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DERIVATIVES OF NAPHTHALIMIDE: NEW POTENT CONFORMATIONALLY RESTRICTED ANTAGONISTS OF 5-HT$_3$ RECEPTORS.

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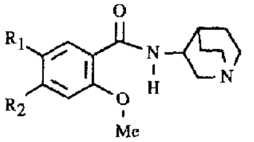
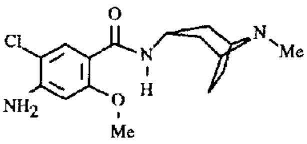
***Abstract:** New potent 5-HT$_3$ antagonists were synthesized from naphthalic anhydride and racemic or (R) and (S) 3-aminoquinuclidines. In contrast to zacopride, the activity resided essentially in the (R) enantiomer. Conformational studies demonstrated the presence of a locked structure. The reduction of one carbonyl function yielded equipotent compounds with a loss of enantioselectivity.*

Interest in the research on antagonists of 5-HT$_3$ receptors has been stimulated recently by the discovery of numerous pharmacological properties of this class of compounds in the field of central nervous and gastro intestinal systems[1]. Indeed, these compounds exhibit, in various animal models, anxiolytic[2], promnesic[3], antidepressant[4] and antiemetic[5]properties. Several studies on the structural analysis of the main antagonists of this class of receptor have already been reported[6,7] and they agree upon the existence of three structural elements implicated in the binding to the receptor site: an aromatic moiety, a carbonyl function or a bioisosteric group and a basic nitrogen atom. From these studies, it was clear that the carbonyl function was mandatory for recognition by the receptor, but the nature of the aromatic system and the basic nitrogen were ambiguous since potent compounds can be obtained from structures as diverse as an indole ring or benzamide with a basic nitrogen atom in an aromatic heterocycle as an imidazole or a heterocycle such as a quinuclidine. To improve the structural analysis of the pharmacophore of the 5-HT$_3$ receptor, it seemed to us essential to design a potent antagonist with a locked structure which should allow us to define the position of the basic nitrogen atom with regard to the aromatic plane and we were incited by a recent patent[8] to report our preliminary results[9] in this field.

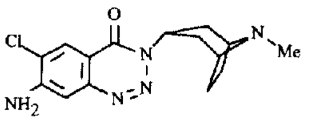
Zacopride **1**, a member of the benzamide family[10], is a very potent antagonist of this class of receptor and could be used as a molecular model to reach our goal. It possesses two structural parameters which had not been considered in the previous structural analysis: a virtual ring formed by the intramolecular bond between the amidic NH and the ortho methoxy group, a characteristic shared by the members of the benzamide family[11], and a chiral center providing an enantioselectivity for the affinity for the 5-HT$_3$ receptor, the (S) enantiomer being one order of magnitude more potent than the (R) isomer[12]. The essential role of the virtual ring had been clearly demonstrated for pharmacological activity in the class of antidopaminergic benzamides[11], but few studies have been reported with the 5-HT$_3$ receptor antagonist benzamides. The only such study published so far comes from King[13], who used BRL 24682 **3** to design a benzotriazinone **4** which can be regarded as a rigid analogue of the benzamide structure. Following a similar approach, we synthesized derivatives of 2-4-quinazolinediones **5** and **6** as analogues of cyclised benzamides of 3-aminoquinuclidine. These compounds should provide important structural informations about the suitable orientation of the lone pair of the basic nitrogen with regard to the aromatic plane needed to fit well into the receptor site. They were prepared through reaction between isatoic anhydride and 3-aminoquinuclidine followed by a cyclisation with ethyl chloroformate[14]. The antagonist activity for the 5-HT$_3$ receptor was evaluated in vitro by binding experiments with [^{3}H]-BRL 43694[15] using rat posterior cortex and by inhibition of the Bezold-Jarisch reflex[16] in the anesthetized rat (K_i (nM) and ID_{50} (μg/Kg/iv) respectively, (see footnotes Table I). The data obtained with compounds **5** and **6** (K_i = 80 and 30 nM and ID_{50} = 103 (52-205) and 80 µg/Kg/iv respectively) showed a loss of activity in comparison with the simple ortho methoxy benzamide **2** (K_i = 22 ± 3.7 nM and ID_{50} = 25(12-55) µg/Kg/iv). The drop in the affinity for the 5-HT_3 receptor could be due to the decrease of electronic density in the aromatic system with regard to the benzamide **2**. We have reported[17] recently the influence of this parameter on the affinity for the 5-HT_3 receptor and emphasized the favorable role of the naphthalene ring to obtain potent ligands. The design of structures such as the naphthalimide derivatives **7** could combine the favorable effect of a 10 π aromatic moiety and the rigid structure of 2-4-quinazolinedione compounds. These derivatives were directly synthesized from naphthalic anhydride and an amino derivative, R_4-NH_2[18]. The first results obtained with **10** (Table I) showed that the pharmacological activity was greatly increased with regard to compounds **5** and **6** and that the potency resided mainly with the quinuclidine framework. The reported data show the unfavorable effect on the activity of para substitution to the carbonyl function by halogen atoms (**11**, **12**) whereas it was increased to a value close to that of zacopride with the amino derivative **14**. It was noted also that the activity of the nitro compound **13** to inhibit the Bezold-Jarisch reflex was unrelated to its affinity for the 5-HT_3 receptor.



1 R_1= Cl, R_2= NH_2
2 R_1= R_2= H



3 BRL 24682



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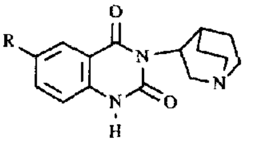
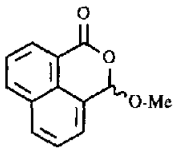


5 R= H
6 R= Cl



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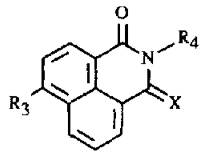
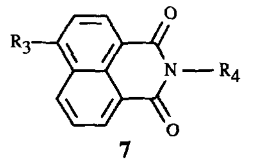


7 X= O
16 X= H_2, R_3= H, R_4= 3-quinuclidinyl

The influence of both carbonyl groups on the biological activity was evaluated with the reduced compound **16**. It was synthesized from the derivative **15** which was prepared by reduction of naphthalic anhydride and treatment with methanol[18]. **15** reacted slowly with 3-aminoquinuclidine to afford an amino ether which was reduced with $NaBH_4$ to yield the derivative **16**[19]. This compound was equipotent to **10** (K_i = 2.7 ± 0.4 nM in the binding test and ID_{50} = 8.5 (1.4-49)µg/Kg/iv in the Bezold-Jarisch), indicating that only one carbonyl function was implicated in the binding to the receptor.

The influence of the chirality of the quinucline moiety on the affinity for the 5-HT_3 receptor was studied with the enantiomers of **10** ((R), α_D^{25}= +24.6°, (c = 0.8, H_2O); (S), α_D^{25}= -26.4° (c = 0.8, H_2O)), **14** and **16** ((R), α_D^{25}= + 2.4°, (c = 1, H_2O); (S), α_D^{25}= -2.3°, (c = 1, H_2O)) prepared from (R) or (S)-3-aminoquinuclidine. As can be seen from Table I, the pharmacological data for the naphthalimide enantiomers, **10** and **14**, depended largely on the chirality of the quinuclidine framework, but we observed an inversion of the enantioselectivity with regard to the zacopride enantiomers since the (R) derivatives were much more active than the (S) derivatives in the both tests. In contrast, the enantiomers of the reduced compound **16** displayed a complete lack of enantioselectivity in the binding test (K_i= 2.3 ± 0.4 nM and 2.2 ± 0.4 nM for (R)-**16** and (S)-**16** respectively). These data seem to be important both to obtain a better insight into the recognition rules for the basic nitrogen atom by the 5-HT_3 receptor, and also to acquire information on the influence of the virtual ring in the benzamide series, since compound **16** and its enantiomers could be regarded as cyclised analogues of benzamide.

5-HT$_3$ SEROTONIN RECEPTOR ANTAGONIST ACTIVITIES



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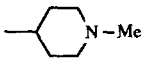
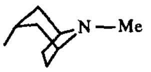
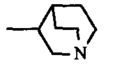
Compounds	R_3	R_4	$K_i \pm$ SEM,nM^a	ID$_{50}$µg/Kg/iv^b
8	H		15 ± 4	500
9	H		374 ± 26	>250
10	H		2.7 ± 0.4	11(1.7-74)
(R)-**10**	H	" "	0.9 ± 0.5	5.7(3.8-8.7)
(S)-**10**	H	" "	61 ± 4.9	224(81-615)
11	Cl	" "	14 ± 5	125(82-190)
12	Br	" "	41 ± 4	>250
13	NO_2	" "	31 ± 4.7	5.25(3-8)
14	NH_2	" "	0.5 ± 0.04	3(2.5-3.9)
(R)-**14**	"	" "	0.15 ± 0.04	1.6(0.7-3.6)
(S)-**14**	"	" "	23.5 ± 4	21(8-55)
(R)-zacopride **(S)-zacopride**			2.6 ± 0.4 0.2 ± 0.04	1.0(0.3-3.4) 0.2(0.05-0.75)

Table 1. a)[^{3}H]-BRL-43694 was used in the radiolabelled ligand assays[15]. Binding assays were carried out using rat posterior 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) Antagonism of the bradycardia induced by IV injection of 5-HT(10-30 µg/Kg). Compounds were administered 3 mn before 5-HT[16]. ID$_{50}$ values represent the dose of antagonist inhibiting 50% of the effect of serotonin and were calculated from three doses with $n \geq 3$ at each dose by linear-regression analysis.

A preliminary conformational analysis of the naphthalimide structure was made using the mechanic program of the ALCHEMY software[20]. Rotation around the C'3, N2 bond (figure 1) showed the existence of a strong steric hindrance between the oxygen atoms O1 and O3 of the carbonyl groups of **10** and the hydrogen atoms H4, H8a, H2b and H7a of the quinuclidine framework. The evaluation of the internal energy by a 360° rotation suggested the existence of stable conformations for a range of values for the dihedral angle C3-N2-C'3-C'2 between +130° and +140° and indicated for **10** a limited number of permissible conformations to interact with the receptor. X-Ray crystallography confirmed this analysis since the value found for the torsion angle was 141°[21]. This is the first example of a potent 5-HT$_3$ antagonist with a relatively locked structure. The reduction of one carbonyl group in the compound **10** caused an increase in the degrees of freedom giving, doubtless, more energetically stable conformers. At this step of the study, the causes of the loss of enantioselectivity for **16** were not clear and are currently under investigation.

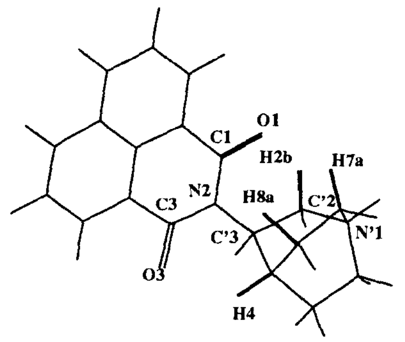


Figure 1. View with ALCHEMY of an energetically stable conformation of **10** and the atoms implicated in the steric hindrance.

To summarize, the above reported data demonstrate that naphthalimide derivatives with a quinuclidine framework constitute a new, important class of 5-HT_3 receptor antagonists with a locked conformation and an important enantioselectivity. They should contribute much information about the structural parameters of the 5-HT_3 pharmacophore.

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