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DERIVATIVES OF QUINUCLIDINE AS 5-HT3 RECEPTOR ANTAGONISTS: INFLUENCE OF AN ADDITIONAL CARBONYL GROUP ON THE RECOGNITION OF CHIRALITY BY THE RECEPTOR.

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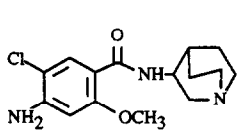
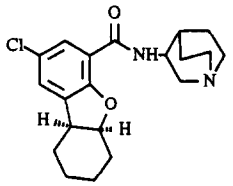
Abstract: *The influence of the aromatic moiety on the recognition of the (R) or (S) chirality of the quinuclidine framework by 5-HT$_3$ receptors was evaluated in a series of simple o-alkoxy benzamide and naphthalene derivatives. In all the derivatives, the highest potency resided in the (S) isomers, as seen with zacopride, and depended upon the hydrophobic properties of the substituent. It was demonstrated that an intramolecular hydrogen bond was not implicated in the activity and the introduction of an additional carbonyl function, as in 1,2-naphthalimide, brought about an inversion of the enantioselectivity. The existence of a secondary hydrogen donor group in the receptor site was suggested.*

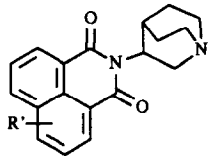
A number of 5-HT$_3$ receptor antagonists contain a quinuclidine ring as a basic moiety and almost all of them are potent ligands indicating a good fit with the receptor site. The first compounds described were members of the benzamide family, such as zacopride[1] or RG 12915[2]. An enantioselectivity was reported for these compounds, the (S) enantiomer being the more potent[3]. Recently, we synthesized from the 1,8-naphthalimide moiety a new family of 5-HT$_3$ receptor antagonists[4],**1** , possessing the quinuclidine framework. The compounds were very potent ligands for rat entorhinal cortex 5-HT$_3$ receptors and potent antagonists of 5-HT-induced bradycardia in the rat. We observed, with regard to previous benzamides, an inversion and an increase in the enantioselectivity, the (R) enantiomer being 2 orders of magnitude more potent than the (S) compound. These derivatives had been designed as cyclised analogues of the benzamide



Zacopride



RG 12915



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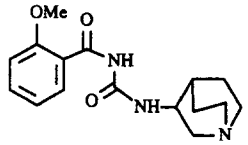

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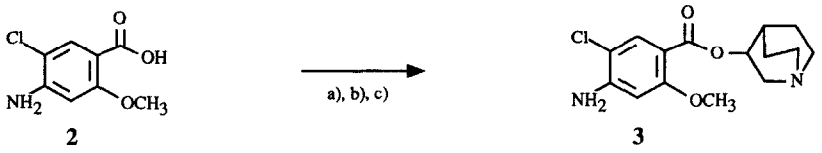
compounds where a virtual ring, formed by a hydrogen bond between the oxygen atom of the methoxy group and the NH amidic, may be implicated in the biological activity of this class of compounds[5]. Thus, a reappraisal of the role of the hydrogen bond and the influence of the o-alkoxy group on the recognition of the chirality of the quinuclidine ring by 5-HT$_3$ receptors seemed worthwhile in this class of benzamide derivatives.

Firstly, the existence of an intramolecular hydrogen bond was demonstrated by examination of the ^{1}H NMR spectra of zacopride (^{1}H NMR 200 MHz $CDCl_3$, δ_{NH}= 7.78) . We observed in DMSO-d_6 a linear variation, depending upon the temperature, of the chemical shift of the NH amidic (^{1}H NMR 300 MHz, T=293°K, δ_{NH}= 7.94; T= 350°K, δ_{NH}= 7.85), whereas no change occured in the other peaks. This finding has previously been reported for a D_2 antagonist benzamide series[6]. These data support the presence in solution of the conformer with the supposed virtual ring formed by a hydrogen bond. Moreover, conformational analysis with the molecular mechanics program of ALCHEMY software[7] showed the conformer to be energetically stable (E_{min}= - 6.3 Kcal/Mol). However, a 180° rotation of the phenyl ring around the C_{1ar}-C(=O) bond also gave the energetically equivalent non-hydrogen-bonded rotamer (E_{min}= - 6.5 Kcal/Mol) which cannot be rejected as a potentially bioactive conformation which could fit into the receptor[8]. For this purpose, and to evaluate the role of the hydrogen bond, it was important to compare the affinity for 5-HT$_3$ receptors of the enantiomers of zacopride and the corresponding esters. (R) and (S) zacopride were prepared according to the previously described process[9] and the esters were synthesized from (R) and (S) 3-quinuclidinol[10] by the synthetic route shown in scheme 1.



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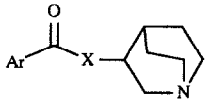
SCHEME 1. a) trityl chloride, pyridine. b) CDI, DBU, 3-quinuclidinol, THF, 60°C. c) HCl, $CHCl_3$.

The acid **2** was tritylated and condensation with the alcohol was only effective in the presence of 1,1'-carbonyldiimidazole (CDI) and DBU in THF or DMF. After purification by chromatography, treatment with HCl in $CHCl_3$ gave the esters **3**. The affinity for 5-HT_3 receptors was evaluated in vitro by binding assays with [^{3}H]-BRL 43694 using rat entorhinal cortex[11]. The results reported in table I show the equipotency of the amides and esters and a similar enantioselectivity, emphasizing the lack of influence of the hydrogen bond on the affinity values and the recognition of the chirality by the receptor. However, it is probable that the o-alkoxy substituent is implicated in the recognition of the (S) enantiomer in the benzamide series and, to evaluate this hypothesis, some simple benzamides of (R) and (S) 3-aminoquinuclidine[12] **4**, **5**, **6** and **7** were synthesized by classical methods.

5-HT_3 RECEPTOR BINDING AFFINITY DETERMINED IN RAT ENTORHINAL CORTEX



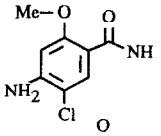
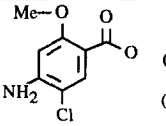
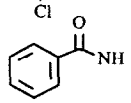
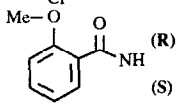
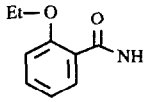
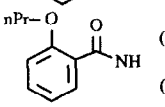
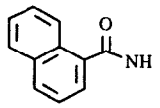
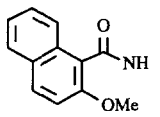
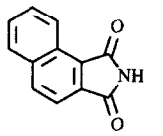
Compound	Ar-CO-X		α^{25}(°)[c]	K_i±SEM,nM[a]	Compound	Ar-CO-X		α^{25}(°)[c]	K_i±SEM,nM[a]
Zacopride		(R)		2.6±0.4	3		(R)	-36.8	3.6±0.4
		(S)		0.2±0.04			(S)	+40.6	0.7±0.04
4		(R)	-9.3	1890	6		(R)	-4.7	409±49
		(S)	+8.7	147			(S)	+5.1	23.4±5.2
5		(R)	-6.0	100±34	7		(R)	-6.0	71.6±6
		(S)	+6.0	18.9±5.9			(S)	+6.0	11.2±5.3
8		(R)	-14.8	$>10^{-6}$	9				342±42[b]
		(S)	+13.9	32±4.7					
10		(R)	+2.3	6±0.2					
		(S)	-2.2	119±41					

Table I. a) [^{3}H]-BRL-43694 was used in the binding assays which were carried out using rat entorhinal cortex (30 min-27°C) and seven concentrations of the competing compound. 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 formula. b) **9** was a racemic mixture. c) The optical rotation was measured by using the Hg ray in MeOH (C=1) except for **10** where the D-ray was considered and H_2O was used as the solvent. The compounds were hydrochloride salts except for **4** which was a base.

The binding data for these compounds reported in table I show a clear increase in potency from the unsubstituted compound **4** to the o-propoxy derivative **7** which can be explained by the hydrophobic properties of the substituent. From these results, it can also be inferred that only the benzamide moiety is involved in the enantioselectivity since it is already present in the unsubstituted compound **4**. To get a better insight into the role of this substituent, we compared previous data for the activity of the (S) and (R)

enantiomers of the naphthalene derivatives **8**. Indeed, the naphthalene ring has been reported by us[13] to be an important aromatic moiety in the field of 5-HT_3 receptor recognition and the existence of only syn configuration of the carbonyl group and the distal ring of the amides **8** and **9** was demonstrated by ^1H NMR spectra and the mechanics molecular calculations. Thus, the carbonyl group in such a conformation brings about a deshielding of the hydrogen in position 8 with regard to the other aromatic protons (^1H NMR 200 MHz, $CDCl_3$, δ = 8.18, H-8 for **8** and δ = 7.82, H-8 for **9**) and examination of the different conformers with MAD software[20] also only gave the syn configuration for every conformer found in the range of 30 Kcal/Mol above the energy of the most stable conformation. The affinity values reported in table I show similar enantioselectivity and equipotency for the (S) compounds **5**, **6** and **8**, but, on the other hand, an unexpected drop in the affinity of the racemic 2-methoxy derivatives **9**, which only exists as H-bonded conformer, was observed. These data suggest an unfavorable interaction of the methoxy group with this part of the receptor and question the existence of the H-bonded conformer of benzamide compounds as a biologically active conformation at the 5-HT_3 receptor site. Thus, we propose to explain the binding of **5**, **6**, **7** and **8** to the 5-HT_3 receptor site by the occupancy of a common hydrophobic area for the alkoxy substituent and the distal ring of the naphthalene moiety. Consequently, the active conformation for an antiserotonergic benzamide should be the rotamer where the hydrogen bond is not involved, contrary to the antidopaminergic benzamides where it is a structural requirement essential for biological activity[14]. The size of the hydrophobic pocket can be inferred from the large lipophilic cyclic substituent of the potent antagonist, RG 12915, which cannot exhibit the hydrogen bond-stabilized conformer. This is due to an out-of-plane orientation of both electron pairs of the ortho-oxygen which has been demonstrated elsewhere for similar compounds[6]. Moreover, during our research on conformationally restricted antagonists of 5-HT_3 receptors, we synthesized the 1,2-naphthalamides **10** from (R) and (S)-3-aminoquinuclidine and the corresponding anhydride was prepared by a previously described process[15] for biological comparison with the amides **8** and **9**. With regard to compound **8**, an increase in the affinity and an inversion of the enantioselectivity were observed; indeed, the affinity for the 5-HT_3 receptor of (R)-**10** is several hundred-fold higher than that of the (R)-**8** enantiomer. The large increase in the potency of the (R) enantiomer is due to an inversion of the chiral recognition, indicating, doubtless, the existence of a new, important bond between the compound and the receptor site. The additional carbonyl group was implicated in this inversion of stereospecificity. A similar effect, recently reported by us, was observed with compounds derived from 1,8-naphtalimide[4] **1** where the (R)-4-amino enantiomer (**1**, R'= 4-NH_2) was equipotent as a 5-HT_3 receptor antagonist ($K_i\pm$ SEM = 0.15±0.04 nM) compared to (S) zacopride. Compound **10** (R) was tested as a 5-HT_3 receptor antagonist in the rat by assessment of the inhibition of the Bezold-Jarisch effect[16] (ID_{50} = 18.6 (6.6-52.5) µg/Kg/iv).

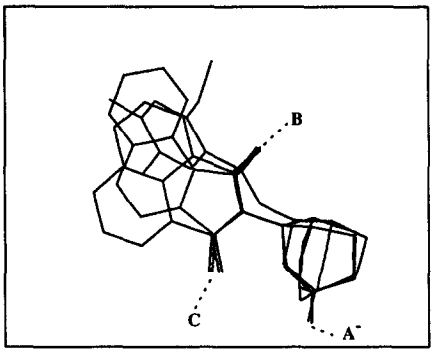


Figure 1. Superimposition of (S) zacopride and the (R) enantiomers of the 1,8 and 1,2-naphthalimide derivatives **1** and **10**. A^- is a putative negative charge, B is a hydrogen donor group and C is the polar group suggested by the (R) naphthalimide derivatives. RMS(Å) is, with regard to (S) zacopride, 0.45 for **1** and 0.48 for **10**.

The energetically stable conformers of (S) zacopride and the (R) enantiomers of **1** (R'= H) and **10** were superimposed in a fit operation with ALCHEMY[7] using as reference atoms the carbonyl functions of zacopride and those carried by the C1 and C8 aromatic atoms of **10** and **1** and the lone pair of the basic nitrogen atom (fig.1). Indeed, many data[17] suggest the existence of a hydrogen bond between the carbonyl group and a hydroxyl function of the receptor site, but also imply that there is an ionic bond between an

anionic group of the protein chain and the basic nitrogen heterocyclic atom. The model in figure 1 displays a suitable fit between both anchorage points for the considered compounds and emphasizes the putative existence of a second polar site for the binding of the additional carbonyl function of the naphthalimide derivatives. The improved recognition of the (R) enantiomers of **1** and **10** could be due to a strong interaction with this site which would oblige the aromatic moieties to occupy a different area to that of zacopride. Thus, two different aromatic binding sites could be implicated in the 5-HT_3 receptor site. Recent results highlight and support the essential role of an additional carbonyl function in the enantioselectivity. Thus, in a series of 2-alkoxybenzoylureas **11**, some potent 5-HT_3 antagonists were obtained[18] where the (R) enantiomers were more potent. On the other hand, the reduction of one carbonyl function in the 1,8-naphathalimide series resulted in the loss of the enantioselectivity[4], the (R) enantiomer being equipotent to the (S) compound.

The preliminary data reported in this paper emphasize the interest of derivatives of 1,2-naphthalimide as new 5-HT_3 receptor antagonists. Additional information on the mapping of the 5-HT_3 receptor binding site published recently[17] is also provided, particularly on the role of a second polar group in the receptor site which could be involved in the atypical properties of (R) zacopride compared to the reference compounds[19]. Several studies are in progress to design compounds capable of interacting only with this site.

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