Communications to the Editor

(1-Butyl-4-piperidinyl)methyl 8-Amino-7-chloro-1,4-benzodioxane-5-carboxylate Hydrochloride: A Highly Potent and Selective 5-HT$_4$ Receptor Antagonist Derived from Metoclopramide

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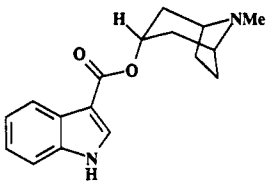
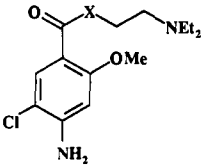
Functional serotonin 5-HT$_4$ receptors have been identified in the central nervous system (CNS) and periphery and in several animal species including human, pig, guinea pig, rat, mouse[1] and dog.[2-4] The first antagonist reported to act at this receptor was the 5-HT$_3$ receptor antagonist ICS 205930 (tropisetron, 1),[5] and this compound was subsequently used to characterize 5-HT$_4$ receptor-mediated pharmacological responses in several preparations. More recently, other indole-based 5-HT$_4$ receptor antagonists have been described.[6,7] The gastric prokinetic activity of metoclopramide (2) has been attributed to its ability to activate 5-HT$_4$ receptors, but its corresponding ester, SDZ 205-557 (3), is a competitive 5-HT$_4$ receptor antagonist.[8]

Chart I



1 ICS 205930, tropisetron



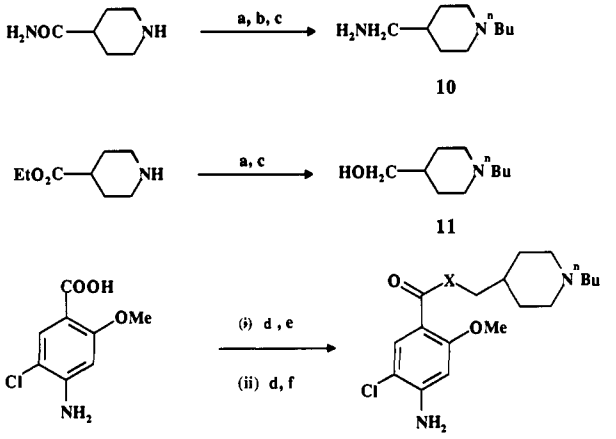
2 X = NH metoclopramide

3 X = O SDZ 205-557

In our search for potent and selective 5-HT$_4$ receptor antagonists we used the guinea pig distal colon longitudinal muscle myenteric plexus preparation[9] (LMMP) to identify the amide 4, originally derived from metoclopramide. Changing to an ester linkage to give 5 increased the antagonist potency. Compounds 6-8 were also targeted for synthesis in order to investigate the effect of incorporating the methoxy oxygen atom within a cyclic structure. Introduction of the 8-amino and 7-chloro substituents led to 9.

The methods of preparation of the benzamide 4 and benzoate 5 are shown in Scheme I. (1-Butyl-4-piperidinyl)-methylamine (10) was prepared from 4-piperidinecarboxamide in 81% overall yield. Alkylation of 4-piperidinecarboxamide with 1-bromobutane was carried out in ethanol under reflux using potassium carbonate as base. Dehydration to the nitrile using phosphorus pentoxide followed by lithium aluminum hydride reduction gave the required amine 10. Coupling of 10 to 4-amino-5-chloro-2-methoxybenzoic acid was effected *via* the imidazolide in a mixture of acetonitrile and *N,N*-dimethylformamide to give 4.

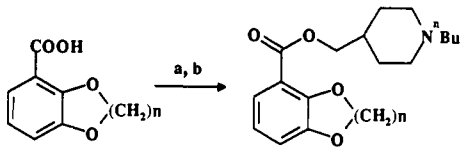
Scheme I[a]



4 (i) X = NH 5 (ii) X = O

[a] Reagents: (a) ^{n}BuBr, K_2CO_3, EtOH, Δ; (b) P_2O_5, 180 °C; (c) $LiAlH_4$, Et_2O; (d) 1,1′-carbonyldiimidazole, MeCN, DMF; (e) 10, MeCN; (f) 11, ^{n}BuLi, THF.

Scheme II[a]



n = 1 6
n = 2 7
n = 3 8

[a] Reagents: (a) 1,1′-carbonyldiimidazole, MeCN; (b) 11, MeLi, THF.

The intermediate for the synthesis of the ester 5, (1-butyl-4-piperidinyl)methanol (11), was prepared in 80% overall yield from ethyl 4-piperidinecarboxylate by N-alkylation with 1-bromobutane followed by lithium aluminum hydride reduction of the intermediate.

4-Amino-5-chloro-2-methoxybenzoic acid was activated as the imidazolide in acetonitrile and reacted *in situ* with the lithium alkoxide derived from 11 to give 5. Compounds 6-8 were prepared analogously from the corresponding carboxylic acids[10-12] (Scheme II). Compound 9 was prepared according to Scheme III. 8-Acetamido-1,4-benzodioxane-5-carboxylic acid,[12] 12, was treated with chlorine in acetic acid to give 13. Base hydrolysis of this amide followed by coupling of the acid 14 via the imidazolide as for 5 gave the ester 9, which was isolated as the hydrochloride salt.

Compounds 4-9 were evaluated in the guinea pig distal colon LMMP for their ability to block the 5-HT-evoked, 5-HT$_4$ receptor-mediated contractions. Structure-activity relationships were determined using pIC$_{50}$ values (the

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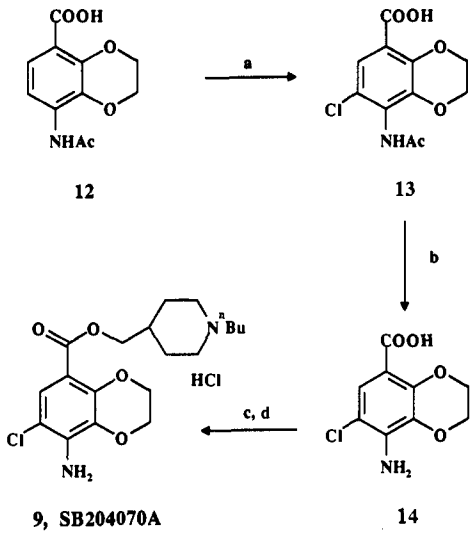
Scheme III[a]



[a] Reagents: (a) Cl_2, AcOH; (b) NaOH, H_2O, Δ; (c) 1,1'-carbonyldiimidazole, MeCN; (d) 11, MeLi, THF.

Table I. Potency of Compounds 1 and 3–9 in the Guinea Pig Distal Colon LMMP

compd	pIC_{50} (mean ± sem) (n)	compd	pIC_{50} (mean ± sem) (n)
1	5.5 ± 0.04 (5)	6	7.2 ± 0.4 (3)
3	6.6 ± 0.06 (6)	7	8.2 ± 0.1 (3)
4	8.0 ± 0.1 (3)	8	7.3 ± 0.3 (3)
5	9.0 ± 0.3 (4)	9	10.1 ± 0.7 (5)

negative logarithm of the concentration of test compound required to reduce the response evoked by the approximate EC_{50} concentration of 5-HT by 50%, Table I). Benzamide 4 was 500 and 50 times more potent than 1 or 3, respectively, and a further 10-fold increase in potency was observed with the corresponding ester 5. Results obtained with 6, 7, and 8 showed that incorporation of the oxygen atom within a six-membered ring was optimum for activity. Introduction of the chlorine and amino substituents gave the highly potent benzodioxan 9 which was 10-fold more potent than the corresponding *o*-methoxy compound, 5.

On the basis of these results, compound 9 was selected for further evaluation in the guinea pig distal colon LMMP. In the presence of methiothepin (10^{-7} M, to eliminate 5-HT_1 and 5-HT_2 effects) and granisetron (10^{-6} M, to eliminate 5-HT_3 effects), 5-HT (10^{-11}–10^{-6} M) produced a monophasic, concentration-dependent contraction with a pEC_{50} of 9.2 ± 0.08 (n = 38, Figure 1 control). At low concentrations (10^{-11}, 3×10^{-11}, and 10^{-10} M), 9 produced a concentration-dependent rightward shift of the 5-HT curve yielding an apparent pA_2 of 10.8 ± 0.1. At higher concentrations (10^{-10} M and above), a reduction in the maximum was also observed. Because of this, the onset and recovery of the antagonist effects of 9 were investigated. Immediately after a control response to the approximate EC_{50} concentration of 5-HT (generally 10^{-9} M, 30-s contact), 9 was added to the bathing solution and was left in contact with the tissues for 30 min, during which time 5-HT was added twice. The bathing solution was then replaced with compound-free Krebs solution and the tissue challenged with the same concentration of 5-HT every 15 min until responses returned to control levels. At all concentrations of 9 investigated (10^{-10}, 3×10^{-10}, and 10^{-9} M) the responses to 5-HT recovered to control levels with $t_{1/2}$(off) values of 36 ± 5, 46 ± 5, and 70 ± 7 min,

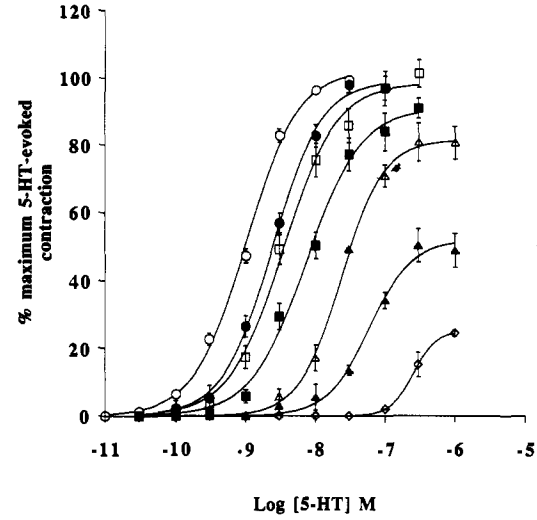


Figure 1. Effects of 9 on 5-HT-evoked contractions in the guinea pig distal colon LMMP (n = 6): (○) control; (●) 10^{-11} M; (□) 3×10^{-11} M; (■) 10^{-10}; (△) 3×10^{-10} M; (▲) 10^{-9} M; (◇) 3×10^{-9} M.

Table II. Receptor Binding Profile of 9[a]

receptor	affinity (pK_i)	receptor	affinity (pK_i)
5-HT_{1A}	<6	D_2	<5
5-HT_{1D}	<5	D_3	<6
5-HT_{1E}	<5	adrenergic α_1	<6
5-HT_{2A}	<6	adrenergic α_2	<6
5-HT_{2C}	6.9, 7.1	adrenergic $\beta 1$	<6
5-HT_3	6.6, 6.7	adrenergic $\beta 2$	<6
D1	<6		

[a] Radioligand binding assays were performed as previously described[14,15] with the following exceptions: [^{3}H]-5-HT was used to radiolabel the cloned human 5-HT_{1E} receptor. Dopamine D_2 and D_3 receptor affinities were determined using cloned human receptors expressed in CHO cells, radiolabeled with [^{125}I]iodosulpride, and adrenergic α_1 cells were radiolabeled with [^{3}H]-7-methoxyprazosin.

respectively, indicative of reversible blockade. In addition, in this model 9 did not affect contractions evoked by the nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, 3×10^{-6}–3×10^{-4} M) at concentrations up to and including 10^{-6} M and showed >5000-fold selectivity for the 5-HT_4 receptor when compared with affinities obtained at 5-HT_{1A}, 5-HT_{1D}, 5-HT_{1E}, 5-HT_{2A}, 5-HT_{2C} (formerly 5-HT_{1C}), 5-HT_3, D_1, D_2, D_3 dopamine receptors and $\alpha 1$, $\alpha 2$, $\beta 1$, and $\beta 2$ adrenoceptors (Table II). Thus the nonsurmountable antagonism observed was not due to either irreversibility or lack of selectivity but may be a consequence of the high lipophilicity of 9 (log p = 4.86, based on pK_a 10.4). This issue is the subject of a pending publication.[13]

The in vivo activity of SB 204070A will be reported elsewhere.

In conclusion 9, SB 204070A is a highly potent and selective 5-HT_4 receptor antagonist in the guinea pig distal colon and as such is a useful tool for further characterizing this receptor.

Supplementary Material Available: Experimental procedures, including analytical and spectral data, for the preparation of 4–9 (7 pages). Ordering information is given on any current masthead page.

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