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N-(1-Methyl-5-indolyl)-N'-(3-methyl-5-isothiazolyl)urea: A Novel, High-Affinity 5-HT$_{2B}$ Receptor Antagonist

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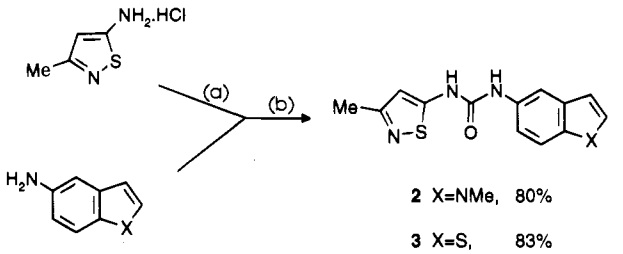
The 5-HT$_2$ family of receptors is comprised of three sub-types, namely 5-HT$_{2A}$, 5-HT$_{2B}$, and 5-HT$_{2C}$. These receptors have been grouped in the same class on the basis of molecular structure, signal transduction characteristics, and pharmacology.[1] 5-HT$_{2B}$ receptors are expressed in rat stomach fundus longitudinal muscle, where they mediate a contractile response to 5-HT,[2,3] and also in rat jugular vein, where they mediate relaxation.[4] The mRNA transcript for 5-HT$_{2B}$ receptors has been reported to occur in human brain, retina, liver, heart, kidney, small intestine, and other organs,[5] and a human 5-HT$_{2B}$ receptor has recently been cloned.[5,6]

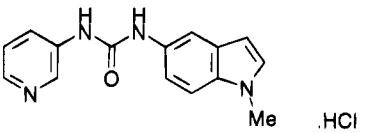
The 5-HT$_{2B}$ receptor is very closely related to the 5-HT$_{2C}$ receptor, with which it shares a high degree of sequence homology[2a,b] and very similar pharmacology.[3,7] In view of this close similarity between 5-HT$_{2B}$ receptors and 5-HT$_{2C}$ receptors, it is possible that some of the biological effects previously ascribed to activation or antagonism of 5-HT$_{2C}$ receptors could in fact be due to actions at 5-HT$_{2B}$ receptors, and, for example, it has recently been suggested that 5-HT$_{2B}$ receptors may be involved in the pathophysiology of migraine.[8] Unfortunately, there is a complete lack of selective agents which could be used to probe the functional role of 5-HT$_{2B}$ receptors. No selective agonists have been reported, and although the antagonists yohimbine and rauwolscine do show some selectivity for 5-HT$_{2B}$ over 5-HT$_{2C}$ receptors,[3] they are not particularly useful as pharmacological tools as both compounds possess significant affinity at certain other 5-HT and non-5-HT receptors.

Scheme 1[a]



[a] Reagents: (a) CDI, Et_3N, CH_2Cl_2, 1 h, 0 °C; (b) DMF, 1 h, 120 °C.

The pyridylurea **1** (SB 200646A) has recently been reported as having mixed antagonist properties at cloned rat 5-HT$_{2C}$ receptors and at 5-HT$_{2B}$ receptors in the rat stomach fundus, but possessing selectivity over rat 5-HT$_{2A}$ and a range of other receptors.[9-11] In order



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to identify the important physicochemical properties required for 5-HT$_{2C/2B}$ affinity in this novel series, we have undertaken a comprehensive investigation of structural variations around **1**. We now report the synthesis of closely related isothiazolylureas, one of which displays marked selectivity for 5-HT$_{2B}$ over 5-HT$_{2C}$ and 5-HT$_{2A}$ receptors.

Compounds **2** and **3** were prepared as shown in Scheme 1. 5-Amino-3-methylisothiazole hydrochloride was treated with 1,1'-carbonyldiimidazole (CDI) in the presence of triethylamine to give an intermediate which, after removal of the solvent, was reacted with either a 5-aminoindole[9] or a 5-aminobenzothiophene[13] in dimethylformamide (DMF) to give the isothiazolylureas.

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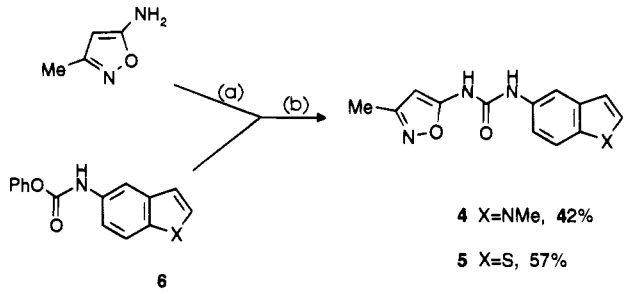
Table 1. 5-HT_{2B}, 5-HT_{2C}, and 5-HT_{2A} Receptor Affinities for Compounds **1**–**5**

compd	mp, °C	pA_2 5-HT_{2B}[a,b]	pK_i 5-HT_{2C}[a,c]	selectivity[e]	pK_i 5-HT_{2A}[a,d]
1	184–185	7.41 ± 0.06 (8)	6.96 ± 0.10 (3)	3	<5.2 (2)
2	188–190	7.95 ± 0.11 (8)	5.82 ± 0.02 (4)	135	<5.2 (4)
3	221–224	7.82 ± 0.15 (4)	6.38 ± 0.12 (4)	27	5.2 ± 0.06 (3)
4	198–201	7.16 ± 0.12 (6)	6.46 ± 0.10 (3)	5	<5.2 (3)
5	>184 dec	7.33 ± 0.08 (3)	7.05 ± 0.11 (6)	2	<5.2 (3)

[a] All values represent means ± SEM (number of determinations). [b] Determined in rat stomach fundus preparation.[3] [c] Binding affinity (human clone expressed in 293 cells; [^{3}H]mesulergine); see ref 14 for assay conditions. [d] Binding affinity (human clone expressed in 293 cells; [^{3}H]ketanserin); see ref 15 for assay conditions. [e] K_i(5-HT_{2C})/A_2(5-HT_{2B}).

Scheme 2[a]



[a] Reagents: (a) 2 NaH, DMF, 20 min, room temperature; (b) DMF, 16 h, room temperature.

For compounds **4** and **5** a different procedure was necessary since the above approach was surprisingly unsuccessful using 5-amino-3-methylisoxazole in place of the isothiazole. The aminoisoxazole was treated first with 2 equiv of sodium hydride in DMF, and the resulting anion was reacted with a phenyl carbamate **6**, prepared from either 5-amino-1-methylindole or 5-aminobenzothiophene and phenyl chloroformate, to give the isoxazolylureas (Scheme 2).

Antagonist affinities for 5-HT_{2B} receptors were determined in rat stomach fundus as described by Baxter *et al.*,[3] and affinities for 5-HT_{2C} and 5-HT_{2A} receptors were determined using cloned human receptors (Table 1). It was found that the isothiazolylurea **2** had significantly reduced affinity for 5-HT_{2C} receptors as compared with the pyridylurea **1**, while retaining good affinity for 5-HT_{2B} receptors, resulting in marked selectivity for 5-HT_{2B} receptors. Replacement of the indole moiety in **2** by benzothiophene as in compound **3** results in an increase in 5-HT_{2C} affinity, with little change in 5-HT_{2B} affinity, and hence reduced selectivity. In contrast, replacement of the isothiazole ring moiety by isoxazole (compounds **4** and **5**) provides an increase in affinity for 5-HT_{2C} receptors but with a concomitant decrease in 5-HT_{2B} affinity, resulting in complete loss of selectivity. In view of the close structural similarity between **2** and **4**, the observed differences in selectivity must be attributable to subtle differences in physicochemical parameters, such as steric or electronic effects. Further studies on structure–activity relationships in this series are ongoing. It is worth noting that all compounds in Table 1 have very low affinity for 5-HT_{2A} receptors. Compound **2** represents the first 5-HT_{2B} antagonist selective within the 5-HT_2 family, and this compound **2** (SB 204741) was further evaluated in a range of other receptor binding assays. As shown in Table 2, it was found that **2** has low affinity for all other neurotransmitter binding sites investigated, and as such represents the first truly selective 5-HT_{2B} antagonist. However, it should be noted that selectivity data presented are based on comparison of a functional assay (5-HT_{2B}) with a receptor binding assay (5-HT_{2C}), and it remains to be seen whether the affinity of the above compounds at cloned human 5-HT_{2B} receptors parallels that seen in the rat stomach fundus.

Table 2. Receptor Binding Profile of **2**[a]

receptor	affinity (pK_i)
5-HT_{1A}	<5.0
5-HT_{1D}	<5.0
5-HT_{1E}	<4.0
5-HT_3	<5.0
5-HT_4	<5.0
dopaminergic D_1	<5.0
dopaminergic D_2	<4.5
dopaminergic D_3	<4.5
histamine H_1	<5.0
adrenergic α_1	<5.5
adrenergic α_2	<6.0
$GABA_A$	<5.0

[a] Tissues and radioligands used in binding assays: 5-HT_{1A} (rat cortex; [^{3}H] 8-OH-DPAT);[16] 5-HT_{1D} (unpublished data); 5-HT_{1E} (cloned human receptors in CHO cells; [^{3}H]-5-HT);[18] 5-HT_3 (rat hippocampus and entorhinal cortex; [^{3}H]granisetron);[19] 5-HT_4 (piglet hippocampus; [^{125}I]-SB 207710);[20] dopaminergic D_1 (cloned human receptors in LTK cells; [^{3}H]SCH 23390);[21] dopaminergic D_2 (cloned human receptors in CHO cells; [^{125}I]iodosulpride);[21] dopaminergic D_3 (cloned human receptors in CHO cells; [^{125}I]iodosulpride);[22] histamine H_1 (guinea pig cerebellum; [^{3}H]mepyramine);[23] adrenergic α_1 (rat cortex; [^{3}H]prazosin);[24] adrenergic α_2 (rabbit cortex; [^{3}H]-RX821002);[25] $GABA_A$ (rat cortex; [^{3}H]muscimol).[26]

In conclusion, we report the synthesis and biochemical profile for a selective 5-HT_{2B} antagonist which represents the first truly selective ligand for this receptor. This selective biological tool should greatly facilitate the evaluation of the, at present ill-defined, functional role of human 5-HT_{2B} receptors both in the periphery and in the central nervous system. The further exploitation of this series of diarylureas as a rich source of selective 5-HT_2 antagonists will be the subject of future publications.

Supplementary Material Available: Experimental procedures, including spectral data, for the preparation of **2** and **4** (1 page). Ordering information is given on any current masthead page.

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