6-Chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]-indoline (SB-242084): The First Selective and Brain Penetrant 5-HT$_{2C}$ Receptor Antagonist

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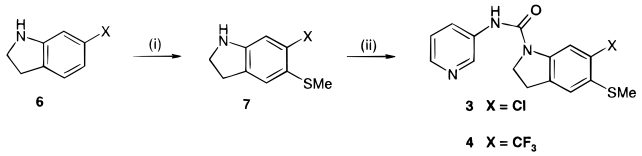
The 5-HT$_2$ receptor family currently consists of 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$ subtypes which have been grouped together on the basis of primary structure, secondary messenger system, and operational profile.[1] Sequence analysis indicates approximately 80% amino acid identity in the predicted seven transmembrane domains, and it is therefore not surprising that many compounds once thought to be selective for the 5-HT$_{2A}$ (classical 5-HT$_2$) receptor also bind with high affinity to the 5-HT$_{2B}$ and 5-HT$_{2C}$ sites. Consequently there remains a need to identify selective ligands to more fully elucidate the functional role of the different subtypes.

Our interest in the 5-HT$_{2C}$ receptor stems principally from the finding that the moderately selective 5-HT$_{2C/2B}$ agonist (*m*-chlorophenyl)piperazine (mCPP) causes behavioral indications of anxiety both in animal models and in humans, implying that selective 5-HT$_{2C/2B}$ antagonists might be useful anxioloytic agents without the side effect burden of current therapies.[2] We have recently reported the synthesis and biological activity of the pyridylurea **1** (SB-206553) which is a 5-HT$_{2C/2B}$ receptor antagonist with 160-fold selectivity over the closely related 5-HT$_{2A}$ site.[3] This compound blocked the hypolocomotion in rats produced by mCPP, thus demonstrating oral activity in a centrally mediated model of 5-HT$_{2C}$ function.[4] It also exhibited significant anxiolytic activity in several different animal models of anxiety, providing strong support for our original hypothesis.[5]

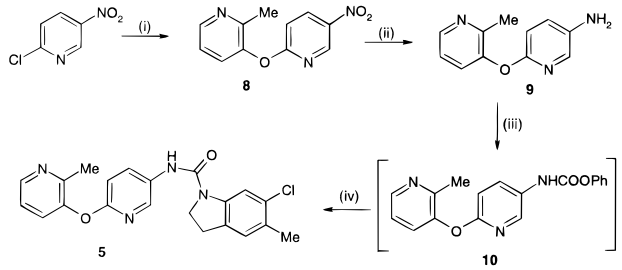
Unfortunately, the 1-methylindole moiety of **1** was subject to metabolic demethylation to a nonselective compound, and so **1** was not progressed.[6] We recently reported the investigation of analogues containing classical indole isosteres: these compounds maintained affinity but not selectivity.[7] In further attempts to circumvent the metabolic liability of **1**, we have now investigated the replacement of the pyrroloindole of **1** with simple substituted indolines as in **2**−**4**. Although these compounds were found to be potent and selective 5-HT$_{2C/2B}$ receptor antagonists,[8] they were also discovered to be very potent inhibitors of a number of human cytochrome P450 enzymes,[9] which precluded their development as potential anxiolytic agents. Structural modification of the 3-amidopyridyl ring of **4** revealed that the unhindered pyridine nitrogen was responsible for the inhibitory activity and led to bipyridyl ethers such as **5** (SB-242084) with increased 5-HT$_{2C}$ affinity, while successfully abolishing the P450 inhibitory activity and affording at least 100-fold selectivity over both the 5-HT$_{2A}$ and 5-HT$_{2B}$ receptor subtypes.[10]

Scheme 1. Synthesis of Substituted 1-(3-Pyridylcarbamoyl)indolines **3** and **4**[a]



[a] (i) (a) KSCN/Br$_2$, MeOH, 0 °C to rt, 4 h; (b) aqueous KOH, 40 °C, 0.5 h; (c) MeI, 10 °C to rt, 1.5 h (61% overall yield); (ii) nicotinic acid azide, toluene, reflux, 0.5 h (82%).

Scheme 2. Synthesis of 6-Chloro-5-methyl-1-[[2-[(2-methyl-3-pyridyl)oxy]-5-pyridyl]carbamoyl]indoline **5** (SB-242084)[a]



[a] (i) 3-Hydroxy-2-methylpyridine/NaH, DMF, 0 °C to rt, 18 h (95%); (ii) SnCl$_2$, EtOH/cHCl, 50 °C, 1 h (86%); (iii) PhOCOCl/NEt$_3$, DCM, −20 °C, 1 h; (iv) 6-chloro-5-methylindoline, NEt$_3$/DMF, 100 °C, 1 h (79%).

The synthetic methods used in the preparation of compounds **3**−**5** are shown in Schemes 1 and 2. The 5-(methylthio)indolines **7** were most conveniently prepared by a one-pot thiocyanation,[11] hydrolysis, and alkylation procedure from the 6-monosubstituted indolines **6**.[10] The disubstituted indolines **7** were converted to the final ureas **3** and **4** by treating them with 3-pyridyl isocyanate, which was generated *in situ* from the azide. Compound **2** was similarly prepared from 6-chloro-5-methylindoline.[8] The bipyridyl ether **5** was prepared by coupling 6-chloro-5-methylindoline with the phenyl carbamate **10**, itself prepared by treating the anion of 3-hydroxy-2-methylpyridine with 2-chloro-5-nitropyridine followed by reduction of the nitrobipyridyl ether **8** and treatment of the resultant aniline **9** with phenyl chloroformate.[10]

The biological data for compounds **1**−**5** are shown in Table 1. In binding assays 6-chloro-5-methyl-1-(3-pyridylcarbamoyl)indoline **2** had affinity comparable to that of **1** at the 5-HT$_{2C}$ receptor but demonstrated only 30-fold selectivity over the 5-HT$_{2A}$ receptor. Encouragingly, **2** exhibited potent oral activity with an ID$_{50}$ of 0.6 mg/kg, which compares very favorably with that of **1** (ID$_{50}$ 5.5 mg/kg po). The 6-chloro-5-(methylthio)-indoline **3** and the 6-(trifluoromethyl)-5-(methylthio)-indoline **4** also had improved 5-HT$_{2C}$ affinities relative to **1** but comparable or improved selectivity over the 5-HT$_{2A}$ receptor. In the rat mCPP-induced hypolocomotion model, **3** showed modest activity whereas **4** was almost as potent (ID$_{50}$ 1.5 mg/kg po) as the 6-chloro-5-

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Table 1. 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} Receptor Binding Affinities[a] and *in Vivo* Activity[e] of Compounds **1**–**5**

compound	5-HT_{2A}[b] (pK_i)	5-HT_{2B}[c] (pK_i)	5-HT_{2C}[d] (pK_i)	selectivity 5-$HT_{2C/2A}$	selectivity 5-$HT_{2C/2B}$	ID_{50}[e] (mg/kg po)
1 (SB-206553)	5.7	7.6	7.9	160	2	5.5 ± 1.7
2	6.8	–	8.2	30		0.6 ± 0.1
3	5.6	–	8.2	420		10 ± 0.1
4	6.4	7.9	8.6	160	5	1.5 ± 0.5
5 (SB-242084)	6.8	7.0	9.0	160	100	2.0 ± 0.5

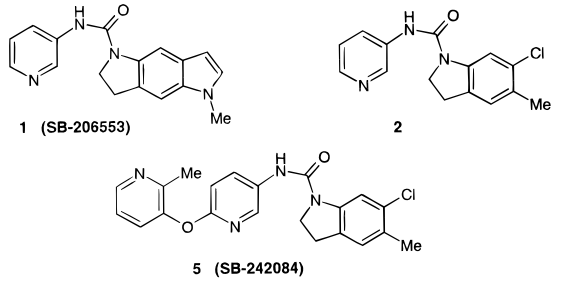
[a] All values represent the mean of at least two determinations, with each determination lying within 0.2 log unit of the mean. [b] Binding affinity (human cloned receptors; HEK 293 cells; [^{3}H]ketanserin). [c] Binding affinity (human cloned receptors; HEK 293 cells; [^{3}H]-5-HT). [d] Binding affinity (human cloned receptors; HEK 293 cells; [^{3}H]mesulergine). [e] Mean dose (± confidence limit) of compound required to reverse mCPP (**7** mg/kg ip administered 30 min pretest) induced hypolocomotion by 50%.

Table 2. Human Cytochrome P450 Inhibitory Potential[a] of Compounds **4** and **5**

compound	IC_{50} 1A2	2C9	2C19	2D6	3A
1 (SB-206553)	0.05	>100	>100	30	>100
4	0.013	>100	>100	0.11	>100
5 (SB-242084)	>100	>100	>100	>100	>100

[a] The cytochrome P450 inhibitory potential was determined using isoform selective assays and heterologously expressed human CYP1A2, CYP2C9, CYP2C19, CYP2D6, and CYP3A4. IC_{50}'s were determined at the substrate K_m as has been previously described[16] [CYP1A2, caffeine N3-demethylation (500 μM); CYP2C9, tobutamide methylhydroxylation (100 μM); CYP2C19, *S*-mephenytoin 4-hydroxylation (100 μM); CYP2D6, bufuralol 1′-hydroxylation (10 μM); CPY3A4, total cyclosporin oxidation (1 μM)]. These values are the mean of duplicate determinations which did not vary by more than 10%.

methyl analogue **2**. The bipyridyl ether **5** demonstrated the most impressive binding profile with nanomolar affinity at the 5-HT_{2C} receptor and 160-fold selectivity over the 5-HT_{2A} receptor. Whereas the 3-pyridylureas **1** and **4** were almost equipotent at the 5-HT_{2C} and 5-HT_{2B} receptors, showing less than 10-fold selectivity, **5** demonstrated 100-fold selectivity. Compound **5** also demonstrated over 100-fold selectivity over all 11 other 5-HT, dopamine, and adrenergic receptors so far tested.[4] In the 5-HT-stimulated phosphinositol (PI) hydrolysis model of 5-HT_{2C} receptor function, using human cloned receptors expressed in HEK 293 cells, **5** was found to be a competitive antagonist with a pK_B of 9.3, which is consistent with the binding affinity. Crucially, whereas **4** was found to be a potent inhibitor of a number of key human cytochrome P450 enzymes *in vitro*, particularly the 1A2 and 2D6 isoforms, **5** showed very little inhibitory activity (Table 2). *In vivo* compound **5** demonstrated excellent activity in the rat hypolocomotion model (ID_{50} 2.0 mg/kg po) and was also found to have significant anxiolytic activity in two different models of anxiety, namely the Geller Seifter conflict test and the



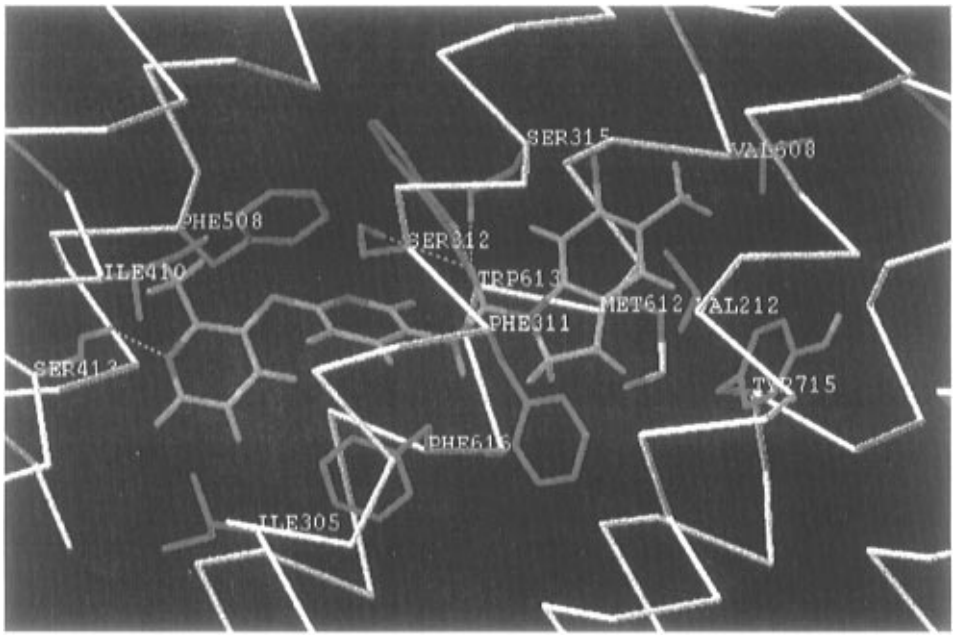


Figure 1. Structure **5** docked into the model of the 5-HT_{2C} receptor.

social interaction test in the rat at doses (0.1−1 mg/kg ip) similar to those that antagonized mCPP-induced hypolocomotion, with no evidence of sedative effects.[4] Significantly, **5** showed no evidence of either proconvulsant (up to 30 mg/kg po) or hyperphagic properties which are characteristic of mutant mice lacking the 5-HT_{2C} receptor.[12] Thus, **5** is the first reported brain penetrant 5-HT_{2C} receptor antagonist able to discriminate between 5-HT_{2C} and 5-HT_{2B} components of central activity.[13] The detailed *in vivo* properties of **5** are the subject of a separate publication.[4]

Docking of 5 into the 5-HT_{2C} Receptor. The bipyridyl ether **5** was manually docked into a model of the 5-HT_{2C} receptor, constructed on the basis of the structure of bacteriorhodopsin as we have previously described,[7] and the ligand−receptor complex was minimized using the CHARMm program[14] (Figure 1). The proposed binding mode is similar to that previously proposed[6] for **1** with the urea carbonyl oxygen double hydrogen bonding to the hydroxyl side chains of Ser312 and Ser315.[15] In addition, the indole NH of Trp613 is also in a position to hydrogen bond to the urea carbonyl. The central pyridyl ring occupies a lipophilic pocket surrounded by Phe508 and Phe616, while the terminal pyridyl ring occupies another lipophilic pocket formed by Ile305 and Ile410. In this mode it is possible that the terminal pyridyl nitrogen is hydrogen bonding to Ser413. This ligand receptor interaction cannot be invoked in the case of the 5-HT_{2B} receptor as the corresponding residue in the protein sequence is alanine, and this difference may contribute to the observed selectivity. The substituted indoline is placed in another pocket, the boundary of which is defined by residues Val212, Val608, Met612, and Tyr715. In the 5-HT_{2B} receptor the corresponding 608 residue is leucine, and in the 5-HT_{2A} receptor both the 212 and 608 residues are leucine. These differences would be expected to lead to binding pockets of reduced size, and it is proposed that these steric differences in the receptors may also account for the observed 5-HT_{2C} selectivity.

In summary, we report the synthesis and biological activity of substituted 1-(3-pyridylcarbamoyl)indolines which illustrates the use of 5,6-disubstitution as a replacement for a fused 5-membered ring in the context of 5-$HT_{2C/2B}$ receptor antagonists. Although compounds such as **3** and **4** were found to be potent and selective 5-$HT_{2C/2B}$ receptor antagonists, they were also very potent inhibitors of a number of human cytochrome P450 enzymes which precluded their development as potential anxiolytic agents. Elaboration of the left hand side pyridyl ring abolished the inhibitory activity, leading to bipyridyl ethers such as **5**, which is the first reported brain penetrant, 5-HT_{2C} receptor antagonist with selectivity over both the 5-HT_{2A} and 5-HT_{2B} receptor subtypes. Furthermore, **5** was found to exert marked anxiolytic-like responses in rat models, confirming that these responses are mediated by 5-HT_{2C} receptor blockade.

Supporting Information Available: Experimental details for the synthesis of **2**−**5** (8 pages). Ordering information is given on any current masthead page.

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