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Structure activity relationship exploration of 5-hydroxy-2-(3-phenylpropyl) chromones as a unique 5-HT_{2B} receptor antagonist scaffold

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ABSTRACT

Antagonists for the serotonin receptor 2B (5-HT_{2B}) have clinical applications towards migraine, anxiety, irritable bowl syndrome, and MDMA abuse; however, few selective 5-HT_{2B} antagonists have been identified. Previous studies from these labs identified a natural product, 5-hydroxy-2-(2-phenylethyl)chromone (5-HPEC, **2**) as the first non-nitrogenous ligand for the 5-HT_{2B} receptor. Studies on 5-HPEC optimization led to the identification of 5-hydroxy-2-(3-phenylpropyl)chromone (5-HPPC, **3**), which showed a tenfold improvement in binding affinity over **2** at 5-HT_{2B}. This study aimed to further improve receptor pharmacology of this unique scaffold. Guided by molecular modeling studies modifications at the C-3′ and C-4′ positions of **3** were made to probe their effects on ligand binding affinity and efficacy. Among the derivatives synthesized 5-hydroxy-2-(3-(3-cyanophenyl)propyl) chromone (5-HCPC, **3d**) showed the most promise with a multifold improvement in binding affinity (pK_i = 7.1 ± 0.07) over **3** with retained antagonism.

The neurotransmitter serotonin or 5-hydroxytryptamine (5-HT (**1**), Fig. 1) plays a role in a vast array of biological functions. Studies on **1** have confirmed its clinical relevance in most physiological systems, including gastrointestinal, cardiovascular and nervous systems.[1] The ability of a single molecule like serotonin to participate in mediating such a diverse array of physiological functions can be attributed to its complex receptor pharmacology. Signaling by **1** is mediated by at least 15 different serotonin receptor subtypes which are classified into 7 receptor subfamilies (5-HT_{1-7}). All of them are G protein-coupled receptors with the exception being the ligand gated ion channel 5-HT_3.[2,3] The 5-HT_2 receptor family presently has three identified subtypes 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C}, and has been shown to regulate sleep, appetite, cardiovascular function, muscle contractions, and the hallucinogenic activity of phenylalkylamines.[4] Accordingly, ligands targeting this receptor family carry significant clinical importance. For example, antagonists for the 5-HT_{2A} and 5-HT_{2C} have shown potential as antipsychotics and antidepressants, respectively.[5,6] Antagonist for 5-HT_{2B} have been explored as treatments for migraines, heart diseases, and substance abuse.[7,8] One of the challenges in realizing the clinical potential of the 5-HT_2 receptor family is a lack of selective ligands for one subtype over the others.[8,9] Thus, developing ligands targeting specific subtypes within the 5-HT_2 receptor family may yield useful scaffolds upon which to design novel chemotherapeutics.

Previous work from our labs identified the natural product, 5-hydroxy-2-(2-phenylethyl)chromone (5-HPEC (**2**), Fig. 1), as an interesting scaffold for the development of novel 5-HT_{2B} selective antagonists.[10] In an effort to understand the structural features affecting the receptor pharmacology of this scaffold at 5-HT_{2B} a small library of analogues were designed and synthesized. Homologation of the C-2 alkyl chain to afford 5-hydroxyl-2-(3-phenylpropyl)chromone (5-HPPC, **3**), improved ligand binding affinity at 5-HT_{2B} ten-fold (pK_i = 5.6, (**2**) to 6.6, (**3**)) while also retaining moderate antagonism (pIC_{50} = 5.05 ± 0.05).[10,11] The work presented here discloses our continued efforts to improve 5-HT_{2B} receptor pharmacology for this class of molecules.

To guide further molecular design, *in silico* modeling studies of **3** within the putative binding pocket of the 5-HT_{2B} receptor (PDB ID 4IB4) were undertaken to identify potential molecular interactions that could be exploited to further improve ligand binding affinity. A genetic algorithm GOLD 5.6 was used to explore docking poses of **3** at 5-HT_{2B}.[12] The generated poses were scored using CHEMPLP and GOLD scores. Clustering of the docking poses for **3** showed one high scoring cluster family and the highest ranked pose is shown in Fig. 2. Within the identified binding pocket, the chromone core of **3** occupies a

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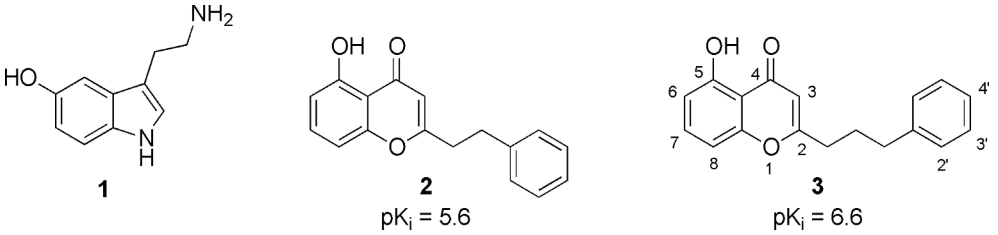


Fig. 1. Molecular structures of 5-HT (**1**), 5-HPEC (**2**), and 5-HPPC (**3**).

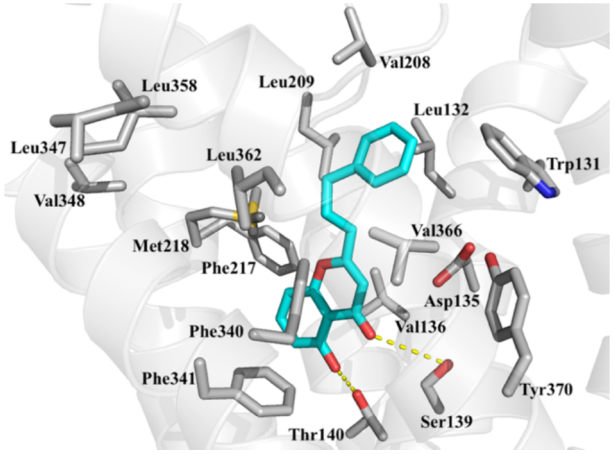


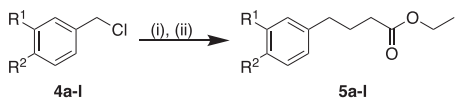
Fig. 2. Plausible ligand binding mode of **3** in the 5-HT_{2B} receptor. 5-HT_{2B} receptor is shown in grey cartoon while the amino acid residues involved in interactions are shown in grey stick representations. **3** is shown in stick representations (cyan). Hydrogen bonding interactions are shown in yellow dashes.

hydrophobic pocket formed by Val136, Phe217, Phe340, Phe341, and Val336. Hydrogen bonding interactions between the C-5 hydroxyl group and the C-4 carbonyl with Thr140 and Ser139, respectively, may help anchor the scaffold within the binding pocket. The pendant phenyl ring of the C-2 alkyl chain seems oriented towards a largely hydrophobic pocket composed of Val366, Trp131, and Leu132. Given such an observation, it was hypothesized that higher hydrophobicity would further improve the binding affinity for this scaffold. Thus, it was determined that initial structural modification on **3** would be focused on the pendant phenyl ring with the substituents being chosen selected from the Craig plot.[13] In accordance with this decision a series of analogues with substituents selected from each quadrant of the Craig plot at either C-3′ or C-4′ of the phenyl ring were implemented.

Chemical syntheses of the newly designed 5-HPPC (**3**) derivatives was accomplished using an established route from our labs.[14] The needed butanoate esters **5a-l** were prepared via Heck reaction between the appropriate benzyl chloride and ethyl acrylate followed by hydrogenation (Scheme 1).[15,16] With the needed esters in hand, Claisen condensation between acetophenone **6** and esters **5a-l** followed by an acid catalyzed cyclization afforded the anticipated 5-HPPC (**3**) derivatives **3a-l** with substituents at C-3′ and C-4′ as outlined in Scheme 2.

Characterization of receptor pharmacology for the newly synthesized derivatives was done in a three-stage process.[17] In the initial stage, each compound was screened for its ability to inhibit radioligand binding at a fixed concentration of 10 μM at each of the 5-HT_2 receptor subtypes. At this concentration significant inhibition was deemed ≥ 50%. Results from this screening are summarized in Table 1. Most compounds maintained inhibitory activity at 5-HT_{2B} comparable to **3**. In general it was observed that the C-3′ derivatives had greater inhibitory activities than the C-4′ derivatives in this assay. From this initial data it was also observed that the more hydrophobic groups substituents such as those in **3a** and **3g** decreased selectivity between 5-$HT_{2B/2C}$ while more polar substituents such as those in **3b** and **3h** provided better selectivity towards 5-HT_{2B}. The C-3′ methoxy analogue **3k** showed greater inhibitory activity (75.3%) at the 5-HT_{2B} than **3** though selectivity seemed to be lost over the 5-HT_{2C} (87.9%). The C-4′ methoxy analogue **3f** showed a similar loss in selectivity indicating larger hydrophobic electron donating groups though potentially tolerated may reduce selectivity. The halogen containing derivatives **3c**, **3e**, **3i** and **3j** with substitutions at either C-3′ or C-4′ positions showed decreased inhibitory activity and loss of selectivity when compared to **3** suggesting that highly electronegative groups may be unfavorable while also supporting previous observations that increased hydrophobicity decreases selectivity. The most promising analogue 5-hydroxy-2-(3-(3-cyanophenyl)propyl)chromone (5-HCPC, **3d**) showed significantly greater inhibitory activity (91.1%) than the parent compound **3** while still maintaining an apparent selectivity for the 5-HT_{2B} over 5-$HT_{2A/2C}$ (7.2%/15.1%). It is interesting that the cyano group in **3d** is electron withdrawing in character like that of the halogens however it is less lipophilic; thus, suggesting that polar hydrophilic groups on C-3′ may be favored for improved binding affinity.

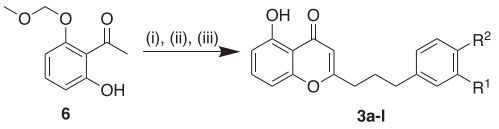
Following the initial screening, compounds showing significant inhibitory activity were moved forward and their binding affinities at the given receptor determined. Binding affinity results are shown in Table 2. Interestingly, the enhanced inhibitory activity of **3k** did not translate into a significant increase in affinity at the 5-HT_{2B} but showed comparable affinity to **3** (pK_i = 6.0). The loss of selectivity inferred from the initial screening was also observed in the secondary assay as **3k** had a pK_i = 6.1 at the 5-HT_{2C}. The most encouraging data from this



4a-l 5a-l

	5a	5b	5c	5d	5e	5f	5g	5h	5i	5j	5k	5l
R^1	CH_3	CF_3	Cl	CN	F	OCH_3	H	H	H	H	H	H
R^2	H	H	H	H	H	H	CH_3	CF_3	Cl	F	OCH_3	OCF_3

Scheme 1. Heck reaction followed by hydrogenation of benzylchlorides yielded esters. Reagents and conditions: (i) ethyl acrylate, $Pd(OAc)_2$, Bu_3N, reflux 15 h,; (ii) H_2, 50 psi, Pd/C, EtOH, rt 2 h. (Yields 23–85%).



	3a	3b	3c	3d	3e	3f	3g	3h	3i	3j	3k	3l
R^1	CH_3	CF_3	Cl	CN	F	OCH_3	H	H	H	H	H	H
R^2	H	H	H	H	H	H	CH_3	CF_3	Cl	F	OCH_3	OCF_3

Scheme 2. Claisen condensation and cyclization of **6** and **5a-l** yielded derivatives **3a-l**. Reagents and conditions: (i) NaH, THF, reflux 1 h; (ii) **5a-l**, THF, reflux 4 h; (iii) HCl, MeOH, reflux 45 min. (Yields 2–67%).

Table 1
Percent inhibition data of compounds **3a-l** obtained from primary radioligand binding assays at 10 μM (n = 4).

Compound	% Inhibition			Compound	% Inhibition		
	5-HT_{2B}	5-HT_{2A}	5-HT_{2C}		5-HT_{2B}	5-HT_{2A}	5-HT_{2C}
2	62.6	14.2	4.8	**3**	70.4	5.4	7.9
3a	57.7	14.4	59.4	**3g**	50	17.2	51.2
3b	65.8	8.7	43.5	**3h**	50	19.5	36
3c	61.7	13.9	44.8	**3i**	50.4	9.3	35.8
3d (5-HCPC)	**91.1**	7.2	15.5	**3j**	49.2	14.2	33.9
3e	52.7	8.4	44.4	**3k**	**75.3**	−1.6	87.9
3f	69.3	9.8	67.4	**3l**	35.3	−5	32.6

Table 2
pK_i values of compounds showing ≥ 50% inhibition at the 5-HT_{2B} in the primary assays.

Compound	pK_i at 5-HT_{2B} (pK_i at 5-HT_{2C})
2	5.6
3	6.6
3a	5.7 (5.5)
3b	6.0
3c	5.7
3d (5-HCPC)	**7.1**
3e	5.6
3f	5.9 (5.7)
3g	5.8 (5.5)
3h	5.6
3i	5.6
3k	6.0 (6.1)

assay was the significant improvement in inhibitory activity observed in the primary assay for **3d** correlated to a 4-fold improvement in binding affinity (pK_i = 7.1 ± 0.07) over **3**.

With 5-HCPC showing improved affinity at 5-HT_{2B}, its functional activity at 5-HT_{2B} was then determined. In the calcium mobilization agonist assay, **3d** did not display any significant agonist activity. The pEC_{50} for **3d** was determined to be 6.3 ± 0.11 compared to the endogenous ligand 5-HT with a pEC_{50} of 10.15 ± 0.05, which is a 40-fold difference in the EC_{50} values. Additionally, the maximal response for **3d** was only 12% of the maximal response at a concentration of 10 μM compared to the maximal response seen by the endogenous agonist **1** at the significantly lower concentration of 3.0 nM. These data taken together suggest that **3d** may not act as an agonist at 5-HT_{2B}. In the antagonist assay an EC_{50} dose of 5-HT (1.6 nM) was challenged with various concentrations of **3d**. It was determined that the pIC_{50} for **3d** is 5.2 ± 0.31 which was a moderate improvement over the original lead (pIC_{50} = 5.05 ± 0.05).

To help rationalize the improved binding affinity of **3d** at 5-HT_{2B}, molecular docking studies were conducted using the same approach as for **3**.[12] **3d** showed very similar binding modes to that of **3**. Its chromone ring occupied the same hydrophobic pocket formed by Val136,

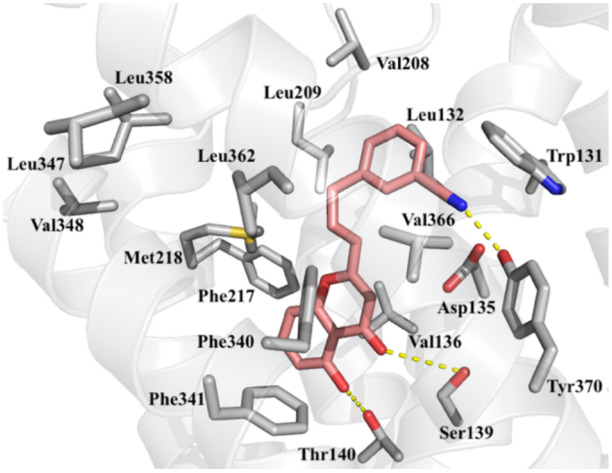


Fig. 3. Plausible ligand binding mode of **3d** in the 5-HT_{2B} receptor. 5-HT_{2B} receptor is shown in grey cartoon while the amino acid residues involved in interactions are shown in grey stick representations. **3d** is shown in stick representations (pink) Hydrogen bonding interactions are shown in yellow dashes.

Val136, Phe217, Phe340, Phe341, and Val336 with the C-5 hydroxyl group showing potential hydrogen bonding interactions with Thr140 and the C-4 carbonyl showing potential hydrogen bonding interactions with Ser139 (Fig. 3). The C-2 alkyl chain of **3d** appeared to be directed towards the cytoplasmic surface of the receptor. The phenyl ring substituents of **3d** seemed positioned within the hydrophobic pocket formed between extracellular loop 2 and helix 3 consisting of Trp131, Leu132, Val208, and Leu209 residues. It seemed that the C-3′ cyano group in **3d** may form hydrogen bonding interaction with the hydroxy group of Tyr370 on TM7. This additional hydrogen bonding interaction could be perceived as a favorable interaction and helped provide an explanation for the increased affinity of **3d** over **3**.

In summary, this study aimed to improve the *in vitro* pharmacology of the 5-HPPC (**3**) scaffold with a focus on the 5-HT_{2B} receptor. A series

of derivatives were synthesized via an efficient route and their receptor pharmacology examined. While several compounds showed comparable results to the original lead compound, one new compound **3d** showed the most promise with a 4-fold increase in binding affinity at the 5-HT_{2B} compared to **3** while retaining antagonist functional activity. Ligand docking studies suggested this improvement in binding affinity may be due to favorable hydrogen bonding interactions of the C-3′ cyano group with the hydroxy group of Tyr370 of the 5-HT_{2B}. Further optimization of this new lead is underway and will be reported in due course.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127511.

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