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DESIGN OF A POTENT 5-HT$_4$ RECEPTOR AGONIST WITH NANOMOLAR AFFINITY

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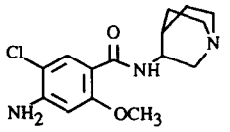
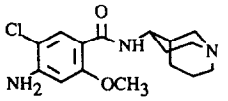
Abstract: *It was demonstrated that potent and selective ligands for the 5-HT$_4$ receptor devoid of 5-HT$_3$ receptor antagonist properties could be designed from the esters of 4-amino-5-chloro-2-methoxybenzoic acid and the conformationally flexible piperidine framework derived from the locked structure of the quinuclidine ring of zacopride. The compounds were evaluated in binding assays with [^{3}H]-GR-113808 and [^{3}H]-BRL-43694 and fonctionally using electrically-evoked contractions in the guinea-pig ileum. Compound 7 exhibited nanomolar 5-HT$_4$ receptor agonist activity.*

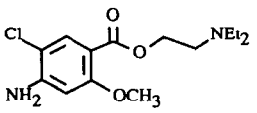
In recent years many agents which increase gastric emptying and intestinal transit have been discovered[1]. For a number of them, it was thought that they mimicked the effect of serotonin (5-HT) on cholinergic transmission in the myenteric plexus[2] and that their gastro-intestinal prokinetic properties were related to their ability to block 5-HT$_3$ receptors[3]. However, recently, support for a 5-HT$_3$ receptor-based mechanism underlying these gastro-intestinal prokinetic effects has decreased because other potent 5-HT$_3$ receptor antagonists lacking prokinetic properties have been designed[4] and, in addition, the 5-HT$_4$ receptor subtype was discovered. Indeed, simultaneously, Bockaert[5] described this new receptor in embryonic mouse colliculi neurones and Clarke[6] demonstrated its presence in the guinea-pig ileum, providing a better mechanism for the action of the prokinetic drugs. Although the 5-HT$_4$ receptor has not yet been cloned, it is, doubtless, a G-protein-coupled receptor since it mediates the stimulation of adenylate cyclase, leading to an increase in the levels of cyclic AMP[7]. Several prokinetic benzamide derivatives such as cisapride, renzapride and zacopride act as agonists[7] at this receptor, shown by their ability to stimulate adenylate cyclase and increase the size of electrically-evoked contractions in isolated guinea-pig ileum. Since then, 5-HT$_4$ receptors have been identified in several peripheral tissues: colon[8], rat oesophagus[9], porcine and human myocardium[10,11] and, recently, they were found in the guinea-pig, rat and human CNS[12]. The characterisation and understanding of the role of this receptor have been made possible by the discovery of antagonists such as tropisetron, SDZ 205-557[13] and GR 113808[14]. Tropisetron, a potent 5-HT$_3$ receptor antagonist, was



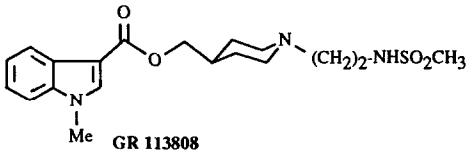
Zacopride



Renzapride



SDZ 205-557



GR 113808

used in the early studies of 5-HT$_4$ receptors for which it was found to possess only a micromolar affinity. However, it has drawbacks because of its weak affinity and lack of selectivity. SDZ 205-557, a close derivative of metoclopramide, was considered as the first useful antagonist available to characterise the 5-HT$_4$ receptor. However, the promising selectivity in the guinea-pig was not observed in the rat where it has equipotent affinity for 5-HT$_3$ and 5-HT$_4$ receptors[15]. A very potent and selective antagonist with subnanomolar affinity for the 5-HT$_4$ receptor, GR-113808[14], was described recently. It

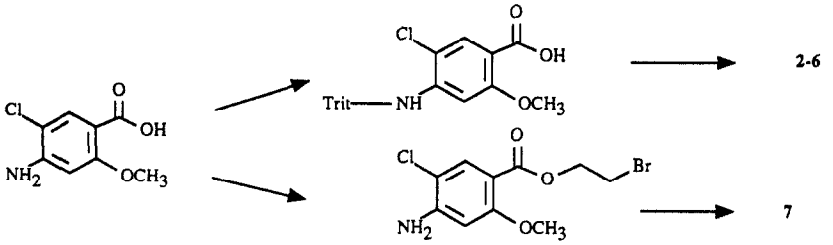
enabled the development of a radioligand binding assay for 5-HT_4 receptors and allowed their localisation in the CNS using autoradiography. Many compounds described as agonists for this receptor are less potent than 5-HT itself and are not specific. A number of them are potent 5-HT_3 antagonists or display more complex pharmacological profiles such as cisapride[16]. The first attempts to design a selective agonist were reported by Flynn[16] and King[17] who prepared several benzamides with a relatively good affinity and selectivity with regard to the reference compounds. Apart from these reports, neither significant structure-activity relationships nor selective compounds with similar potency to 5-HT have been described up to now and we present here the preliminary results in this field from our laboratory. Three structural properties of the reference compounds seemed to us worthwhile for the design of new compounds:

-The unique role of 4-amino-5-chloro-2-methoxybenzoic acid as the aromatic moiety involved in molecular recognition by the 5-HT_4 receptor.

-The advantage of the ester function over the amidic group emphasized by the superior affinity of SDZ 205-557 with regard to metoclopramide.

-The discrepancy between the availibility of the flexible amino moiety of SDZ 205-557 and the locked quinuclidine structure of zacopride which are both recognized by the 5-HT_4 receptor. It seemed to us that more selective molecules for the 5-HT_4 receptor could be obtained by removing the steric constraints of the amino bicyclic framework of the 5-HT_3 receptor antagonist benzamides such as zacopride or BRL 24682[18].

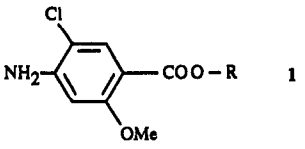
The compounds synthesized and reported in table I are the esters **1** of 4-amino-5-chloro-2-methoxy benzoic acid and of an alcohol such as tropane, 3-quinuclidinol and piperidinol with the hydroxy function located in the 3 or 4 positions. They were prepared according to the following synthetic routes: 4-amino-5-chloro-2-methoxy benzoic acid was N-protected with trityl chloride and condensed with the alcohol in the presence of DBU and carbonylimidazole in THF. Trityl group was removed by HCl in acetone. Compound **7** was prepared by the direct condensation of the acid with 1,2-dibromoethane to give the bromo derivative which reacted with the piperidine.



The pharmacological properties of the compounds were evaluated by radioligand binding assays using [^{3}H]-BRL 43694[19] in the rat entorhinal cortex and [^{3}H]-GR 113808[14] in the rat striatum for the assessment of their affinity for 5-HT_3 and 5-HT_4 receptors respectively. 5-HT_4 receptor agonist or antagonist activity was measured in the electrically-stimulated myenteric plexus and longitudinal muscle of guinea-pig ileum according to the method described by Clarke[9]. The pharmacological data are shown in table I. Examination of the results obtained with the esters SDZ-205557, **2** and **3** which are analogues of the reference compounds metoclopramide, zacopride and BRL 24682 respectively confirmed the favorable role of the ester to increase the potency for the 5-HT_4 receptor. This structural variation induced a mixed agonist-antagonist profile for the enantiomers (R)-**2** and (S)-**2** derived from zacopride, while **3** displayed a marked agonist activity. However, the values for the affinity of the esters for the 5-HT_3 receptor were similar to those of the amidic derivatives as we have already reported[20] and thus have a low selectivity. On the other hand, as we postulated, the less conformationally restricted compounds **4**, **5** and **6** with the piperidine framework displayed an agonist activity in the 20-100 nM range while a drop in their affinity for 5-HT_3 receptors was observed. However, a very large improvement in the potency and selectivity occurred with the totally flexible compound **7** which exhibited full agonist activity at the 5-HT_4 receptor equipotent to serotonin (EC_{50}: 5 nM, 100%) and a weak affinity for 5-HT_3 receptors. It appears to be the first 5-HT_4 receptor agonist which is 2 orders less

potent and selective for the 5-HT_3 receptor.

PHARMACOLOGICAL ACTIVITY AT 5-HT_4 AND 5-HT_3 RECEPTORS OF DERIVATIVES OF COMPOUND 1



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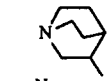
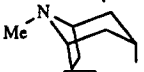
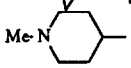
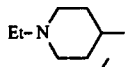
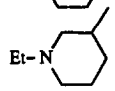
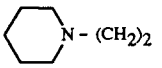
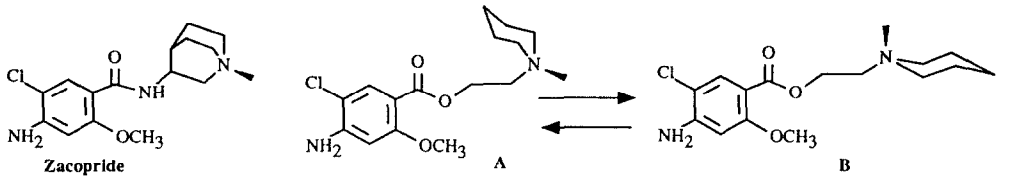
Compounds R	[a,c]Binding assays (K_i, nM) 5-HT_4	[b,c]Binding assays (K_i, nM) 5-HT_3	[d]5-HT_4 receptor activity EC_{50}(nM)	IC_{50} (nM)
Metoclopramide	975±63	443±58	[f]NT	NT
Zacopride	753±59	0.7±0.04	260[190-360] (100%)	[e]ne
BRL 24682	>1000	0.8±0.2	NT	NT
SDZ-205-557	5.3±0.7	205±33	>1000	77
 (R)-2	488±63	3.6±0.4	110[80-140] (75%)	30
(S)-2	91.4±6	0.7±0.04	153[134-174] (54%)	450
 3	64.3±6.5	3.2±0.4	27[20-36] (54%)	ne
 4	23±6	72±3.7	110(53%)	60
 5	10.3±0.5	118±40	22[17-29] (60%)	ne
 6	34.5	NT	44[34-57] (79%)	ne
 7	1.07±0.5	782±67	4[3.7-4.3] (80%)	ne

Table I.[a][^{3}H]-GR-113808 was used as the radioligand and the binding assays were carried out using rat striatum (30 min-25°C) and seven concentrations of the competing drug. The non-specific binding was determined with compound 7 (10 μM).[b][^{3}H]-BRL-43694 was used as the radioligand and the binding assays were carried out using rat posterior cortex (30 min-25°C) and seven concentrations of the competing drug. The non-specific binding was determined with GR 38032F (10 μM). [c]Each assay was done in triplicate and inhibition curves were analyzed by a computer-assisted-curve-fitting program (ALLFIT). K_i values were determined from the Cheng-Prussof equation.[d]Segments of ileum from male guinea-pigs were dissected out, mounted in an organ bath, attached to an isometric transducer and electrically stimulated (0.2 Hz, 15 ms) to produce 50% of the maximum contraction. After obtaining consistent contractions with $3x10^{-8}$M 5-HT and washout, cumulative concentration-response curves were constructed for the test compounds. The agonist activity was assessed as the concentration which gave a 50% increase in the response to the electrical stimulation (EC_{50}, nM) with regard to the maximum. The activity is expressed as % of the maximum 5-HT effect. The antagonist activity (IC_{50}, nM) was calculated as the concentration which produced a 50% reduction of the 5-HT contraction, [..]= 95% confidence limits.[e]ne= not evaluable.[f]Not tested.

Recently, several papers[21,22] reported the 5-HT_4 receptor antagonist activity of derivatives structurally close to our compounds. Nevertheless, the agonist activity of 7 was confirmed by the relaxation of the carbachol-contracted rat oesophageal tunica muscularis mucosae (IC_{50}: 2.4 nM [1.5-3.9]) compared to serotonin (IC_{50}: 3.7nM [3.2-4.2]). Apparently, the structure of the amino moiety of compound 7 is different from the azabicycles described recently by King [17] as 5-HT_4 receptor agonists in which the second ring is "tied back". However, one could postulate that 7 can exist in equilibrium in two conformations A and B and that the relatively high energy conformer A, for which

the spatial position of the nitrogen lone pair is close to that of zacopride, represents the agonist form. The potent agonist activity could be due to a better fit with the 5-HT_4 receptor site and the weak 5-HT_3



receptor affinity to the lack of the locked framework, an important structural requirement for this receptor. Several studies are currently in progress to investigate this important point for the structural relationship in the field of the 5-HT_4 receptor agonists and for the understanding of the surprising observation that compounds with structural similarity such as **7** and SDZ 205-557 possess opposite properties.

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