Communications to the Editor

N-[(1-Butyl-4-piperidinyl)methyl]-3,4-dihydro-2H-[1,3]oxazino[3,2-a]indole-10-carboxamide Hydrochloride: The First Potent and Selective 5-HT$_4$ Receptor Antagonist Amide with Oral Activity

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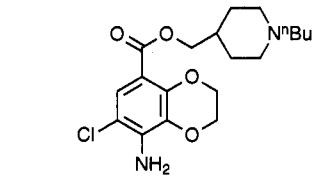
Early characterization of the 5-HT$_4$ receptor was carried out using operational pharmacology in a variety of species.[1] Transduction mechanisms have also been investigated,[1-4] but the cloning of this receptor was only recently reported.[5] Despite the lack of contribution of molecular biology to the definition of the 5-HT$_4$ receptor, its existence and unique properties are now widely accepted.[6] The chemical tools that were used for the initial identification studies were not ideal. Nonselective receptor agonists such as the benzamides, metoclopramide, renzapride, and cisapride were used.[7] The best antagonist available was the nonselective indole ester, ICS 205-930 (tropisetron).[8] Nevertheless, these compounds not only provided valuable information about this receptor but also served as lead structures for medicinal chemists intent on the design of more potent and selective agents.

It is now evident that the most potent and selective 5-HT$_4$ receptor antagonists fall into two distinct structural classes, substituted benzoate derivatives, such as SB 204070[9] (1) and indole esters, such as GR 113808[10] (**2**, Chart 1).

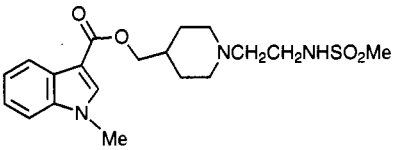
The 5-HT$_4$ receptor is widely distributed in a variety of animal species on specific nerve types within the enteric and central nervous system and on certain smooth and cardiac muscle tissues. Clearly, selective antagonism of this receptor could exert beneficial effects in a number of pathophysiological disorders. Putative indications for this class of compound have recently been reviewed.[11,12]

All of the most potent and selective 5-HT$_4$ receptor antagonists reported are unhindered ester derivatives and as such might be expected to be rapidly metabolized *in vivo*. Thus, their potential as orally active drugs is severely limited.[13] Recently, in an attempt to address this problem, ketone derivatives have been identified[14,15] as 5-HT$_4$ receptor antagonists and oral activity has been demonstrated for one of these, RS-39604.

Chart 1



1 (SB 204070)



2 (GR 113808)

The present study set out to overcome the issue of metabolic lability by identifying potent, selective, and orally active 5-HT$_4$ receptor antagonists and, in particular, targeted amides for synthesis. The guinea pig isolated distal colon longitudinal muscle myenteric plexus preparation (LMMP)[16] was used for the development of structure–activity relationships.

The indole ester **3** (SB 204139)[17] served as the lead for this work. The corresponding amide **4** was also evaluated. Substitution at the indole 2-position of **3** led to identification of the highly potent **5**, and the analogous amide **6** was also investigated. Cyclic derivatives of **5** were then synthesized as exemplified by compounds **7**–**9**.[17] The preparation of the amide analogues **10**–**12**, corresponding to **7**–**9**, led to the identification of **11** as a potent 5-HT$_4$ receptor antagonist.

The methods of preparation of the ester **3** and the corresponding amide **4** are shown in Scheme 1. (1-Butyl-4-piperidinyl)methylamine and (1-butyl-4-piperidinyl)methanol were prepared as previously described.[9] Coupling to the indole nucleus was effected in each case *via* the acid chloride. The 2-methoxy substituent present in both ester **5** and amide **6** was introduced by reaction of the unsubstituted precursor **3** and **4**, respectively, with *N*-chlorosuccinimide in chloroform, followed by treatment of the resulting solution with methanol. The oxazolo-, oxazino-, and oxazepino[3,2-*a*]indole esters[17] and amides **7**–**12** were prepared in an analogous fashion from the same precursors, **3** and **4**, substituting methanol by the appropriate halogenated alcohol. The intermediate halo ethers were cyclized with potassium carbonate in acetone without prior isolation (Scheme 1).

Compounds **3**–**12** were evaluated in the guinea pig distal colon LMMP for their ability to antagonize the 5-HT-evoked contractions mediated through 5-HT$_4$ receptor activation. Results are expressed as pIC$_{50}$ values[18] and are summarized in Table 1. The indole ester **3** was a potent antagonist but the corresponding indole amide **4** was 500-fold less potent. It was hypothesized that an out-of-plane conformation could be favored for interaction at the 5-HT$_4$ receptor, and this might be exploited further in the case of the ester by incorporation of a suitable 2-substituent. Introduction of a 2-methoxy group into **3** to give **5** reinforced this idea

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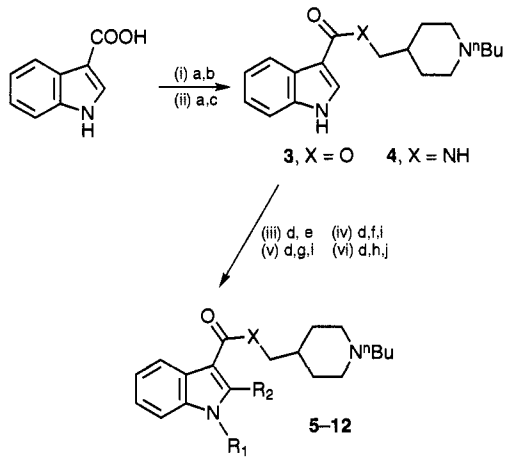
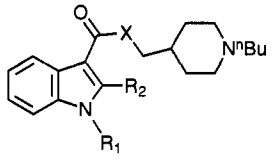
Scheme 1[a]



[a] Reagents: (a) $(COCl)_2$, CH_2Cl_2, DMF; (b) (1-butyl-4-piperidinyl)methanol, MeLi, THF; (c) (1-butyl-4-piperidinyl)methylamine, Et_3N, CH_2Cl_2; (d) NCS, $CHCl_3$; (e) MeOH; (f) $HO(CH_2)_2Br$; (g) $HO(CH_2)_3Br$; (h) $HO(CH_2)_4Cl$; (i) K_2CO_3, Me_2CO; (j) K_2CO_3, NaI, Me_2CO, Δ.

Table 1. Potency of Compounds **3–12** in the Guinea Pig Distal Colon LMMP



compd	method	R_1	R_2	X	pIC_{50} (mean ± SEM) (*n*)
3	i	H	H	O	9.3 ± 0.04 (5)
4	ii	H	H	NH	6.7 ± 0.04 (3)
5	iii	H	OMe	O	10.0 ± 0.1 (3)
6	iii	H	OMe	NH	7.1 ± 0.4 (3)
7	iv	$-(CH_2)_2O-$		O	10.0 ± 0.2 (3)
8	v	$-(CH_2)_3O-$		O	10.6 ± 0.2 (6)
9	vi	$-(CH_2)_4O-$		O	9.8 ± 0.1 (3)
10	iv	$-(CH_2)_2O-$		NH	7.8 ± 0.1 (3)
11	v	$-(CH_2)_3O-$		NH	9.2 ± 0.1 (5)
12	vi	$-(CH_2)_4O-$		NH	8.1 ± 0.2 (6)
SB 204070					10.1 ± 0.7 (5)

and produced a 5-fold increase in potency. In this case, conversion to the analogous amide **6** had an even more detrimental effect on activity, and a 1000-fold reduction in potency was observed. By introduction of both the amide and 2-methoxy substituent, a planar conformation is likely to be evoked, stabilized by an intramolecular hydrogen bond between the amide NH and the 2-methoxy oxygen atom. This result further suggested that an in-plane conformation of the molecule was not favourable for interaction at this particular receptor.

However, our previous work with benzoates had shown that incorporating the oxygen of a 2-methoxy group within a 6-membered ring as in **1** significantly increased potency. Also, derived amides showed good activity.[9,19] Extrapolation of that finding here, to incorporate the oxygen substituent of the 2-methoxy group of **5** into cyclic ester derivatives, led to the identification of the oxazolo-, oxazino-, and oxazepino-[3,2-*a*]indoles[17] **7–9**. These compounds were all very potent 5-HT_4 receptor antagonists in the guinea pig distal colon LMMP, with compound **8** (SB 207058) being particularly so. Although the corresponding amides were still less potent than the esters, in this series a good level of potency was retained and this was especially marked with the oxazino[3,2-*a*]indole **11** which was at least 10-fold more potent than its 5- or 7-membered ring counterparts, **10** and **12**, respectively. A low-energy planar conformation, stabilized through intramolecular hydrogen bonding, can also exist for this compound. The similarities in the chemical shifts of the adjacent peri-hydrogens in the ^1H NMR spectra of **10–12** (free bases, $CDCl_3$ $\delta = 8.2$, 8.4, and 8.4, respectively) and the similar ν for the carbonyl stretching frequencies in their IR spectra ($CHCl_3$, 1625, 1625, and 1635 cm^{-1}, respectively) suggest that the same conformation is allowed for all three compounds. The higher affinity of **11** at the 5-HT_4 receptor is therefore difficult to explain but may be related to an effect of the different direction of the ring oxygen lone pairs.

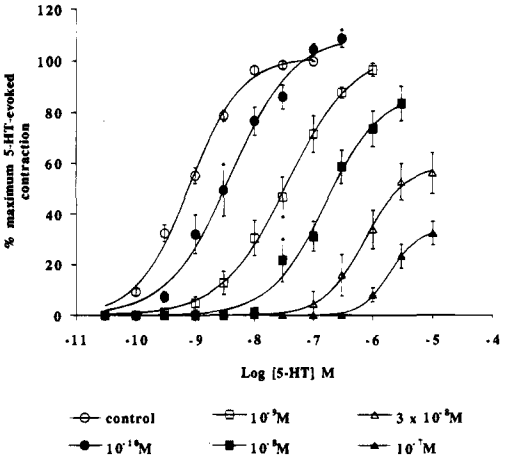


Figure 1. Effects of **11** on 5-HT-evoked contractions in the guinea pig distal colon LMMP ($n = 6$).

Table 2.[a] Receptor Binding Profile of **11**

receptor	affinity (pK_i)	receptor	affinity (pK_i)
5-HT_{1A}	<5	5-HT_3	<6
5-HT_{1D}	<5	5-HT_4	9.3 ± 0.2 (3)
5-HT_{1E}	<5	D_2	<6
5-HT_{2A}	<6	D_3	<6
5-HT_{2C}	<6	H_1	<6

[a] Radioligand binding assays were performed as previously described with the following exceptions: [^{3}H]-5-HT was used to radiolabel the cloned human 5-HT_{1E} receptor. 5-HT_{2C} affinity was determined using cloned rat receptors expressed in 293 cells, radiolabeled with [^{3}H]mesulergine and dopamine D_2 and D_3 receptor affinities were determined using cloned human receptors expressed in CHO cells, radiolabeled with [^{125}I]iodosulpride.

On the basis of its activity in the guinea pig distal colon LMMP, the amide **11** was selected for further evaluation both *in vitro* and *in vivo*.

Using previously described methodology,[16] a full concentration-response curve to 5-HT was constructed (Figure 1, results are mean values ± SEM). At low concentrations, (10^{-8}–10^{-10} M), **11** produced a concentration-dependent rightward shift of the 5-HT curve, yielding an apparent pA_2 of 10.6 ± 0.1 (Schild regression slope confined to unity). At higher concentrations (3×10^{-8} M and above) a reduction in the maximum was also observed. Compound **11** did not affect contractions mediated by the nicotinic agonist 1,1-dimethyl-4-phenylpiperazinium iodide (DMPP, 3×10^{-6} to 3×10^{-4} M) at concentrations up to and including 10^{-6} M. The onset and recovery of the antagonist effects of **11** were also investigated as described previously.[9] At all concentrations of **11** investigated (10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} M), the responses to 5-HT recovered to control

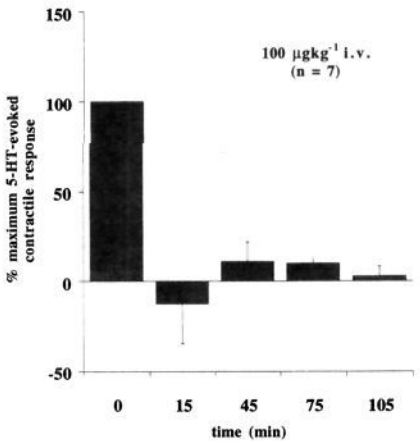


Figure 2. Effects of **11** on the contractile response to 5-HT in the conscious dog Heidenhain pouch. Dogs with previously prepared Heidenhain gastric pouches were fasted overnight. The cephalic vein was cannulated acutely, and 15 min later contractile responses to 5-HT (5 or 10 μg/kg) were recorded from the pouch *via* a pressure transducer, displayed on a chart recorder, and stored on magnetic tape. These doses of 5-HT had been previously determined for each dog as the minimal intravenous dose of 5-HT which evoked a reproducible, cholinergically mediated increase in tonic and phasic contractility. 5-HT was administered every 30 min until two consistent consecutive responses were obtained. Fifteen minutes after the last dose of 5-HT, antagonists or saline were administered either by intravenous injection or, in the case of oral dosing, in a gelatine capsule. Fifteen minutes later, 5-HT was dosed again and subsequently at 30 min intervals for the duration of the experiment (105 min post antagonist or saline dose). The pressure trace was integrated every minute and the total integral calculated 3 min before and 3 min after administration of 5-HT and the difference taken. Responses to 5-HT after administration of antagonist or saline were expressed as a percentage of the control response which was taken as 100%.

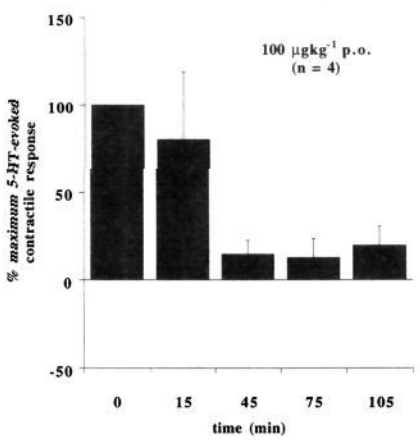


Figure 3. Effects of **11** on the contractile response to 5-HT in the conscious dog Heidenhain pouch. See Figure 2 for procedure.

levels with $t_{1/2}$ (off) values of 12.3 ± 3, 29 ± 4, 58 ± 3, and 70 ± 6 min respectively, indicative of reversible blockade.

The affinity of **11** for the 5-HT_4 receptor was confirmed by its ability to displace the radioligand [^{125}I]-SB 207710[20] from piglet hippocampal membranes with a pK_i of 9.3 ± 0.2. Compound **11** was also shown to be highly selective 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}, 5-HT_3, dopamine D_2, dopamine D_3, and histamine H_1 receptors (Table 2).

The difference between the apparent pA_2 in the guinea pig distal colon and the pK_i obtained from radioligand binding in the piglet hippocampus could possibly be due to accumulation of **11** in the tissue in the former case. The subject of nonsurmountable antagonism with potent 5-HT_4 receptor antagonists in the guinea pig distal colon LMMP functional model is discussed in another publication.[21] Alternatively it may be a consequence of species homologue variation of 5-HT_4 receptors or a real difference in 5-HT_4 receptor subtypes. Further work is necessary to further clarify this issue.

Because of its pharmacological activity *in vitro*, favorable selectivity profile, and potential for metabolic stability, compound **11** was evaluated in conscious fasted dogs for its ability to prevent 5-HT_4 receptor mediated 5-HT-evoked contractile activity in a Heidenhain gastric pouch, separated from the main body of the stomach.[22] At 100 μg/kg iv, compound **11** caused virtually complete abolition of the contractile response to 5-HT over the time course of the experiment (Figure 2, mean values ± SEM). Oral dosing produced a similar effect but with a delayed onset of action (15 min postdosing). (Figure 3, mean values ± SEM.) Full details of the effects of **11** at varying doses in this model, together with duration of action data, will be published separately. In summary, SB 207266A (**11**) has been identified as the first highly potent, selective, and orally active 5-HT_4 receptor antagonist amide. This compound is currently in phase II clinical trials and is being evaluated as a potential new drug for the treatment of irritable bowel syndrome.

Supporting Information Available: Experimental procedures, including analytical and spectral data, for the preparation of **3**–**12** (7 pages). Ordering information is given on any current masthead page.

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