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5-Hydroxy-2-(2-phenylethyl)chromone (5-HPEC): A novel non-nitrogenous ligand for 5-HT_{2B} receptor

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ABSTRACT

Chromones are a class of natural products found in almost every known terrestrial plant with over 4000 naturally occurring derivatives having been isolated and structurally elucidated. Recently, 5-hydroxy-2-(2-phenylethyl)chromone (5-HPEC), isolated from *Imperata cylindrical*, showed neuroprotective activity against glutamate induced excitotoxicity in primary cultures of rat cortical cells. In comparison to other naturally occurring neuroprotective chromones, 5-HPEC contains fewer hydroxyl groups. Here we report our most recent characterization on this interesting natural product against a number of CNS receptors for the purpose to identify the potential molecular targets that may be related to its biological activity. Based on our studies, including radiobinding assays, calcium flux functional assays and molecular modeling studies, 5-HPEC may represent a type of novel nonnitrogenous ligands to the 5-HT2B receptor.

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Neurodegenerative diseases affecting the central nervous system (CNS) are widely spread among the population.[1] Consequently, tremendous research effort has been directed towards the development and application of small molecules to study the proteins involved in these CNS disorders.[2] The role of natural products in these efforts however has not been very significant.[3] Chromones (**1**) are a class of natural products found in almost every known terrestrial plant with over 4000 naturally occurring derivatives having been isolated and structurally elucidated.[4] Perhaps the most widely studied chromones are the flavonoids like Kaempferol (**2**). Flavonoids bear a phenyl ring at the C2 position and are typically polyhydroxylated. The medicinal value of these chromones has been known for centuries.[5] They have demonstrated various biological activity such as antioxidants, antivirals, antifungals, antimicrobials, anti-inflammatories, neurotrophics and neuroprotectives.[6–8] Such a broad range of activity makes chromone type natural products a potential treasure trove for therapeutics. Thus far, most of the research on the therapeutic value of chromones as related to CNS disorders has been correlated to their antioxidant properties.[9] Until recently, little attention has been paid to the ability of chromones to serve as small molecule modulators of enzymes and receptors within the CNS.[10–12]

Lately, 2-(2-phenylethyl)chromones (**3**), a relatively small and under-explored class of chromones have shown promise as potential tools to study CNS related disorders.[13] Unlike the flavonoids, these chromones possess a phenylethyl substituent at C2 and to date less than 100 congeners of 2-(2-phenylethyl)chromone have been isolated and characterized.[14] In 2006 Yoon et al. isolated 5-hydroxy-2-(2-phenylethyl)chromone (5-HPEC, **4**) from *Imperata cylindrical.* They showed that this natural product had neuroprotective activity against glutamate induced excitotoxicity in primary cultures of rat cortical cells.[15] The neurotoxic effects of glutamate have been shown to involve not only glutamate receptors but also non-glutamate receptors, ion channels, and transporters.[16,17] Thus, elucidation of the potential molecular target(s) of 5-HPEC would be necessary in order to further study the role of this type of natural products in their CNS neuroprotection mechanism.

In comparison to other naturally occurring neuroprotective chromones, for example Kaempferol (**2**), 5-HPEC contains fewer hydroxyl groups.[18] Therefore, we hypothesize that the neuroprotective effects of 5-HPEC may be not only due to its antioxidant potential but very possibly relate to its ability recognizing certain receptors and/or ion channels in the CNS. We recently developed an efficient method to synthesize 5-HPEC.[19] Here we report our further characterization on this interesting natural product against a number of CNS receptors in order to identify the potential molecular targets that may be related to its biological activity (Fig. 1).

The initial screening measured the inhibition by 5-HPEC (at 10 μM) to radioligand binding on a series of selected receptors and ion channels. Inhibition of 50% or greater at this concentration was deemed meaningful. As shown in Figure 2, 5-HPEC only

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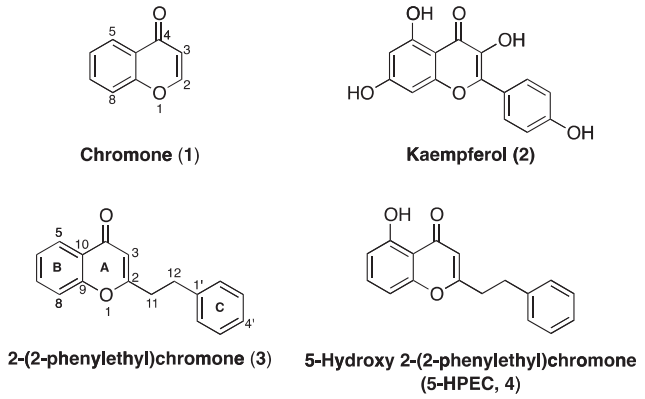


Figure 1. Several natural products bearing the chromone core.

demonstrated notable inhibitory response at several serotonin (5-HT) receptors, namely 5-HT_{1E}, 5-HT_{2B}, and 5-HT_3, with inhibitory activities of 55%, 62%, and 61%, respectively. Interestingly, 5-HPEC showed very low inhibitory effects on other receptors from the 5-HT_2 subfamily, for example 5-HT_{2A} (14.2%) or 5-HT_{2C} (4.8%). This suggested that 5-HPEC may act as a selective ligand for 5-HT_{2B} over other receptors in this subfamily.

Following this initial investigation, the binding affinity of 5-HPEC at these identified receptors was determined (Table 1). The affinity of 5-HPEC at the 5-HT_{1e} receptor seemed to be insignificant. On the other hand, 5-HPEC showed almost equal affinity on both 5-HT_3 and 5-HT_{2B} receptors with pK_i values of 5.60 and 5.61, respectively. These results were very encouraging since serotonergic pathways have been implicated to play an important role in CNS neurorprotection.[20–22] More specifically, both 5-HT_{2B} and 5-HT_3 antagonists have demonstrated certain neuroprotective properties.[23,24]

Table 1
Determined pK_i values for 5-HPEC at 5-HT_{1E}, 5-HT_{2B}, and 5-HT_3

Receptor	pK_i[a]
5-HT_{1E}	<5
5-HT_{2B}	5.61 ± 0.1
5-HT_3	5.60 ± 0.08

[a] pK_i were determined by competitive inhibition of [^{3}H]5-HT, [^{3}H]LSD, [^{3}H]GR65630 at 5-HT_{1E}, 5-HT_{2B}, and 5-HT_3, respectively. Concentration of the radioligand was equal to the K_d of the radioligand which was determined by finding the mean for 3 previously conducted saturation binding assays.

Next the functional activity of 5-HPEC at 5-HT_{2B} was studied. Several reasons directed our research focus toward the 5-HT_{2B} receptor at this stage: (1) the selectivity shown by 5-HEPC at 5-HT_{2B} over 5-HT_{2A} and 5-HT_{2C}; (2) the recently available crystal structure of the 5-HT_{2B} receptor as an advantage to further facilitate our research; (3) the accessibility of functional activity screening methods for the 5-HT_{2B} receptor; and (4) some toxicity concern, that is, should 5-HPEC show any significant agonist activity at the 5-HT_{2B} receptor, enthusiasm for further investigation would be diminished as 5-HT_{2B} agonists are known to play a critical role in valvular heart disease.[25] A preliminary calcium mobilization assay in Flp-In HEK cells using 5-HPEC at 10 μM showed minimal agonist activity (0.6 ± 0.2% of the maximal response) when compared to 5-HT. Conversely, when challenged with an EC_{50} dose (1.6 nM) of 5-HT, 5-HPEC demonstrated modest antagonists activity (6.9 ± 1.9% inhibition). This suggested that 5-HPEC did behave as an antagonist, not an agonist to the 5-HT_{2B} receptor. Following these, a concentration response curve in the presence of an EC_{50} concentration of 5-HT gave a pIC_{50} value of 5.05 ± 0.05 for 5-HPEC at the 5-HT_{2B} receptor.

Figure 3 showed 5-HT (**5**) and three other well-known 5-HT_{2B} antagonists. It has long been assumed that ligands targeting the 5-HT receptors and transporters would contain an amino moiety

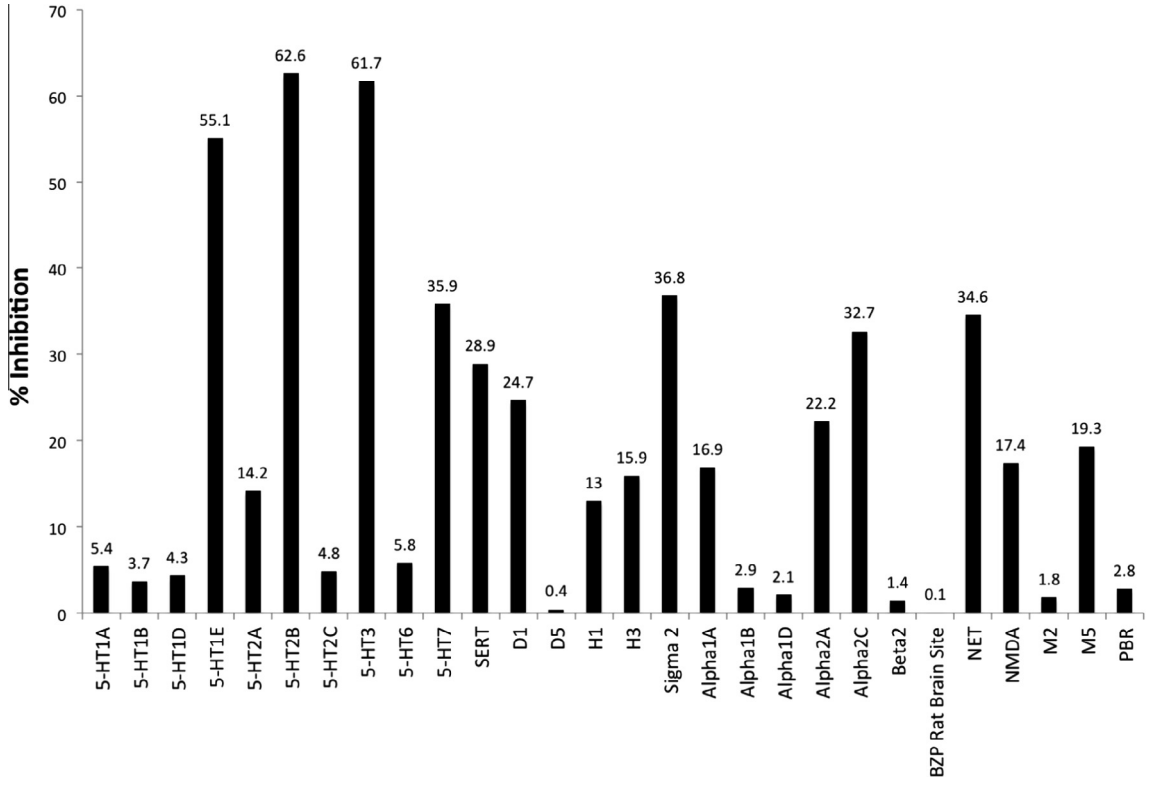


Figure 2. Representative data for the inhibitory responses produced by 5-HPEC at various CNS receptors.

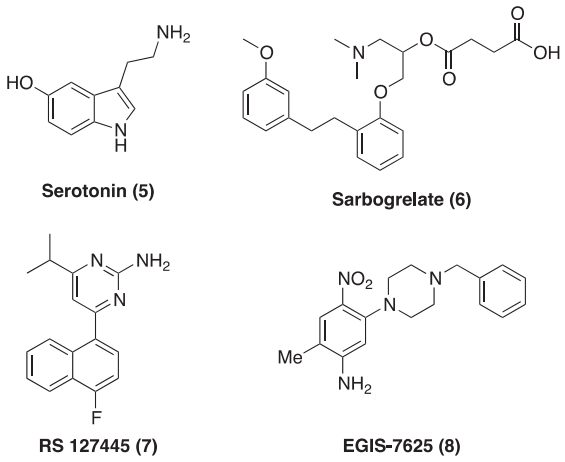


Figure 3. The chemical structures of serotonin and selected 5-HT_{2B} antagonists.

in order to facilitate ligand binding.[26] However, this idea has been challenged recently and a few non-nitrogenous compounds have demonstrated high affinity for the 5-HT transporter.[27] To our knowledge, no such ligand has ever been reported for the 5-HT_{2B} receptor, and in this respect 5-HPEC may represent a structurally unique non-nitrogenous 5-HT_{2B} ligand. To ascertain the putative binding mode of 5-HPEC on the 5-HT_{2B} receptor, an automated docking experiment was performed using the recently available agonist bound X-ray crystal structure of 5-HT_{2B}.[28] A generic algorithm docking program GOLDv51 was used to explore docking poses for both 5-HT and 5-HPEC.[29] The generated binding modes were then re-scored using Hydropathic INTeraction (HINT) that calculates free energy associated with non-bonded interactions based on a natural force field generated by employing experimentally determined partition co-efficients (Table 2).[30] As shown in Figure 4 the highest scored 5-HT binding mode in the receptor indicated that the indole ring of 5-HT located in a hydrophobic pocket between helices 3 and 6. The indole nitrogen atom and the hydroxyl group seemed to be involved in plausible hydrogen bonding interactions with Thr140 and Ser222, respectively. The terminal amino group was shown to interact with Asp135 and Ser139. The above binding mode for 5-HT was in general agreement with previously reported site-directed mutagenesis studies.[31]

In contrast, the best scored docking solution for 5-HPEC showed that its chromone core recognized a similar binding pocket compared to that of 5-HT, that is, between helices 3 and 6 Asp135

Table 2
The optimal docking scores of 5-HT (5) and 5-HPEC (4) in the 5-HT_{2B} receptor crystal structure

Compound	HINT	ChemPLP
5-HT	2669	59.44
5-HPEC	537	50.75

and Ser139 were involved in a potential hydrogen bonding network with the chromone hydroxyl group. Meanwhile, 5-HEPC also seemed to take advantage of a hydrophobic cavity around helix 7 and extracellular loop 2 lined with Leu362 and Leu209 which may interact with the phenylethyl substituent of 5-HEPC. A qualitative comparison of the suggested binding mode of 5-HPEC indicated somehow less hydrogen bonding potential than 5-HT, which was reflected in its lower HINT score. This may explain the lower affinity of 5-HPEC (pK_i 5.60) compared to 5-HT (pK_i 7.87).[32] To be noticed, the binding mode comparison between the endogenous ligand 5-HT (an agonist) and 5-HPEC (an antagonist) in an agonist bound crystal structure may not be ideal; however, recent reports demonstrated that only relatively subtle structural changes within the ligand binding pocket were observed between agonist and antagonist bound protein.[33] Therefore we believe that such an approximation would be tolerable in order to serve the purpose of preliminarily predicting the putative binding mode of 5-HPEC in the 5-HT_{2B} receptor.

To gain some insight into what structural modifications might be tolerated to maintain the binding affinity of 5-HPEC to the receptor, six analogues of 5-HPEC were synthesized (Fig. 5) and their inhibitory activity (at 10 μM) was assessed preliminarily at each 5-HT_2 receptor (Table 3). The design of these analogues was focused the effects of the alkyl chain length and the aromatic 'C' ring on the affinity. Interestingly none of the modifications gave rise to any significant affinity change at the 5-HT_{2A} or 5-HT_{2C} receptors. With respect to 5-HT_{2B} these data suggested that the nature of the alkyl chain may be important. For example, replacing C12 with an oxygen atom (**10**) led to a decrease in activity, which meant that a hydrocarbon chain would be preferred. Additionally, the alkyl chain length seemed to be critical because extension of the alkyl chain by only one methylene group (**12**) led to an increase in activity while any other changes in chain length were detrimental. Finally, exchanging the phenyl ring with a thiophene (**13**) showed improved activity over the parent compound.

In summary, the goal of this study was to identify potential target protein(s) relevant to the biological activity of 5-hydroxy-2-(2-phenylethyl)chromone (5-HPEC). Accordingly 5-HPEC was screened

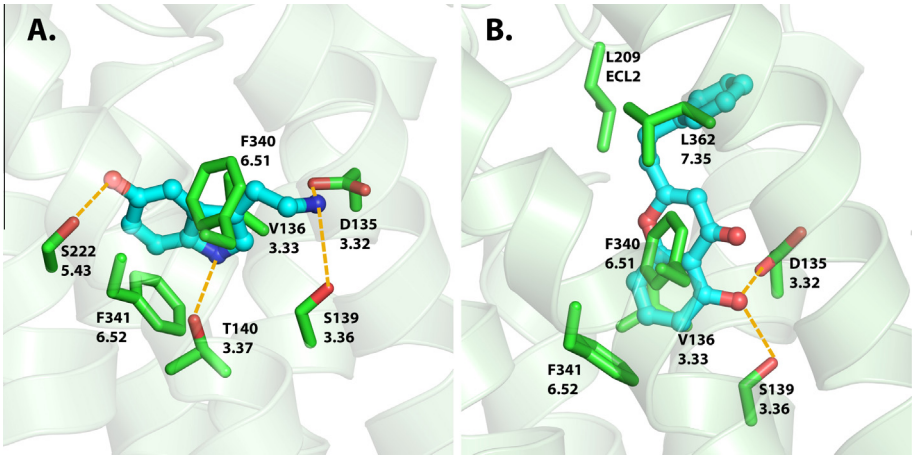


Figure 4. (a) Docking of serotonin (5-HT) in the 5-HT_{2B} receptor. (b) Putative binding mode of 5-HPEC (**4**) in the 5-HT_{2B} receptor. Small molecules were shown in balls and sticks (cyan), residues in sticks (with carbons colored as green). Numbers 'x.yy' referred to Ballesteros–Weinstein numbering. Possible hydrogen bonds were shown with yellow dashed lines.

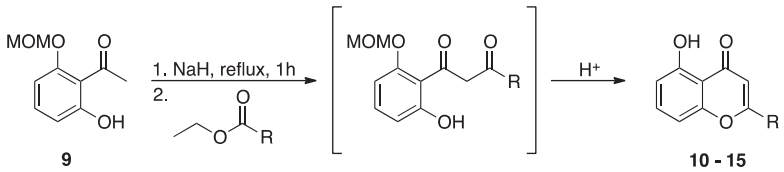
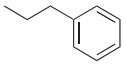
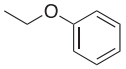
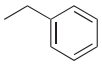
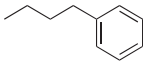
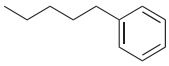
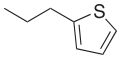


Figure 5. Chemical synthesis route of 5-HPEC analogues.

Table 3
Inhibitory activity of 5-HPEC analogues at the 5-HT_2 family of receptors

Compound	R	% Inhibition		
		5-HT_{2B}	5-HT_{2A}	5-HT_{2C}
4		62.6	14.2	4.8
10		50.0	0.2	7.8
11		30.6	1.4	24.8
12		70.4	5.4	7.9
13		45.2	5.7	14.7
14		31.5	−4.5	23.5
15		74.8	1.4	19.6

for its activity at a number of CNS receptors, transporters, and ion channels. 5-HPEC showed inhibitory activity at 5-HT receptors, namely 5-HT_{1E}, 5-HT_{2B}, and 5-HT_3. 5-HPEC also showed selectivity for 5-HT_{2B} over others in the 5-HT_2 subfamily. In addition 5-HPEC acted as an antagonist at 5-HT_{2B} receptor in a calcium mobilization assay. Furthermore, docking studies utilizing the crystal structure of 5-HT_{2B} revealed a potential binding pocket for this novel ligand. Moreover, six analogs of 5-HPEC were synthesized and suggested that the composition and length of the alky chain may be important and that the aromatic 'C ring can be substituted for the more hydrophobic thiophene. Taken together these suggested that 5-HEPC and it s analogues may act as a novel non-nitrogenous ligands for the 5-HT_{2B} receptor. Further more intensive investigation on the structure–activity relationship of 5-HEPC is underway and may lead to novel and potent 5-HT_{2B} antagonists to help understand the role of this receptor in CNS related disorders.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.029.

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29. *Molecular modeling procedure:* the molecular structures of 5-HT and 5-HPEC were sketched, and their Gasteiger–Hückel charges were assigned before energy minimization (10,000 iterations) with the TAFF within SYBYL-X2.0. The generic algorithm docking program GOLD 5.1 was used to perform the docking studies on X-ray crystal structure of agonist bound 5-HT2B receptor (PDB ID 4IB4) with standard default settings. The binding site was defined to include all atoms within 10 Å of the γ-carbon atom of Asp135. Based on the fitness scores and the binding orientation of each ligand within the binding cavity, the best GOLD-docked solution was selected and merged into the receptor. The combined receptor–ligand structures were then energy-minimized to optimize the interactions between ligand and receptor by removing structural clashes and minimizing strain energy. These optimized models were then subjected to hydropathic analysis with the HINT program. Pictures depicting docking poses were generated using PyMOL Molecular Graphics System, V1.5.0.4.
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