70-fold 5-HT$_{2C}$ selectivity. Molecular docking studies of the most potent compound (**5**) at both receptor subtypes revealed the likely structural basis of its selectivity. Although in both cases, some crucial interactions are conserved, the change of the Ala222$^{5.46}$ residue in the 5-HT$_{2C}$ receptor to the larger Ser242$^{5.46}$ in the 5-HT$_{2A}$ subtype, which is the only structural difference between the orthosteric binding pockets of both receptors, weakens a $\pi-\pi$ stacking interaction between the dibenzofuran moiety and the important Phe$^{6.52}$ residue and breaks a hydrogen bond between the dibenzofuran oxygen and Ser$^{5.43}$, explaining the selectivity of compound **5** for the 5-HT$_{2C}$ receptor. We believe that this effect of the residue at position 5.46 merits further exploration in the search for selective 5-HT$_{2C}$ receptor agonists that are of considerable interest in the treatment of schizophrenia and substance abuse.



1. INTRODUCTION

Serotonin (5-hydroxytryptamine, 5-HT) is a biologically important neurotransmitter that plays key roles in mental states related to mood, sleep and dreaming, appetite, libido, aggression, anxiety, cognition, and pain. It also regulates peripheral functions in the gastrointestinal, cardiovascular, endocrine, and pulmonary systems. The actions of 5-HT are mediated by fourteen different receptor subtypes of which all but 5-HT$_3$ are class A G-protein-coupled receptors (GPCRs). Ligands that bind more or less selectively to these receptors have proven effective in the treatment of migraine, pain, and a wide range of psychiatric and neurological disorders. Among these receptors, the 5-HT$_2$ type is of particular interest because of its affinity for molecules that are therapeutically useful in a variety of conditions or induce altered mental states in humans. The 5-HT$_2$ receptor family consists of three subtypes (2A, 2B, and 2C). 5-HT$_{2A}$ receptor activation is a characteristic effect of classic hallucinogens, while inhibition of this subtype contributes to the activity of some antipsychotic drugs. 5-HT$_{2C}$ receptor agonists are of interest as appetite suppressants and, possibly, as agents for the treatment of erectile dysfunction, drug addiction, and schizophrenia, while antagonists may be useful as antidepressants and/or anxiolytics.[1−3] Finally, 5-HT$_{2B}$ receptors are commonly considered as an "antitarget" because the extended use of compounds that activate them can lead to cardiac valvulopathy, and no clear therapeutic effects of 5-HT$_{2B}$ receptor antagonists have been identified.[4]

The structures of the endogenous monoamine neurotransmitters dopamine, norepinephrine, and serotonin are based on the 2-phenylethylamine (phenethylamine) and 2-(3-indolyl)ethylamine skeletons. Many variations of the substitution patterns on the aromatic moieties of these scaffolds and particularly on the phenyl ring have been synthesized and tested, particularly exploring their psychedelic activity.[5] The activity of these classic hallucinogens is ascribed primarily to their agonist activity at 5-HT$_{2A}$ receptors, and an early generalization was that 2,5-dimethoxy substitution of phenethylamines, plus a small, preferably hydrophobic substituent at C-4 of the phenyl ring is associated with strong receptor binding and functional potency. Moreover, when the orientation of the electron lone pairs on the oxygens is fixed by locking these atoms in dihydrofuran or furan rings, affinity for 5-HT$_2$ receptors (usually with little selectivity among the three subtypes) increases due to postulated interactions with hydrogen bonding residues in the receptor binding site.[6,7] Docking studies have confirmed that these two oxygen atoms tend to function as proxies for the indole NH and 5-hydroxyl groups of serotonin (Figure 1).

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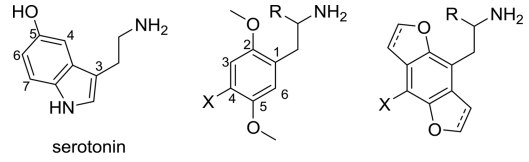


Figure 1. Structures of serotonin and 2,5-dioxygenated-4-substituted serotonin receptor ligands.

Bulky ring systems have been introduced in high affinity ligands of structurally related GPCRs, notably in the successful beta blockers carazolol and carvedilol (Figure 2).

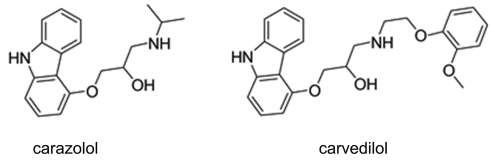


Figure 2. Structures of carbazole-derived beta blockers.

Carazolol has been cocrystallized with the β_2-adrenergic receptor, presumably in an inactive conformation in which the carbazole moiety occupies the orthosteric binding site.[8] There are also a few examples of large anthracene-derived aromatic systems that bind the 5-HT_{2A} receptor with high affinity.[9]

Thus, a set of dibenzofuranylethylamines with different orientations of the dibenzofuran moiety were synthesized and evaluated for binding affinity and functional activity at 5-HT_{2A} and 5-HT_{2C} receptors. Also, models of both receptors were built using the X-ray structure of the ergotamine-bound 5-HT_{2C} receptor, and docking studies were done for all compounds, to shed light on the selectivity of these compounds on both receptors.

2. RESULTS AND DISCUSSION

Two of us recently described the synthesis of a number of dibenzo[*b*,*d*]furan-derived aldehydes, which appeared as obvious candidates for further synthetic elaboration.[10,11] Using these aldehydes as building blocks and assuming that the bioisosteric dibenzofuran core might also fit into the orthosteric binding site of 5-HT_2 receptors, we prepared an initial set of dibenzofuranylethylamine derivatives (**1**−**5**) (Figure 3) as candidates to explore their affinity and functional activity at these receptors and performed docking studies to illuminate the interpretation of our experimental results.

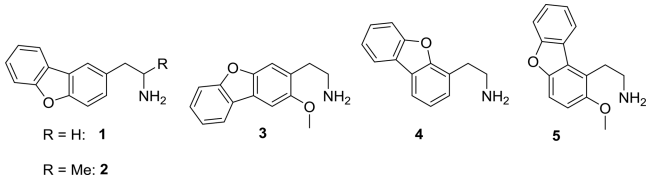
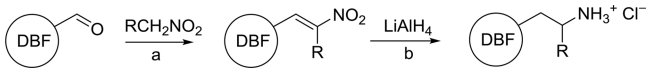


Figure 3. Dibenzofuranylethylamines synthesized and tested in this work.

The aldehydes were condensed with nitromethane, and in one case instead with nitroethane, to afford the nitroalkenyl derivatives, which were reduced to the corresponding amines **1**−**5** using $LiAlH_4$. These were converted into their crystalline, water-soluble hydrochlorides (Scheme 1).

Scheme 1. DBF: Variously Substituted Dibenzo[*b*,*d*]furans[a]



[a](a) AcOH/$AcO^-NH_4^+$, reflux, 4 h, 84−88%. (b) THF, reflux, 16 h, then HCl/acetone, 65−72%.

The affinities of all five compounds for both 5-HT_2 receptor subtypes were determined by radioligand displacement assays ([^{3}H]ketanserin for 5-HT_{2A} and [^{3}H]mesulergine for 5-HT_{2C}), and the functional activities were assessed by a standard fluorescence assay, as Ca^{2+} mobilization. The hydrochlorides of 2-(dibenzo[*b*,*d*]furan-2-yl)ethanamine (**1**) and 2-(dibenzo-[*b*,*d*]furan-4-yl)ethanamine (**3**) had been synthesized by another route in the mid-1900s and only said to be "toxic".[12,13] The results are shown in Table 1.

Amines **1**, **2**, **3**, and **4** failed to displace [^{3}H]ketanserin from the human 5-HT_{2A} receptor by 50% or more at 10 μM concentration suggesting, under the conditions of the assay, that their inhibition constants were greater than 10^{-5} M. Nevertheless, their K_i values at the human 5-HT_{2C} receptor were all submicromolar, suggesting at least modest 5-$HT_{2C/2A}$ selectivity. Compound **5** had a low micromolar K_i at the h5-HT_{2A} receptor, and its affinity for the h5-HT_{2C} receptor, K_i = 35 nM, was particularly striking. In the functional assay, all the compounds were weak 5-HT_{2A} receptor partial agonists, although **4** was somewhat more potent, with a low micromolar EC_{50} and a rather high maximal response (88%) compared to serotonin. Compounds **1** and **3** were extremely weak partial agonists at the 5-HT_{2C} receptor, and **2** and **4** displayed moderately potent full agonist activity at this subtype. Compound **5** stands out with its 222 nM EC_{50} and is a 70 times weaker partial agonist at the 5-HT_{2A} subtype, its potencies closely reflecting its relative affinities for both receptors. Interestingly, the location of the aminoethyl group on the dibenzofuran skeleton does not seem to be a crucial factor, particularly when **4** and **5** are compared, with the amine substituents at C4 and C1, respectively. However, it may be noted that in both **4** and **5** the dibenzofuran oxygen lone pairs appears to be "right" (plus a favorably placed flexible methoxyl group in the latter), and in **3**, it should be "wrong" for hydrogen bonding to donor residues in the active site of the receptors.[6,7]

Amine **5** may be viewed as a structural analog of 1-(5-methoxy-2,3-dihydrobenzofuran-4-yl)propanamine,[7,16] with the α-methyl group removed and annulated with a second benzene ring to form the dibenzofuran system. The 5-HT_{2A} affinities of both compounds are practically identical (though not assayed in the same model), as if the negative effect of allowing the 2-methoxy group to rotate freely was counteracted by the increased hydrophobic and potentially π−π-interacting volume of the added benzene ring. Assuming that **5** binds at the orthosteric site of serotonin receptors, this would imply that the additional ring fits into a hydrophobic pocket and interacts favorably with one or more residues in this area, more particularly of the 5-HT_{2C} receptor, conjectures that we addressed with docking studies.

The human 5-HT_{2C} receptor was modeled on the basis of its crystal structure bound to the agonist ergotamine,[17] and a model of the h5-HT_{2A} receptor was developed by homology. Even though both receptors share a high sequence identity (73% identity between h5-HT_{2C}/h5-HT_{2A} whole protein

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Table 1. Affinity (pK_i) and Functional Activity (EC_{50} and E_{max}) of the Studied Compounds[a]

	pK_i (K_i, nM)			EC_{50} (nM,% E_{max}[b])	
compound	5-HT_{2A}	5-HT_{2C}	5-$HT_{2A/2C}$	5-HT_{2A}	5-HT_{2C}
2C-B[b]	8.16 (6.9 ± 0.8)	7.37 (43 ± 4)	6	2.1 ± 0.8 (92 ± 8)	ND[b]
tryptamine[e]	5.39 (4074)	7.02 (95.50)	43	17.4 (97.60 ± 2.34)	1.2 (107.8 ± 3.43)
5-HT	ND[b]	ND[b]	ND[b]	7.26	0.41
1	≪ 5	6.13 ± 0.04 (736.6 ± 67.2)	> > 14	22,600 ± 8800 (66.09 ± 2.79)	NA[f] (49.1 ± 8.85)[b]
2	≪ 5	6.32 ± 0.03 (473.9 ± 33.1)	> > 21	NA[f] (64.03 ± 1.9)[b]	3520 ± 740 (111.62 ± 0.12)
3	< 5	6.69 ± 0.02 (204.1 ± 10.2)	> 49	32,400 ± 7200 (58.18 ± 3.05)	53,200 ± 13,500 (54.02 ± 1.13)
4	< 5	6.84 ± 0.06 (140.5 ± 21.1)	> 71	3330 ± 830 (88.21 ± 2.79)	1380 ± 190 (98.03 ± 12.27)
5	5.59 (2549 ± 183.2)	7.45 ± 0.03 (35.3 ± 2.47)	72	14,500 ± 860 (58.99 ± 2.41)	222 ± 41 (106.35 ± 11.89)
risperidone	(0.51)	(15.5)	0.03	ND[b]	ND[b]

[a]Values represent the mean ± range of two independent assays with duplicate measurements. [b]%maximum response (5-HT) at 100 μM. [c]Data from Luethi et al., 2018.[14] [d]ND, not determined. [e]Data from Toro et al., 2019.[15] [f]NA, not active. Compound with partial agonist activity but not sufficiently active to obtain an EC_{50} value.

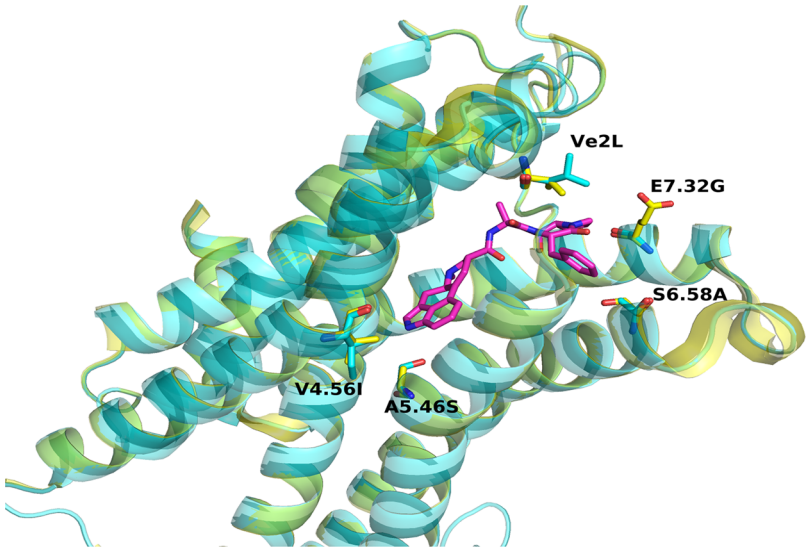

Figure 4. Overlaid crystal structure of the 5-HT_{2C} receptor (cyan) and model of the 5-HT_{2A} receptor (yellow-tan). The bound ergoline in the crystal is shown in magenta.[17] Nonconserved residues are shown as sticks.

sequences), their binding sites exhibit at least five differences, namely, at positions 5.46 (Ballesteros–Weinstein notation:[18] A222/S242, 5-HT_{2C}/5-HT_{2A}, respectively), 4.56 (V185/I206), 6.58 (S334/A346), 7.32 (E347/G359), and 5.29 (V208/L228). Of these differences, the last three lie far away from the binding site of the ergoline moiety described for ergotamine in the h5-HT_{2C} crystal structure. Figure 4 shows overlaid models of both receptors, with the ergotamine structure in its crystallographically determined pose.

Docking studies using the crystal structure of the human 5-HT_{2C} receptor bound to the agonist ergotamine,[17] and a model of the 5-HT_{2A} receptor based on this template, supported the hypothesis that our compounds can bind in the orthosteric site. In the 5-HT_{2C} receptor, aside from the usual ionic interaction with D3.32, **5** forms hydrogen bonds with T3.37 (at 2.8 Å) through its methoxy group and S5.43 (at 3.1 Å) through the dibenzofuran oxygen bridge. In addition, the two benzene rings of the dibenzofuran moiety participate in face-to-edge $\pi-\pi$ interactions with F6.51 and F6.52 (at 4.7 and 5.6 Å, respectively). The binding region of the 5-HT_{2C} receptor compared to the 5-HT_{2A} receptor differs in the exchange of an alanine residue (A5.46) for a serine (S5.46). According to our docking results, this replacement seems to be pivotal for the different affinities displayed by **5** at the two receptor subtypes. In the 5-HT_{2A} receptor, the greater bulk of a serine (S5.46) residue is sufficient to push the dibenzofuran system away from one of the TM6 phenylalanines, thus increasing the distance to F6.52 (from 4.7 to 5.8 Å) and disrupting the hydrogen bond between the furan oxygen and the S5.43 residue. The dominant ionic bond between the protonated amine nitrogen and D3.32 and the hydrogen bond between the methoxyl group and T3.37 located in the 5HT_{2C} receptor are conserved, even though the flat dibenzofuran ring of compound **5** adopts a different angle when binding in the orthosteric site (Figure 5).

The better than 70-fold selectivity of compounds **4** and **5** for the 5-HT_{2C} receptor, together with the steric effect of replacing $A222^{5.46}$ in the 5-HT_{2C} receptor by $S242^{5.46}$ in the 5-HT_{2A} receptor suggest that unwanted 5-HT_{2A} agonism might be subdued by introducing bulky extensions of the aromatic moieties in phenethylamine analogs. However, the micromolar or only slightly better functional potencies of **4** and **5** are not sufficiently attractive to warrant preclinical studies, and additional compounds will have to be synthesized and tested. It should be noted that both the 5-HT_{2B} and 5-HT_{2C} receptors have identical orthosteric binding sites, both with alanine at the 5.46 position, which would not favor selectivity between these subtypes on the basis of the mechanism we are proposing here.

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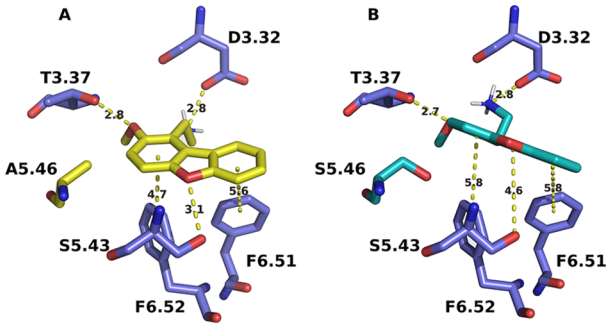

Figure 5. Key interactions of **5** in the orthosteric binding site of 5-HT_{2C} and 5-HT_{2A} receptors (colored slate blue). Nonconserved residues in 5-$HT2_{C/2A}$ receptors at position 5.46 (yellow for $A222^{5.46}$ in 5-HT_{2C} (A) and cyan for $S242^{5.46}$ (B) in 5-HT_{2A}, respectively). According to our docking results, this difference results in a significant weakening of the $\pi-\pi$ interaction with F6.52 and of the hydrogen bond between the dibenzofuranyl oxygen atom and S5.43.

It is therefore possible that any compounds of interest that might be synthesized would also exhibit high potency at the "antitarget" 5-HT_{2B} receptor. Consequently, future studies of this family will have to envisage such a possibility by including affinity and functional assays at all three 5-HT_2 receptor subtypes.

It should be mentioned that the ionic bond between the protonated amine nitrogen and D3.32 seems to be the dominant interaction in all amine ligands of class A GPCRs. S5.43 also forms a characteristic hydrogen bond with the C5 oxygen of the hallucinogenic 2,5-dioxygenated phenethylamines and phenylisopropylamines (see Figure 1), both S3.36 and T3.37 form hydrogen bonds to the C2 oxygen, and F6.52 interacts with the benzene ring of these smaller psychedelic molecules, while F6.51 does not.[19,20] In contrast, F6.51 forms an edge-to-face interaction with the additional benzene ring of the "superpotent" 25X-NBOMe compounds.[21,22] We found all these interactions in the docking pose adopted by **5** in the 5-HT_{2C} receptor's orthosteric site, but there is no C4 substituent to interact with a key hydrophobic pocket in the receptor, which makes an important contribution to the affinities of classic phenethylamine psychedelics.[19] This lack presumably explains the relatively low affinity of **5** compared to the 25X-NBOMes. It may also be pointed out that they are reminiscent of the interactions observed in the β_2 receptor crystal structure where the carbazole moiety of the antagonist carazolol interacts with D3.32, F6.51, and F6.52.[8]

Regarding the weakly binding compound **3**, which is practically devoid of (partial) agonist activity at both receptor subtypes, it may be seen as a cyclized version of the 5-HT_{2A} antagonist 2-(2,5-dimethoxy-4-phenylphenyl)ethanamine (2C-phenyl, compound **7** in Trachsel et al., 2009).[23] However, the orientation of the oxygen lone pairs on the dibenzofuran ring is "wrong" for hydrogen bonding,[6,7] as corroborated by the higher affinities of 2C-phenyl and the "Fly" and "Dragonfly" compounds with "correct" orientations and pK_i values of 6.11 and greater than 8, respectively, at the 5-HT_{2A} receptor (the 5-HT_{2C} affinities of 2C-phenyl and the "Fly/Dragonfly" compounds are not available, nor are their functional activities at this receptor). Nevertheless, it should be noted, however, that while 2C-H-Fly elicited a positive drug discrimination response in LSD-trained rats, suggesting that it is a 5-HT_{2A} agonist, and **3** is a very weak partial agonist, 2C-phenyl is an antagonist at this receptor (Figure 6).

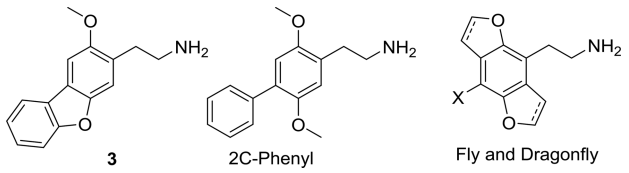

Figure 6. Comparison of the structures of compound **3**, 2C-phenyl, and "Fly" (with dihydrofuran rings) and "Dragonfly" compounds (with furan rings).

3. CONCLUSIONS

In conclusion, we have shown that arylethylamines incorporating the dibenzo[*b*,*d*]furan ring system as the aromatic moiety bind to the orthosteric site of 5-HT_{2A} and 5-HT_{2C} receptors. In doing so, they can establish ionic, hydrogen-bonding, and $\pi-\pi$ stacking interactions involving the same amino acid residues as their simpler phenethylamine analogs and the *N*-benzyl derivatives of the latter (including the N-BOMes). Furthermore, the $\pi-\pi$ stacking interactions in which the dibenzofuran moiety participates are analogous to those of the carbazole moiety of the β-adrenergic antagonist carazolol, cocrystallized with the β_2-adrenergic receptor, highlighting the bioisosteric character of these two heterocyclic systems. However, in vitro studies showed that their K_i values at the 5-HT_{2C} subtype are all submicromolar and as low as 35 nM in the most favorable case (**5**), exhibiting at least modest 5-HT_{2C} selectivity. Moreover, **5** and **4**, its next highest affinity analog, are full agonists at the 5-HT_{2C} receptor. In contrast, our dibenzofuranylethylamines are partial agonists with worse or much worse than micromolar affinities for the 5-HT_{2A} receptor. The 5-HT_{2C} selectivity of **5** is explained by a single difference in the orthosteric site, that is, A5.46 in this receptor and the bulkier and less hydrophobic S5.46 in the 5-HT_{2A} subtype, which displaces the dibenzofuran moiety in the 5-HT_{2A} receptor, weakening or abolishing its interactions with other amino acid residues.

4. EXPERIMENTAL SECTION

4.1. Receptor Modeling and Docking Methodology. The human 5-HT_{2C} receptor crystal structure was retrieved from the Protein Data Bank (ID: 6BQG, agonist bound state).[17] The h5-HT_{2A} receptor was modeled using the h5-HT_{2C} crystal structure as a template by means of SWISS-MODEL.[24] Even though both receptors share a high sequence identity (73% identity between h5-HT_{2C}/h5-HT_{2A} whole protein sequences), their binding sites exhibit at least five differences, namely, at positions 5.46 (Ballesteros–Weinstein notation:[18] Ala222/Ser242, 5-HT_{2C}/5-HT_{2A}, respectively), 4.56 (Val185/Ile206), 6.58 (Ser334/Ala346), 7.32 (Glu347/Gly359), and 5.29 (Val208/Leu228). Of these differences, the last three are far away from the ergoline moiety binding site described for ergotamine in the 5-HT_{2C} crystal structure.[17]

All the dibenzofuranylethyl structures were optimized at the DFT level of theory using the B3LYP functional and the 6-31G(d,p) basis set as implemented in the Gaussian 09 package of programs.[25] RESP charges for all compounds were calculated prior to docking studies.[26] Docking studies were performed by means of AutoDock 4.2 in the orthosteric

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binding site of the 5-$HT_{2A/2C}$ receptors.[27] Grid maps were calculated using AutoGrid4 centered on Asp3.32 (numbering according to Ballesteros and Weinstein),[18] defining a volume of 40 $Å^3$ with a 0.375 Å grid spacing. The AutoTors option of AutoDockTools was used to define rotatable bonds. Genetic Lamarckian algorithm was used under the following conditions: population size 50, maximum number of evaluations 2,500,000, maximum number of generations 27,000, rate of mutation 0.02, and rate of crossover 0.08. The calculations were performed with dielectric as the default setting. The most stable conformation for each compound was chosen according to the best docking score, the population of the conformation, and the activity reported here.

4.2. Synthesis of Dibenzofuranylethylamines. Solvents were purchased commercially (Merck) and dried prior to use according to standard protocols. Additional reagents were from Sigma-Aldrich. Melting points were measured with a Stuart SMP 10 melting point apparatus and are uncorrected. NMR spectra were recorded on a Bruker Avance III HD 400 spectrometer (9.4 T, 400.13 MHz for ^1H, and 100.62 MHz for ^{13}C) in appropriate solvents using TMS or solvent peaks as internal standards, and the chemical shifts are shown in the δ scale. All the NMR spectra were indicative of greater than 95% purity. An ESI-MS Exactive Plus Orbitrap high-resolution mass spectrometer, Thermo Fisher Scientific (Bremen, Germany), was used for the final derivatives. All the experiments were monitored by thin-layer chromatography (TLC) performed on silica gel GF_{254} precoated plates, and silica gel finer than 200 mesh was used for column chromatography. Yields refer to chromatographically homogeneous materials.

4.2.1. General Procedure for the Synthesis of Nitroalkenyl Dibenzofuran Derivatives. To a solution of each dibenzofuran aldehyde (10 mmol) in acetic acid, ammonium acetate (15 mmol) and nitromethane or nitroethane (13 mmol) were added, and the reaction mixture was refluxed for 4 h. After reaching RT, the reaction mixture was left aside for a sufficient time (>6 h) to allow the product to precipitate and collect it in good yield (>84%) by filtration, as a yellow solid.

4.2.1.1. 2-(E)-(2-Nitroethenyl)dibenzo[b,d]furan. Yield: 88%; yellow solid.

^1H NMR (400 MHz, $CDCl_3$) δ 8.15 (d, J = 13.4 Hz, 1H), 8.10 (s, 1H), 7.96 (d, J = 7.5 Hz, 1H), 7.69−7.56 (m, 4H), 7.52 (t, J = 7.3 Hz, 1H), 7.41 (t, J = 7.3 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.2, 156.7, 139.2, 136.2, 128.2, 128.0, 125.4, 124.8, 123.4, 123.0, 122.0, 120.9, 112.7, 112.0.

4.2.1.2. 2-(E)-(2-Nitroprop-1-en-1-yl)dibenzo[b,d]furan. Yield: 85%; yellow solid.

^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (s, 1H), 8.03 (s, 1H), 7.98 (d, J = 7.5 Hz, 1H), 7.67−7.59 (m, 2H), 7.57−7.49 (m, 2H), 7.40 (t, J = 7.5 Hz, 1H), 2.55 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.6, 146.9, 133.7, 129.2, 127.9, 127.0, 124.9, 123.3, 123.2, 122.4, 120.7, 12.1, 111.8, 14.1.

4.2.1.3. 4-(E)-(2-Nitroethenyl)dibenzo[b,d]furan. Yield: 84%; yellow solid.

^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, J = 13.6 Hz, 1H), 8.10 (d, J = 13.6 Hz, 1H), 7.99 (d, J = 6.8 Hz, 1H), 7.92 (d, J = 7.5 Hz, 1H), 7.61 (d, J = 8.3 Hz, 1H), 7.54−7.45 (m, 2H), 7.42−7.32 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 155.9, 154.1, 139.6, 134.0, 130.3, 128.0, 125.2, 124.1, 123.6, 123.3, 123.0, 120.8, 115.2, 111.9.

4.2.1.4. 2-Methoxy-1-(E)-(2-nitroethenyl)dibenzo[b,d]furan. Yield: 86%; yellow solid.

^1H NMR (400 MHz, $CDCl_3$) δ 8.82 (d, J = 13.2 Hz, 1H), 8.21−8.12 (m, 2H), 7.59−7.54 (m, 2H), 7.52−7.47 (m, 1H), 7.39 (t, J = 8.0 Hz, 1H), 7.04 (d, J = 9.0 Hz, 1H), 4.00 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.2, 156.2, 150.3, 140.2, 131.0, 128.1, 125.5, 123.1, 122.7, 114.8, 113.1, 112.0, 110.0, 56.2.

4.2.1.5. 2-Methoxy-3-(E)-(2-nitroethenyl)dibenzo[b,d]furan. Yield: 84%; yellow solid.

^1H NMR (400 MHz, $CDCl_3$) δ 8.27 (d, J = 13.6 Hz, 1H), 7.96−7.87 (m, 2H), 7.60 (s, 1H), 7.58−7.49 (m, 2H), 7.42 (s, 1H), 7.39−7.34 (m, 1H), 4.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.7, 155.9, 150.1, 138.0, 135.5, 128.8, 128.5, 123.5, 123.0, 121.1, 118.4, 113.8, 112.0, 102.2, 56.1.

*4.2.2. General Procedure for the Synthesis of Dibenzofuran Phenethylamine (**1**−**5**) Hydrochlorides.* A solution of a dibenzofuran nitroethenyl derivative (4 mmol) in anhydrous THF was added carefully and drop by drop to a suspension of $LiAlH_4$ (20 mmol) in THF at 0 °C. After addition, the solution was refluxed for 16 h. The reaction mixture was then cooled to 0 °C and quenched with a solution of sodium potassium tartrate, and NaOH solution was added to maintain basicity. The resulting reaction mixture was filtered through Celite. The filtrate was concentrated and purified by silica gel column chromatography eluting with $MeOH/CH_2Cl_2/25\%NH_3$ 10:88:2 to yield the free amines (**1**−**5**). These were converted into their corresponding hydrochlorides in moderate to good yields (65−72%) by adding conc HCl to the free bases in acetone and precipitating the salts by the addition of excess ethyl ether followed by filtration.

4.2.2.1. 2-(Dibenzo[b,d]furan-2-yl)ethanamine Hydrochloride (1-HCl).[12,13] Yield: 72%; colorless solid; mp 225−227 °C.

^1H NMR (400 MHz, DMSO-d_6) δ 8.30 (br s, 2H), 8.11 (d, J = 7.5 Hz, 1H), 8.05 (s, 1H), 7.71−7.62 (m, 2H), 7.51 (t, J = 7.3 Hz, 1H), 7.45−7.37 (m, 2H), 3.11 (br s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.6, 154.3, 132.2, 128.1, 127.4, 123.6, 123.3, 122.9, 121.0, 120.9, 111.5, 111.5, 40.1, 32.6. ESI-HRMS (m/z): Calcd for $C_{14}H_{14}NO$ $[M - Cl]^+$ 212.1075, found: 212.1067.

4.2.2.2. 1-(Dibenzo[b,d]furan-2-yl)propan-2-amine Hydrochloride (2-HCl). Yield: 68%; white powder; mp 260−262 °C.

^1H NMR (400 MHz, DMSO-d_6) δ 8.48 (br s, 2H), 8.11 (d, J = 7.5 Hz, 1H), 8.02 (s, 1H), 7.71−7.60 (m, 2H), 7.50 (t, J = 7.5 Hz, 1H), 7.43−7.32 (m, 2H), 3.54−3.41 (m, 1H), 3.34−3.25 (m, 1H), 2.93−2.82 (m, 1H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.6, 154.3, 131.7, 128.6, 127.5, 123.6, 123.3, 122.9, 121.5, 121.0, 111.5, 111.4, 48.2, 39.6, 17.3. ESI-HRMS (m/z): Calcd for $C_{15}H_{16}NO$ $[M - Cl]^+$ 226.1232, found: 226.1224.

4.2.2.3. 2-(Dibenzo[b,d]furan-4-yl)ethanamine Hydrochloride (4-HCl).[12,13] Yield: 70%; white powder; mp 232−234 °C.

^1H NMR (400 MHz, DMSO-d_6) δ 8.40 (br s, 2H), 8.14 (d, J = 7.5 Hz, 1H), 8.03 (d, J = 7.5 Hz, 1H), 7.72 (d, J = 8.3 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.44−7.32 (m, 3H), 3.36−3.15 (m, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.1, 153.8, 127.7, 127.5, 123.6, 123.4, 123.2, 123.0, 121.1, 121.0, 119.6, 111.5, 38.2, 27.2. ESI-HRMS (m/z): Calcd for $C_{14}H_{14}NO$ $[M - Cl]^+$ 212.1075, found: 212.1068.

4.2.2.4. 2-(2-Methoxydibenzo[b,d]furan-1-yl)ethanamine Hydrochloride (5-HCl). Yield: 65%; white powder; mp 285−286 °C.

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^{1}H NMR (400 MHz, DMSO-d_6) δ 8.55−8.44 (br m, 3H), 7.76 (d, J = 8.0 Hz, 1H), 7.59−7.49 (m, 2H), 7.38 (t, J = 7.5 Hz, 1H), 7.22 (d, J = 9.0 Hz, 1H), 3.88 (s, 3H), 3.51−3.41 (m, 2H), 3.05−2.92 (m, 2H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 156.0, 153.1, 149.9, 127.4, 123.1, 122.9, 122.8, 119.2, 111.5, 111.4, 110.1, 56.5, 37.6, 24.3. ESI-HRMS (m/z): Calcd for $C_{15}H_{16}NO_2$ [M − Cl]$^+$ 242.1181, found: 242.1174.

4.2.2.5. 2-(2-Methoxydibenzo[b,d]furan-3-yl)ethanamine Hydrochloride (3-HCl). Yield: 67%; white solid; mp 175−176 °C.

^{1}H NMR (400 MHz, DMSO-d_6) δ 8.34 (br s, 2H), 8.11 (d, J = 7.5 Hz, 1H), 7.76 (s, 1H), 7.63 (d, J = 8.0 Hz, 1H), 7.55 (s, 1H), 7.46 (t, J = 8.0 Hz, 1H), 7.35 (t, J = 7.5 Hz, 1H), 3.92 (s, 3H), 3.11−3.00 (br s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 155.7, 153.6, 149.6, 126.9, 125.8, 123.9, 122.6, 122.5, 120.8, 112.7, 111.4, 102.4, 22.9, 38.3, 28.4. ESI-HRMS (m/z): Calcd for $C_{15}H_{16}NO_2$ [M − Cl]$^+$ 242.1181, found: 242.1173.

4.3. Biological Assays. *4.3.1. Binding Studies.* Binding experiments were carried out by means of standard radioligand displacement protocols using CHO-h5-HT$_{2A}$ cell membranes (cells were kindly provided by Prof William P. Clarke from the University of San Antonio, Texas, USA) (receptor expression = 200 fmol/mg protein, protein concentration = 4910 μg/mL) against [^{3}H]-ketanserin (47.3 Ci/mL, 1 mCi/mL, PerkinElmer NET791250UC) and HeLa-5-HT$_{2C}$ cell membranes (cell line was generated in-house) (receptor expression = 150 fmol/mg protein, protein concentration = 2041 μg/mL) against [^{3}H]-mesulergine (84.7 Ci/mL, 1 mCi/mL, PerkinElmer NET1148250UC). K_D values obtained for [^{3}H]-ketanserin and [^{3}H]-mesulergine at human 5-HT$_{2A}$ and 5-HT$_{2C}$ receptors were 1.21 and 0.67 nM, respectively. Briefly, membrane suspensions (60 μg/well for 5-HT$_{2A}$, 3 μg/well for 5-HT$_{2C}$) were coincubated (30 min, 37 °C for 5-HT$_{2A}$; 60 min, 37 °C for 5-HT$_{2C}$) with radioligands (1 nM [^{3}H]-ketanserin, 1.25 nM [^{3}H]-mesulergine), test compounds, and standard in assay buffer (50 mM Tris−HCl, pH = 7.4 for 5-HT$_{2A}$, 7.5 for 5-HT$_{2C}$, V_i = 250 μL/well) in polypropylene 96-well microplates. Nonspecific binding was determined in the presence of methysergide 1 μM (5-HT$_{2A}$) or mianserin 10 mM (5-HT$_{2C}$). After the incubation time, 200 μL of the reaction mixture was treated with binding buffer and filtered through either GF/B (5HT$_{2A}$) or GF/C (5-HT$_{2C}$) multiscreen plates (Millipore Iberica, Spain) pretreated with 0.5% PEI. Filters were washed with ice-cold wash buffer (6 × 250 μL of 50 mM Tris−HCl, pH = 7.4 for 5-HT$_{2A}$ or 4 × 250 μL of 50 mM Tris−HCl, pH = 7.5 for 5-HT$_{2C}$), and 35 μL of Universol Scintillation cocktail (PerkinElmer, Alcobendas, Spain) was added to each well. Radioactivity was detected in a microplate beta scintillation counter (Microbeta Trilux, PerkinElmer, Madrid, Spain). Data were adjusted to nonlinear fitting using Prism V2.1 software (Graph Pad Inc., Chicago, USA), and K_i values were calculated using the Cheng−Prusoff equation.

4.3.2. Functional Study. Functional activities were assessed by measuring Ca^{2+} release in CHO-5-HT$_{2A}$ or HeLa-5-HT$_{2C}$ cells. The day before the assay, 2000 (5-HT$_{2A}$) or 10,000 (5-HT$_{2C}$) cells were seeded in 384-well black plates (Greiner 781,091). Using the Fura-2 QBT calcium kit (Molecular Devices), the cells were incubated with 25 μL of dye loading buffer supplemented with 5 mM probenecid (Invitrogen) for 1 h at 37 °C. Changes in fluorescence due to intracellular Ca^{2+} mobilization (λ_{ex} = 340 nm, λ_{ex} = 380 nm; λ_{em} = 540 nm) were measured using a calcium imaging plate reader system (FDSS7000EX, Hamamatsu) every second after the establishment of a baseline. The agonist Ca^{2+} peak in response to agonist addition occurred from 10 to 20 s following stimulation (Figure S1 in the Supporting Information).

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.9b03430.

Time-course effect of six different concentrations in the range 0.1 nM−10 μM serotonin (A and B) and compound **5** (C and D) on the calcium levels as measured by FURA-2 fluorescence ratio at human 5-HT$_{2A}$ (A and C) and human 5-HT$_{2C}$ (B and D) receptors (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Gerald Zapata-Torres − *Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago 8380494, Chile;* orcid.org/0000-0002-8090-1153; Phone: +56 22 9782843; Email: gzapata@uchile.cl.

Bruce K. Cassels − *Department of Chemistry, Faculty of Sciences, University of Chile, Santiago 7800003, Chile;* orcid.org/0000-0002-0082-0661; Email: bcassels@uchile.cl

Authors

Thirumal Yempala − *Department of Chemistry, Faculty of Sciences, University of Chile, Santiago 7800003, Chile*

José Brea − *USEF, CIMUS, Universidad de Santiago de Compostela, Santiago de Compostela 15782, Spain*

María Isabel Loza − *USEF, CIMUS, Universidad de Santiago de Compostela, Santiago de Compostela 15782, Spain*

Douglas J. Matthies − *Faculty of Chemical and Pharmaceutical Sciences, University of Chile, Santiago 8380494, Chile*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.9b03430

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

5-HT, 5-hydroxytryptamine; GPCR, G-protein-coupled receptors

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