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SEROTONINERGIC 5-HT$_3$ AND 5-HT$_4$ RECEPTOR ACTIVITIES OF DIHYDROBENZOFURAN CARBOXYLIC ACID DERIVATIVES.

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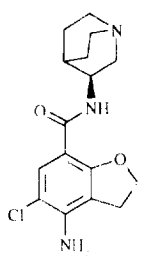
Abstract: Structure-activity relationships in a series of dihydrobenzofuran derivatives related structurally to the 5-HT$_3$ antagonist and 5-HT$_4$ agonist ADR 932 (**1**) were investigated. Members of this series, such as (**18**) and (**23**), were found to be 5-HT$_4$ agonists or partial agonists endowed with remarkably high affinity and selectivity.

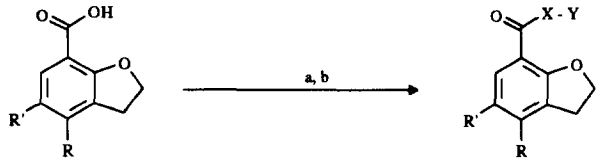
Serotonin (5-HT) is a neurotransmitter involved in a wide range of pharmacological effects. Four broad classes of 5-HT receptors are currently recognised (5-HT$_1$, 5-HT$_2$, 5-HT$_3$ and 5-HT$_4$), and several new receptor clones, termed 5-HT$_5$, 5-HT$_6$ and 5-HT$_7$, have been identified.[1] Antagonists of the 5-HT$_3$ receptor subtype are used clinically for the treatment of chemotherapy or radiotherapy-induced nausea and vomiting[2]. Further therapeutic indications such as control of pain, anxiety and depression, memory impairment and drug withdrawal are being currently investigated.[3,4] Pharmacological responses mediated by the 5-HT$_4$ receptor subtype have been identified in the CNS,[5] the gastrointestinal tract,[6] and the heart.[7] In addition to the gastric prokinetic activity of the benzamide derivatives such as cisapride, renzapride and zacopride, which has been related to the activation of the 5-HT$_4$ receptor,[8] a wide range of other therapeutic targets for modulators of this receptor can be envisaged, including migraine, IBS (irritable bowel syndrome), arrhythmias, anxiety, and memory and/or learning dysfunctions.[9] In the course of our work on the synthesis and evaluation of novel serotonergic agents, ADR-932 (**1**) was identified as a potent, long-lasting and orally active 5-HT$_3$ antagonist.[10]



(**1**)

Similarly to other 5-HT$_3$ antagonists of its generation, ADR-932 also shows relatively potent agonist activity at the 5-HT$_4$ receptor (Table 1). ADR-932 significantly increases gastric emptying when orally or intravenously administered to dogs. This paper describes the discovery of a novel series of 5-HT$_4$ agonists endowed with very high affinity and selectivity following a systematic manipulation of the structure starting from the dual activity of **1**. Structure-activity relationships were explored by variation of the substituents at positions 4, 5 and 7 of the dihydrobenzofuran ring and some requirements for the activities became apparent.

Amides and esters reported in Tables 1 and 2 were prepared from the known carboxylic acids[11,12] **2-5** according to the following scheme by reaction of the corresponding acyl imidazolides with the appropriate amines and alcohols.

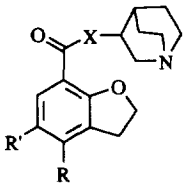


2 R' = H R = H
3 R' = H R = NH_2
4 R' = Cl R = H
5 R' = Cl R = NH_2

a) CDI, THF or DMF, 40°C, 1h. b) Y-OH, DBU, DMF, 40°C, 2h or Y-NH_2, THF, 40°C, 2h.
Satisfactory analytical and spectral data were obtained for new compounds.[13]

Quaternised compounds **16** and **17** were obtained by refluxing **1** and **6** with *n*-BuBr in ethyl alcohol.

Table 1

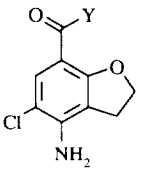


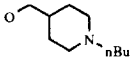
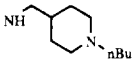
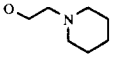
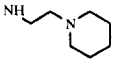
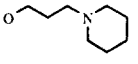
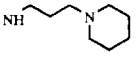
Cpd.	R	R'	X	Enant.	Binding assays K_i(nM)[a]		5-HT_3R activity[d]	5-HT_4R activity[e]	
					5-HT_3[b]	5-HT_4[c]	K_b(nM)[f]	EC_{50}(nM)[g]	K_b(nM)[f]
1	NH_2	Cl	NH	S	0.10±0.02[h]	17±2	3.80±0.75	18.6±1.4	--[i]
6	NH_2	Cl	NH	R	1.00±0.08	115±14	33.9±19	195±51	--
7	NH_2	H	NH	S	1.00±0.10	>1000	44.6±20.7	>10000	>100
8	NH_2	H	NH	R	20.0±1.8	>1000	NT[l]	>1000	>1000
9	H	Cl	NH	S	1.00±0.08	170±9	21.8±12.3	>10000	>100
10	H	Cl	NH	R	1.00±0.07	280±21	38.0±7.0	>1000	>1000
11	H	H	NH	S	1.40±0.09	>1000	40.7±5.4	>10000	>1000
12	H	H	NH	R	14.0±0.9	>10000	NT	>1000	>1000
13	NH_2	Cl	O	S	0.20±0.02	8.3±0.9	6.91±1.60	11.1±1.13	--
14	NH_2	Cl	O	R	1.00±0.09	93.0±7.2	19.0±6.7	385±55	--
15	(S)-zacopride				0.11±0.01	131±15	2.40±0.50	55.9±5.2	--

a) Each compound was tested at 10 concentrations for determining K_i values. b) [^{3}H]-BRL-43694 was used as the radioligand and the binding assays were carried out using rat entorhinal cortex according to the method of Nelson *et al.*.[14] c) [^{3}H]-GR-113808 was used as the radioligand and the binding assays were carried out using rat striatum according to the method of Grossman *et al.*.[15] d) 5-HT_3 activity was evaluated *in vitro* in guinea pig ileum preparations according to Eglen *et al.*.[16] ; none of the tested compounds showed any agonistic activity at concentrations up to 10^{-4} M. e) 5-HT_4 agonist and antagonist activities were evaluated *in vitro* by receptor-mediated relaxation of rat, carbachol-precontracted oesophageal tunica muscularis mucosae (TMM) as reported by Baxter *et al.*.[6] f) Each compound was tested at least in triplicate at 1 concentration for determining the apparent affinity K_b according to the method of Furchgott[17] *et al.*. g) EC_{50} values were determined by a concentration-response curve; each determinant was tested in triplicate. h) values are means ± standard errors of triplicate determinations. i) No activity up to 10^{-4} M concentration. l) Not tested.

A preliminary SAR investigation (Table 1) in the series of the quinuclidine derivatives showed that the 4-amino-5-chloro substitution pattern as well as the S configuration are required to achieve high affinity toward both the receptor subtypes (**1** - **12**), and that the simple ester-amide replacement does not induce significant changes in selectivity (**1**, **13**). As reported for the known 5-HT_3 antagonist renzapride[18], the *n*-butyl quaternisation of the azabicycle both reduced 5-HT_3 receptor affinity and increased 5-HT_4 receptor agonist potency to give the relatively potent and selective 5-HT_4 agonist **16**.

Table 2



Cpd.	Y	Binding assays K_i(nM)[a]		5-HT_4R activity[e]
		5-HT_3[b]	5-HT_4[c]	EC_{50}(nM)[g]
16		73±5[h]	1.9±0.1	8.50±1.76 (i.a. = 0.52)[m]
17		10.0±0.9	420±37	1360±100
18		250±13	0.13±0.02	1.85±0.14 (i.a. = 1.0)
19		57±3	1.9±0.2	4.49±0.93 (i.a. = 0.59)
20		110±6	0.94±0.10	2.02±0.61 (i.a. = 0.71)
21		380±42	41±3	35.4±9.1 (i.a. = 0.65)
22		1180±130	4.5±0.4	7.43±1.38 (i.a. = 0.70)
23		>10000	9.6±1.0	19.3±5.0 (i.a. = 0.60)
24	Metoclopramide	490±52	900±86	1920±500 (i.a. = 0.63)
25	5-HT	87±6	37.4±2.9	17.1±6.5 (i.a. = 1)

See footnotes of Table 1. m) Intrinsic activity relative to 5-HT.

As most of the known 5-HT_4 ligands are characterised by the presence of a conformationally flexible amino moiety,[19-23] a series of 4-amino-5-chloro-dihydrobenzofuran-7-carboxylic acid derivatives bearing variously substituted piperidines were synthesised and tested (Table 2). All these new compounds turned out to be selective 5-HT_4 agonists or partial agonists endowed with good to excellent affinity (**18-23**). Compounds **18** (FCE 29029) and **23** (FCE 29034) showed the most interesting preliminary pharmacological profile of those tested. FCE 29029 is one of the most selective (about 2000 fold over the 5-HT_3 receptor) and potent 5-HT_4 full agonists reported so far. Nevertheless, the presence of the metabolically labile ester linkage will presumably limit the utility of this compound for *in vivo* experiments. On the contrary, the amidic compound FCE 29034 can be regarded as a useful tool to evaluate a selective 5-HT_4 partial agonist *in vivo* as it still retains a nanomolar affinity and high selectivity (> 10^3 fold over the 5-HT_3 receptor). FCE 29034 is at present being evaluated in animal models of memory dysfunctions.

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13. Selected analytical data for compound **18** (hydrochloride): mp 238-240°C; ^{1}H NMR (200 MHz, DMSO) δ: 0.89 (3H, t, J=7.0Hz), 1.29 (2H, m), 1.4-2.0 (7H, m),2.7-3.1 (6H, m,), 3.47 (2H, m), 4.01 (2H, d, J=5.9Hz), 4.61 (2H, t, J=8.8Hz); FAB MS : 367 (100, $[M+H]^+$), 196 (40), 154 (79).
    Selected analytical data for compound **23** (hydrochloride, monohydrate): mp 192-194°C; ^{1}H NMR (200 MHz, DMSO) δ: 1.2-2.0 (8H, m), 2.7-3.1 (6H, m), 3.2-3.5 (4H, m), 4.70 (2H, t, J=8.8Hz), 5.89 (2H, s), 7.45 (1H, s), 7.60 (1H, t, J=5.7Hz), 9.7 (1H, bs); FAB MS : 338 (100, $[M+H]^+$), 253 (18), 196 (31).
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