Communications to the Editor

N-(1-Methyl-5-indolyl)-N'-(3-pyridyl)urea Hydrochloride: The First Selective 5-HT$_{1C}$ Receptor Antagonist

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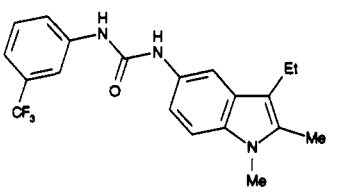
The 5-HT$_{1C}$ receptor was first characterized by Pazos[1] in 1984. It is present in high levels in the choroid plexus epithelial cells with at least 10-fold lower densities in other brain regions of both rat[2] and humans[3,4] and is linked to the phosphatidyl inositol hydrolysis secondary messenger system[5,6] as is the 5-HT$_2$ receptor.[5,7] In addition to sharing the same secondary messenger system, both the 5-HT$_{1C}$ and 5-HT$_2$ receptors have similar gene sequences with 79% homology in the seven transmembrane domains in rat[8] and 80% in humans.[9] This accounts for the very similar pharmacological profile of both receptors. Thus most of the "classical" 5-HT receptor antagonists such as methysergide, metergoline, mianserin, and cyproheptadine have similar affinities for both receptors, while some, such as spiperone, ketanserin, and pirenperone, are 5-HT$_2$ selective.[7] Only two antagonists have been reported to show modest 5-HT$_{1C}$ receptor selectivity compared with the 5-HT$_2$ site: 1-naphthylpiperazine (1-NP) and LY 53857 (10- and 5-fold, respectively).[7] The former compound exhibits considerable affinity for 5-HT$_{1A}$ and 5-HT$_{1B}$ sites[7] as well.

The 5-HT$_{1C}$ receptor also shows considerable sequence homology with the 5-HT receptor present in the rat stomach fundus[10] (RSF), a receptor as yet unidentified in brain tissue.[11] Indeed, the contraction of the RSF has been proposed as being 5-HT$_{1C}$ mediated[12] since many nonspecific 5-HT$_{1C}$/5-HT$_2$ receptor antagonists, but not specific 5-HT$_2$ receptor blockers, are potent antagonists of the site.[13,14] In 1986, Fludzinski[15] reported that the [3-(trifluoromethyl)phenyl]urea 1 had approximately 100-fold greater potency as an antagonist in the RSF than of the 5-HT$_2$-mediated rat jugular preparation.[16] We have recently undertaken the synthesis of certain pyridylureas and tested them for 5-HT$_{1C}$ receptor antagonist properties.[17]



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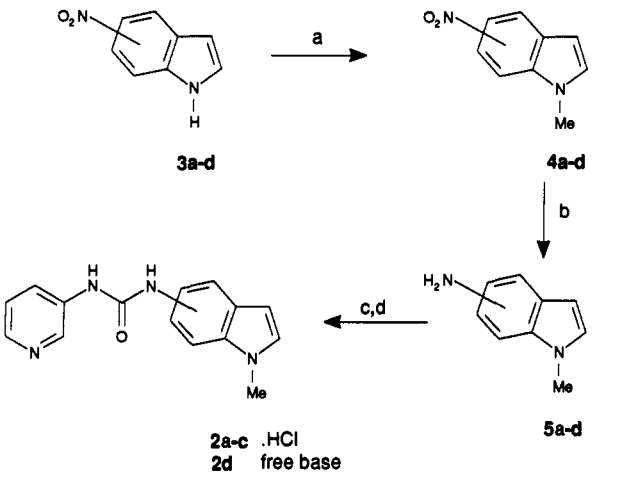
The synthesis of **2a–d** is illustrated in Scheme I. Alkylation of the commercially available nitroindoles **3** with methyl iodide afforded the corresponding *N*-methyl derivatives **4** in high yield, which were then hydrogenated at 60 psi using 10% palladium on charcoal as catalyst. The resultant aminoindoles **5a–d** were smoothly converted to the desired ureas by coupling with 3-pyridyl isocyanate, freshly prepared by thermal rearrangement of 3-pyridinecarbonyl azide in toluene. Isolation of the crude products was achieved simply by filtration of the precipitates, and further purification of **2a–c** was accomplished by hydrochloride salt formation.

A modified procedure was used to prepare the 2- and 4-pyridyl analogues **6** and **7** (Scheme II). Reaction of

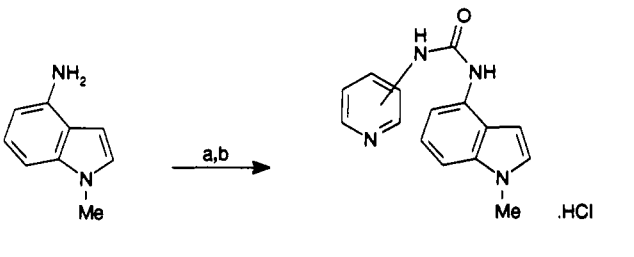
Scheme I[a]



a = 4-indolyl; **b**= 5-indolyl; **c** = 6-indolyl; **d** = 7-indolyl

[a] Reagents: (a) K_2CO_3 (or NaH), MeI, DMF, 20 °C, 24 h (92–100%); (b) H_2, (60 psi), 10% Pd–C, EtOH, (45–97%); (c) 3-pyridyl isocyanate, PhMe, CH_2Cl_2, 20 °C, 17 h (68–95%); (d) HCl, EtOH (salt formation as indicated).

Scheme II[a]



[a] Reagents: (a) $COCl_2$, Et_3N, CH_2Cl_2, 0 °C, 0.5 h; Et_3N, 2- or 4-aminopyridine, 25 °C, 18 h (60–61%); (b) HCl, EtOH.

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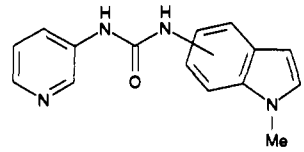
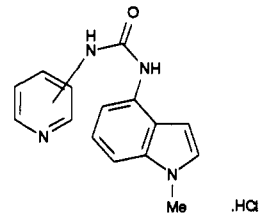
Table I. 5-HT_{1C} and 5-HT_2 Receptor Affinities[a] for Indolyl-3-pyridylureas



compound	indole position	mp, °C	pK_I 5-HT_{1C}[b]	pK_I 5-HT_2[c]	selectivity[e]
2a (HCl salt)	4	238–239	6.54 ± 0.13	<5.3	>17
2b (HCl salt)	5	184–185	6.86 ± 0.07	5.18 ± 0.07	48
2c (HCl salt)	6	215–216	6.47 ± 0.16	<5.2	>19
2d (free base)	7	189–190	<5.3	nd[d]	

[a] All values represent means ± SEM; n = 3 determinations. [b] Binding affinity (rat clone expressed in 293 cells; [^{3}H]mesulergine); see ref 1 for assay conditions. [c] Binding affinity (rat frontal cortex; [^{3}H]ketanserin[18]). [d] Not determined. [e] K_I(5-HT_2)/K_I(5-HT_{1C}).

Table II. 5-HT_{1C} and 5-HT_2 Receptor Affinities[a] for 4-Indolylpyridylureas



compound	pyridine position	mp, °C	pK_I 5-HT_{1C}[b]	pK_I, 5-HT_2[c]	selectivity[d]
2a	3	238–239	6.54 ± 0.13	<5.3	>17
7	4	260 dec	7.18 ± 0.22	5.53 ± 0.15	45
6	2	208–209	6.49 ± 0.06	<5.2	>19

[a] All values represent means ± SEM; n = 3 determinations. [b] Binding affinity (rat clone expressed in 293 cells; [^{3}H]mesulergine); see ref 1 for assay conditions. [c] Binding affinity (rat frontal cortex; [^{3}H]ketanserin[18]). [d] K_I(5-HT_2)/K_I(5-HT_{1C}).

aminoindole **5a** with 1.5 equiv of phosgene at 0 °C, followed by treatment with 2- or 4-aminopyridine, led to the formation of the desired ureas in good yield, which were purified by hydrochloride salt formation. Subsequent investigation has shown that phenyl chloroformate or carbonyldiimidazole function better as phosgene equivalents in the coupling stage.

The in vitro 5-HT_{1C} and 5-HT_2 binding affinities for the compounds **2a–d**, **6**, and **7** are shown in Tables I and II. The effects of varying the position of attachment of the urea moiety on both the pyridine and indole rings have been investigated. As shown in Table I, the 4-, 5-, and 6-indolyl-3-pyridylureas all display reasonable affinity for the 5-HT_{1C} receptor, with the 5-isomer **2b** having the greatest selectivity compared to 5-HT_2. In contrast, the 7-indolyl analogue is significantly less active on 5-HT_{1C} binding. The effect of varying the pyridine substitution pattern was initially investigated in the 4-indolyl series. As shown in Table II, the 2-pyridyl isomer **6** has a very similar profile to the 3-pyridyl isomer **2a**, whereas the 4-pyridyl analogue **7** is somewhat more potent at the 5-HT_{1C} site.

From these results it appears that 5-indolyl/3-pyridyl and 4-indolyl/4-pyridyl substitutions confer the best affinity and selectivity profile in this series of isomeric pyridylindolylureas, and isomer **2b** was selected as a prototype analogue for further in vitro evaluation as detailed below. Energy and conformational studies on the above indolylurea isomers are currently in progress to provide information about the active urea conformation and the required spatial relationship between the two heterocyclic rings for 5-HT_{1C} receptor affinity.

Compound **2b** (SB 200646A) was evaluated in a functional model of 5-HT_{1C} receptor activation, namely 5-HT-stimulated phosphoinositide (PI) hydrolysis.[5,6] This was carried out in slices of piglet choroid plexus,[19] and radiolabeled inositol phosphates were separated using SAX Bond Elute columns.[20] The effect of **2b** at a concentration approximately 100-fold above its binding affinity on the concentration effect curve was determined and the pK_B calculated. Under the conditions used, **2b** produced a parallel shift in the PI response to 5-HT with no loss of maximal response (Figure 1). Indeed, a significant increase in the apparent maximum response to 5-HT was observed, which can be accounted for by the fact that in the presence of **2b** there is an increased retention of radiolabeled inositol 1-phosphate on the SAX columns used for the chromatographic separation. The effect of **2b** on the PI response is consistent with competitive inhibition, and the pK_B value (7.03) is in accord with the compound's affinity for the 5-HT_{1C} receptor.

The selectivity of **2b** for the 5-HT_{1C} receptor was tested by determining its affinity for other receptor sites (Table III). In addition to showing 48-fold selectivity for 5-HT_{1C} over the 5-HT_2 receptor, the compound was inactive at concentrations up to 10 μM at 5-HT_{1A}, 5-HT_{1D}, 5-HT_3, adrenergic α_1, α_{2A}, α_{2B}, and dopamine D_1 receptors. It was also inactive at concentrations up to 30 μM on dopamine D_2 receptors and up to 100 μM on adrenergic β_1 and β_2 receptors.

For reasons stated above, the action of **2b** on the rat stomach fundus was also investigated. Mucosa denuded strips (1.5–20 mm) of longitudinal muscle were obtained from the stomach fundus of male Sprague–Dawley rats (200–350 g) and suspended under an initial resting tension of 1 g in oxygenated (95% O_2/5% CO_2) Tyrode's solution at 37 °C. Experiments were conducted in the presence of indomethacin (3 μM) and after tissues had been exposed to pargyline (100 μM for 15 min). Two concentration effect curves to 5-HT were constructed in each preparation, the

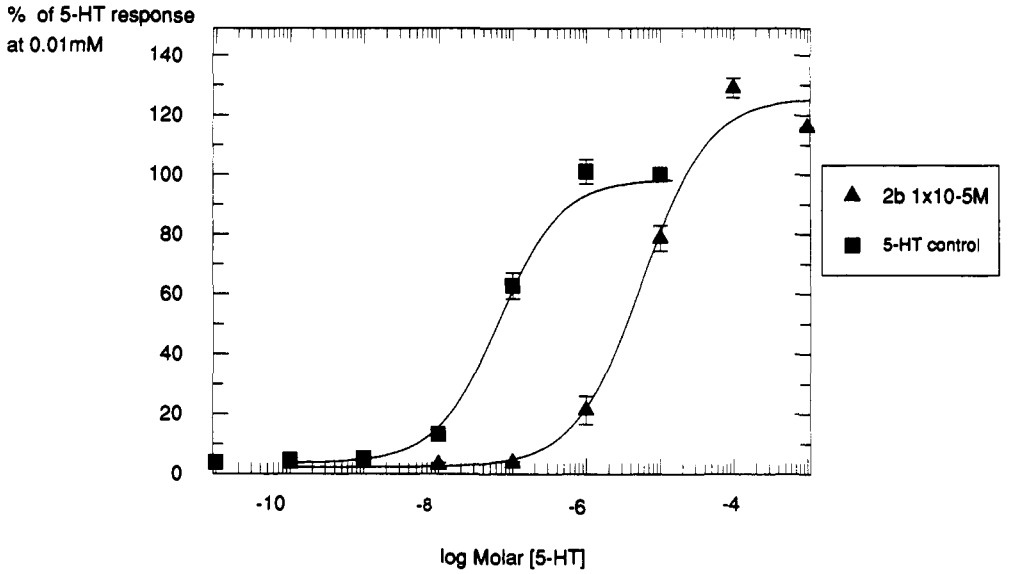


Figure 1. Effect of **2b** on 5-HT-induced phosphoinositide hydrolysis in piglet choroid plexus.

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

receptor	affinity (pK_I)	receptor	affinity (pK_I)
5-HT_{1C}	6.86	adrenergic α_{2A}	<5.0
5-HT_2	5.18	adrenergic α_{2B}	<5.0
5-HT_{1A}	<5.0	adrenergic β_1	<4.0
5-HT_{1D}	<5.0	adrenergic β_2	<4.0
5-HT_3	<5.0	dopaminergic D_1	<5.0
adrenergic α_1	<5.0	dopaminergic D_2	<4.5

[a] Tissues and ^{3}H-radioligands used in binding assays: 5-HT_{1C} and 5-HT_2 (see Table I); 5-HT_{1A} (rat hippocampus; [^{3}H]-8-OH-DPAT);[21] 5-HT_{1D} (guinea pig cortex; [^{3}H]-5-HT);[22] 5-HT_3 (rat hippocampus and entorhinal cortex; [^{3}H]granisetron);[23] adrenergic α_1 (rat cortex; [^{3}H]-7-methoxyprazosin);[24] adrenergic α_{2A} (human platelets; [^{3}H]rauwolscine);[24] adrenergic α_{2B} (rat neonatal lung; [^{3}H]rauwolscine);[24] adrenergic β_1 (cloned rat β_1 receptors expressed in CHO cells; [^{125}I]iodocyanopindolol); adrenergic β_2 (cloned rat β_2 receptors expressed in CHO cells; [^{125}I]iodocyanopindolol); dopaminergic D_1 (rat striatum; [^{3}H]-SCH 23390), dopaminergic D_2 (cloned human D_2 receptors expressed in CHO cells; [^{125}I]iodosulpiride).

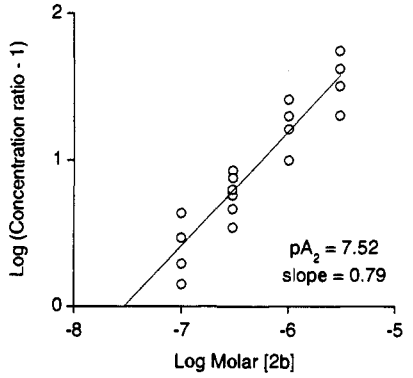


Figure 2. Schild regression analysis of **2b** versus 5-HT in rat stomach fundus.

first in the absence and the second, 1 h later, in the presence of **2b**. It was found that **2b** caused a concentration-dependent dextral displacement of contractile concentration effect curves to 5-HT, with no depression of the maximum response. A pA_2 value of 7.52 associated with a slope of 0.79 (Figure 2) was determined using Schild regression analysis.[25] The relevance of the affinity of **2b** for the RSF receptor is as yet unknown since, although the sequence is present in human genomic DNA, initial experiments have failed to identify it in rat brain tissue.[11]

In conclusion, pyridylindolylureas afford a novel series of potent and selective 5-HT_{1C} receptor antagonists, with **2b** being the most selective compound reported to date. It has previously been reported that the 5-HT_{1C} receptor *agonist* (*m*-chlorophenyl)piperazine[5,19,26] causes anxiety, both in rats[27] and in humans.[28] In rats this has been attributed to stimulation of the 5-HT_{1C} receptor,[27] which implies that specific 5-HT_{1C} receptor antagonists may be anxiolytic as suggested by the effects of nonspecific 5-HT_{1C} receptor antagonists in two rat models of anxiety.[29,30] The in vivo properties of **2b** are therefore of great interest and will be the subject of a future publication.

Supplementary Material Available: Experimental procedures, including analytical and spectral data, for the preparation of **2b** and **7** (3 pages). Ordering information is given on any current masthead page.

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