



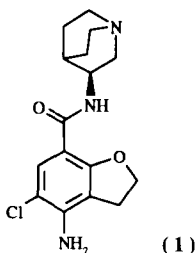
S0960-894X(96)00002-9

SEROTONINERGIC 5-HT₃ AND 5-HT₄ 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-HT₃ antagonist and 5-HT₄ agonist ADR 932 (**1**) were investigated. Members of this series, such as (**18**) and (**23**), were found to be 5-HT₄ agonists or partial agonists endowed with remarkably high affinity and selectivity.

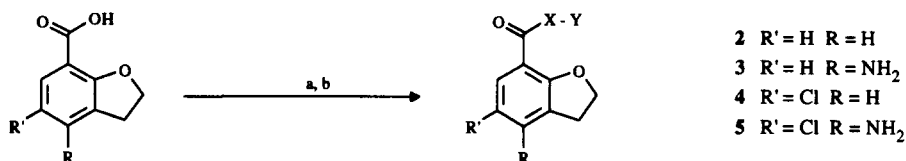
Serotonin (5-HT) is a neurotransmitter involved in a wide range of pharmacological effects. Four broad classes of 5-HT receptors are currently recognised (5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄), and several new receptor clones, termed 5-HT₅, 5-HT₆ and 5-HT₇, have been identified.¹ Antagonists of the 5-HT₃ receptor subtype are used clinically for the treatment of chemotherapy or radiotherapy-induced nausea and vomiting². 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-HT₄ 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-HT₄ 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-HT₃ antagonist.¹⁰



(1)

Similarly to other 5-HT₃ antagonists of its generation, ADR-932 also shows relatively potent agonist activity at the 5-HT₄ 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-HT₄ 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.

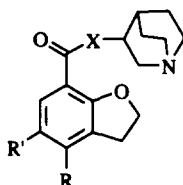
Amides and esters reported in Tables 1 and 2 were prepared from the known carboxylic acids^{11,12} **2-5** according to the following scheme by reaction of the corresponding acyl imidazolides with the appropriate amines and alcohols.



a) CDI, THF or DMF, 40°C, 1h. b) Y-OH, DBU, DMF, 40°C, 2h or Y-NH₂, 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 ₃ R activity ^d	5-HT ₄ R 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) [³H]-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) [³H]-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-HT₃ 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-HT₄ 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 Furchgott¹⁷ *et al.* g) EC₅₀ 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-HT₃ antagonist renzapride¹⁸, the *n*-butyl quaternisation of the azabicyclo both reduced 5-HT₃ receptor affinity and increased 5-HT₄ receptor agonist potency to give the relatively potent and selective 5-HT₄ agonist 16.

Table 2

Cpd.	Y	Binding assays K_i (nM) ^a		5-HT ₄ R activity ^c
		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-HT₄ 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-HT₄ 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-HT₃ receptor) and potent 5-HT₄ 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-HT₄ partial agonist *in vivo* as it still retains a nanomolar affinity and high selectivity (> 10³ fold over the 5-HT₃ 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; ¹H 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; ¹H 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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