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SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF A MACROCYCLIC BENZAMIDE.

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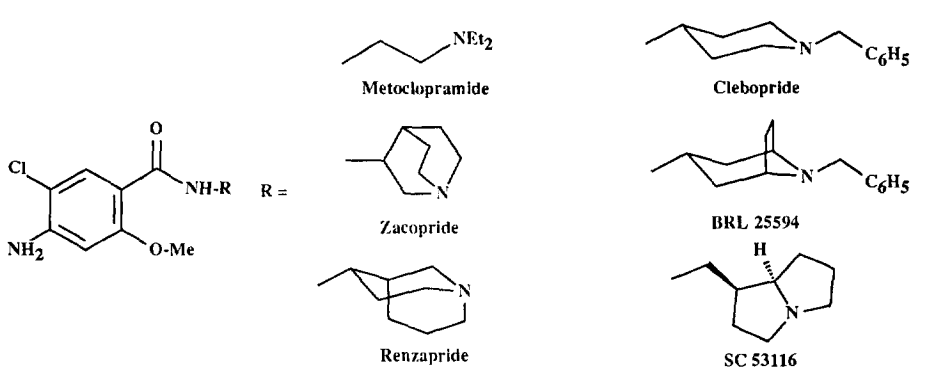
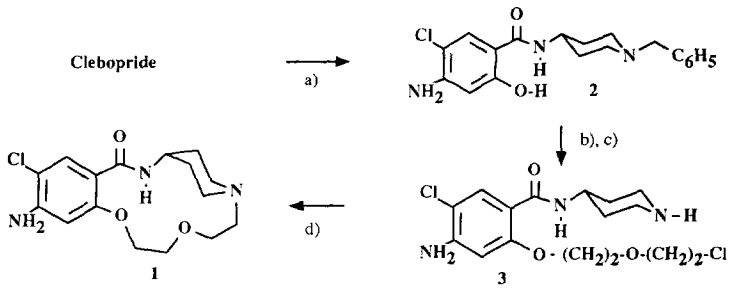
Abstract: a macrocyclic benzamide was synthesized and its affinities for 5-HT_3, 5-HT_4 and D_2 receptors were evaluated in binding assays. It was compared to reference benzamides possessing a piperidine ring with various orientations of the N-substituent. It was clearly demonstrated that the orientation of the basic nitrogen atom lone pair is an essential structural parameter in the recognition of 5-HT_3 and 5-HT_4 receptors.

A number of potent compounds with various pharmacological activities have been designed from the generic benzamide family[1]. Thus, zacopride[2] was described as a potent antagonist of 5-HT_3 receptors and has been used in man for the inhibition of cisplatin-induced emesis[3]. On the other hand, BRL 25594[4], clebopride[5] and eticlopride[6] were found to be potent antagonists of D_2 and D_3 dopaminergic receptors and constitute a new family of antipsychotic drugs which were supposed to possess less clinical side-effects than existing compounds. Recently, other compounds such as renzapride[7] and SC 53116[8] were shown to be potent agonists for 5-HT_4 receptors[9] located in the gastro-intestinal system and their gastro-kinetic effect are now thought to involve a 5-HT_4 receptor-based mechanism. These gastro-kinetic, antiemetic and neuroleptic properties are also seen with metoclopramide[10], the parent molecule of this family.



Almost all of these compounds possess a 4-amino-5-chloro-2-methoxy benzamide group as the aromatic moiety and a basic framework which seems to be implicated in the affinity and selectivity of the compounds for the different receptor types. Thus, the weak selectivity of metoclopramide can be explained by the large number of permissible conformers of the basic chain. On the other hand, the basic, rigid, folded bicyclic structure of zacopride, renzapride and SC 53116 gives compounds with good selectivity for 5-HT_3 or 5-HT_4 receptors[11], while the compounds with a hydrophobic substituent on the nitrogen atom and an extended shape such as BRL 25594 and clebopride have high affinity for D_2 receptors[12]. Thus, hydrophobic parameters, the relative position of the aromatic ring and the basic heterocycle and the orientation of the basic nitrogen atom lone-pair seem to be crucial parameters for recognition by 5-HT_3 and 5-HT_4 receptors or by D_2 receptors. The synthesis and pharmacological study of a conformationally-locked 4-amino-5-chloro-2-methoxy benzamide could provide important structural information on the parameters required for recognition by one or other receptor and constitute a step for the design of new selective, potent ligands. The synthesis of the macrocyclic

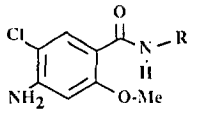
benzamide **1** with the piperidine framework enclosed within a macrocycle structure is shown in Scheme 1. The synthetic pathway was similar to that described by Markovic[13] for an analogue compound. Clebopride, prepared according to a classical method, was demethylated by BBr_3 in methylene chloride to give the hydroxy compound **2**. It was monoalkylated by 2-chloroethyl ether in the presence of K_2CO_3 in DMF and then debenzylated by catalytic hydrogenation with $Pd(OH)_2$ to compound **3**. It was easily cyclized to the macrocyclic benzamide **1** (yield: 69%) by heating in DMF with K_2CO_3. The structure of **1** was identified by ^1H and ^{13}C NMR spectra and mass spectrometry. Several structurally-closely related benzamides (Table 1), possessing the piperidine ring in the basic framework were synthesized according to classical methods.

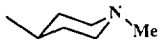
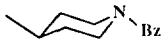
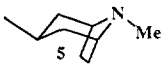
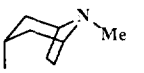
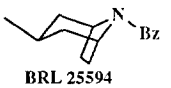


SCHEME 1. a) BBr_3, CH_2Cl_2, r.t. b) $(ClC_2H_4)_2O$, DMF 100°C, KI, K_2CO_3 c) H_2, AcOH, $Pd(OH)_2/C$ 20% d) K_2CO_3, KI, DMF 100°C.

The affinities of compound **1** and the other benzamides reported in Table 1 were determined using binding assays: for 5-HT_3 and 5-HT_4 receptors, competition for [^{3}H]-BRL-43694[14] and [^{3}H]-GR-113808[15] binding sites in the rat posterior cortex and rat striatum respectively was used, while affinity for D_2 receptors was evaluated with [^{3}H]-spiperone in rat striatum[16]. The structural differences between the compounds stem from the hydrophobic nature of the nitrogen substituent or the stereochemistry of the benzamide moiety which occupies the equatorial or axial position on the piperidine framework. The data clearly demonstrate the role of the conformationally-restricted amino chain for the selectivity of the compounds. Metoclopramide, with a flexible aminoethyl chain, does not display any selectivity and has moderate affinity for the three receptor types, while the other compounds possess potent affinity for at least one receptor. Thus, as has already been reported[12], the role of the N-benzyl substitution and the extended shape of the molecules were shown to be important for the recognition of the D_2 receptor by clebopride and BRL 25594 which both had high affinity for this receptor. On the other hand, the 5-HT_3 receptor antagonist binding site prefers compounds with a more compact basic framework such as zacopride, BRL 24682, **4** and **5**. However, the affinity of the piperidine derivatives depends upon the stereochemistry of the benzamide group and is particularly marked with the axial compound BRL 24682, as has already been reported[17]. Moreover, the 5-HT_3 receptor antagonist benzamides are not selective and posssess a fairly good affinity for 5-HT_4 receptors[18], related to their gastro-kinetic properties. However, the K_i(5-HT_3)/K_i(5-HT_4) ratios for almost all of the 5-HT_3 receptor antagonists indicate better recognition of 5-HT_3 receptors than 5-HT_4 receptors. On the other hand, the data reported herein for **1** demonstrated an inversion of this ratio with a superior affinity for 5-HT_4 receptors (K_i= 29.7 ± 6.8), as it has been already reported for SC 53116[8], indicating a better fit with this receptor. Further studies[20] confirmed the interest of **1** which showed a partial agonist profile for 5-HT_4 receptors. In order to understand this difference, a conformational study of **1** was performed on an IRIS INDIGO XS400 workstation with SYBYL 6.03[19] with the Random Search option. The minimal energy conformer was characterized by the chair conformer of the piperidine ring with the benzamide function in the axial position and, consequently, it was structurally related to BRL 24682. These two compounds were fitted with a minima energy conformer of zacopride, selected as the active form[22], with regard to the carbonyl group and the basic nitrogen which were considered as the binding points in the receptor site (fig. 1). The values of RMS (0.25 and 0.27 for the fit of **1** with zacopride and BRL 24682 respectively) showed that the compounds were well superimposed, indicating a very structurally-closely related pharmacophore for 5-HT_3 and 5-HT_4 receptors.

BINDING PROFILE FOR 5-HT$_3$, 5-HT$_4$ AND D$_2$ RECEPTORS OF VARIOUS 4-AMINO-5-CHLORO-2-METHOXY BENZAMIDES AND COMPOUND 1



R	K_i, nM (5-HT$_3$)[a]	K_i, nM (5-HT$_4$)[b]	K_i, nM (D$_2$)[c]
 Metoclopramide	443 ± 58	974 ± 63	285 ± 25
 4	267 ± 59	832 ± 54	> 1000
 Clebopride	> 1000	104 ± 58	11.9 ± 3.8
 5	41.8 ± 5.3	> 1000	> 1000
 BRL 24682	0.8 ± 0.2	48 ± 5.6	> 1000
 BRL 25594	> 1000	233 ± 60	0.28 ± 0.04
1	53.2 ± 4.7	29.7 ± 6.8	> 1000
(S)-Zacopride	0.2 ± 0.04	383 ± 64	>1000

TABLEI. a) [^{3}H]-BRL-43694 was used as the radioligand and the binding assays were carried out using rat posterior cortex (30 min-25°C). Non-specific binding was determined with GR 38032F (10 μM). b) [^{3}H]-GR-113808 was used as the radioligand and the binding assays were carried out using rat striatum (30 min-25°C). Non-specific binding was determined with the 5-HT$_4$ agonist recently described by us[11]. c) [^{3}H]-Spiperone was used as the radioligand and the binding assays was carried out using rat striatum (25°C-30 min). Non specific binding was determined with butaclamol. d) 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.

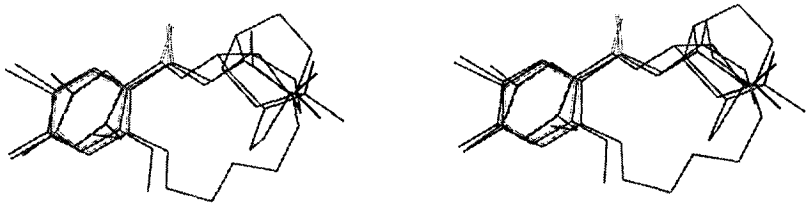


Fig.1. Stereoview of the fit of **1**, zacopride and BRL 24682 with regard to the aromatic carbons, the CO groups and the basic N atoms.

However, examination of fig. 1 shows that the orientation of the lone pairs of the basic nitrogen atom of **1**, BRL 24682 and zacopride is orthogonal. The relative equipotency of **1** and BRL 24682 for 5-HT$_4$ receptors can be explained by the similar orientation of the lone pair. On the other hand, the high affinity of BRL 24682 for 5-HT$_3$ receptors, which is equipotent to zacopride, can be due to the possible inversion of the methyl group of the tropane framework in the axial position[24], bringing about the suitable orientation of the lone pair for this binding site.

In summary, these data can suggest a similar binding site for the pharmacophores for 5-HT$_3$ and 5-HT$_4$ receptors but with difference between the spatial positions of the anionic head in the receptors. The benzamides

with a locked conformation such as **1** provide useful tools for improving the understanding of the role of structural parameters in the recognition of these compounds by D_2, 5-HT_3 and 5-HT_4 receptors and studies are in progress for the synthesis of new macrocyclic benzamides.

Acknowledgements

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