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1-[ω-(4-ARYLPIPERAZIN-1-YL)ALKYL]-3-DIPHENYLMETHYLENE-2,5-PYRROLIDINEDIONES AS 5-HT$_{1A}$ RECEPTOR LIGANDS: STUDY OF THE STERIC REQUIREMENTS OF THE TERMINAL AMIDE FRAGMENT ON 5-HT$_{1A}$ AFFINITY/SELECTIVITY

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Abstract: In the present paper, we report the synthesis and the binding profile on 5-HT$_{1A}$, α_1 and D_2 receptors of a new series of imide-arylpiperazines **3**. The study of the length of the alkyl chain and the imide substructure allows us to suggest some important differences between the no-pharmacophoric sites of both 5-HT$_{1A}$ and α_1-adrenergic receptors, which could be of great importance in order to design new selective ligands.

Most of ligands with affinity for the 5-HT$_{1A}$ receptor exhibit high level of undesired affinity for the α_1-adrenergic receptor, due to these receptors having a high degree of similarity (45%) in their amino acid sequence.[1] Even for the long-chain arylpiperazines with an amide or imide moiety, which represent the class of the 5-HT$_{1A}$ receptor ligands most thoroughly studied up to date, the structural features that decide their selectivity *versus* α_1-adrenergic receptor are not yet clear.[2] The pharmacophore interaction with the 5-HT$_{1A}$ receptor active site has been described in detail.[3] The influence of the length of the spacer on 5-HT$_{1A}$ affinity is also well known,[2b,4] in contrast to the role of the amide substructure. Some authors have reported that the presence of the terminal amide fragment plays an important role in the stabilization of the 5-HT$_{1A}$ receptor-ligand complex and this interaction can be lipophilic,[2h,3d] electronic[5] or steric,[6] while another hypothesis[4a,7] has suggested that the amide function is not required for binding with the 5-HT$_{1A}$ receptor.

In previous papers[8] we have reported the synthesis and quantitative structure-activity relationships (3D-QSAR) of a series of bicyclohydantoin-arylpiperazines **1**, which showed affinity for 5-HT$_{1A}$ and α_1 receptors. These studies give us a better understanding of the α_1/5-HT$_{1A}$ selectivity of the arylpiperazine substitution and lead us to conclude that the hydantoin moiety and the side chain length seem to modulate not only the affinity but also the α_1/5-HT$_{1A}$ selectivity. In a recent work,[9] we described new derivatives **2**, which are devoid of the terminal amide fragment

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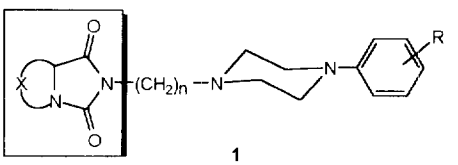
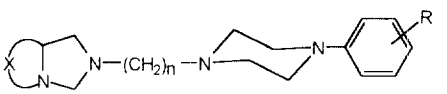
present in related 5-HT_{1A} ligands, but which preserve the steric requirements of this moiety. SAR studies suggest that there is influence of electronic factors on the no-pharmacophoric part of the α_1 receptor site, however they have no influence on the stabilization of the 5-HT_{1A} receptor-ligand complex. In order to gain insight into the role of the amide moiety of this kind of ligand in the affinity and selectivity for 5-HT_{1A} receptors, we have considered a new series of arylpiperazines **3**, in which we have explored some steric requirements by modifying the size of the amide portion, with respect to the bicyclohydantoins **1**. We have taken into account a significant increase in the van der Waals volume of the no-pharmacophoric part (ΔV_W approximately 100 Å^3). In the present paper, we report the synthesis and the binding profile on 5-HT_{1A}, α_1 and D_2 receptors of compounds **3**, where the length of the spacer is 1-4 methylenes, and the arylpiperazines are present in compounds **1** with the highest affinity for the 5-HT_{1A} receptor.



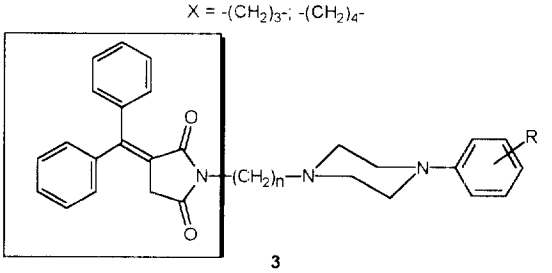
1

X = -$(CH_2)_3$-; -$(CH_2)_4$-



2

X = -$(CH_2)_3$-; -$(CH_2)_4$-



3

n = 1, 2, 3, 4

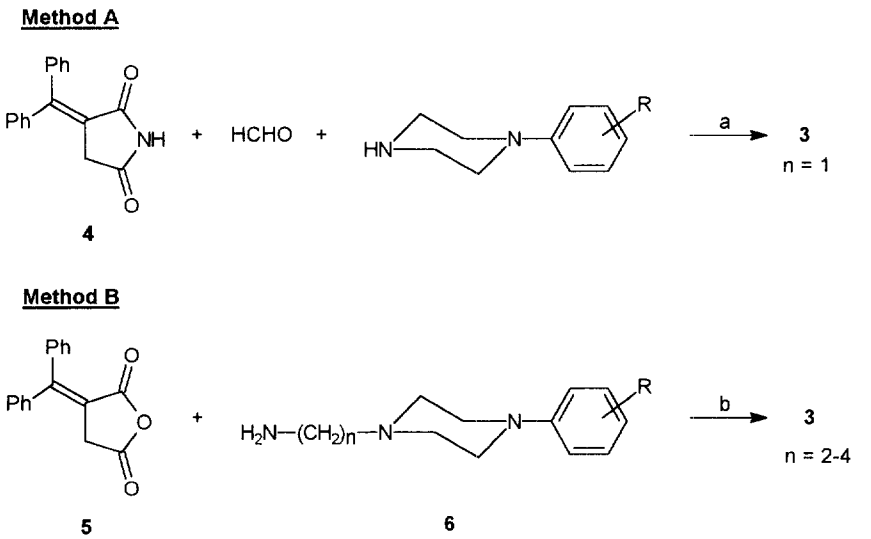
R = H, *o*-OCH_3, *m*-Cl, *m*-CF_3, *p*-F

Chemistry

Two pathways (Scheme 1) were used in order to prepare the imide-derivatives **3**. Compounds **3** (n = 1) were prepared by Mannich reaction of the imide **4**[10] with formaldehyde and the appropriate arylpiperazines (Method A). The desired compounds **3** (n = 2-4) were obtained through Method B, by reaction of the anhydride **5**[11] with the corresponding 1-(ω-aminoalkyl)-4-arylpiperazines **6** in acetic acid as solvent. The 1-(ω-aminoalkyl)-4-arylpiperazines **6** were prepared following standard procedures[12] by reduction of the corresponding nitriles with lithium aluminium hydride in THF. Hydrochloride salts of the target compounds **3** were prepared as samples for biological assays. A

new compounds were characterized by IR, ^1H and ^{13}C NMR spectroscopy and gave satisfactory combustion analyses (C, H, N).[13]

Scheme 1



Reagents and conditions: (a) EtOH, 100 °C, 1-3 h; (b) acetic acid, reflux, 24-30 h.

Results and Discussion

The compounds were evaluated for *in vitro* 5-HT_{1A}, α_1 and D_2 receptors affinity by radioligand binding assays, using the following specific ligands and tissue sources: (a) serotonin 5-HT_{1A} receptors, [^{3}H]-8-OH-DPAT, rat cerebral cortex membranes;[14] (b) adrenergic α_1 receptors, [^{3}H]prazosin, rat cerebral cortex membranes;[15] (c) dopamine D_2 receptors, [^{3}H]raclopride, rat striatum membranes.[16] The receptor binding affinities (K_i, nM) are shown in Table 1. Most of compounds **3** demonstrated moderate to high affinity for 5-HT_{1A} and α_1 receptor binding sites, and have no affinity for D_2 receptors.

The study of the results presented in Table 1 shows that the imide substructure together with the length of the alkyl chain play an important role in the affinity and selectivity for the 5-HT_{1A} receptor. On the other hand, the influence of the phenylpiperazine ring substitution on the affinity at both receptors is in agreement with our previous reports.[8b,c] One of the most important facts observed in Table 1 is that compounds **3** (n = 1) display moderate affinity for 5-HT_{1A} receptors and are almost inactive at α_1 receptors, so they show a good 5-HT_{1A} selectivity *versus* α_1-adrenergic receptors. Compounds **1**[8a,b] with one carbon alkyl chain showed a similar trend and this structural feature has become one of the few structural modulators of selectivity between 5-HT_{1A} and α_1-adrenergic receptors. On the other hand, the size of the imide substructure has not an important influence on 5-HT_{1A} affinity when there is a one carbon atom spacer. Thus, compound **3c** (R = *m*-Cl) has similar K_i values to the corresponding bicyclohydantoins

1 (X = -$(CH_2)_3$-: K_i (5-HT_{1A}) = 58.4 nM; X = -$(CH_2)_4$-: K_i (5-HT_{1A}) = 57.7 nM), despite the fact that bicyclohydantoins have half the van der Waals volume.

Table 1. Binding Data[a]

compd	*n*	R	K_i (nM) 5-HT_{1A} [^{3}H]-8-OH-DPAT	α_1 [^{3}H]prazosin	D_2 [^{3}H]raclopride
3a	1	H	98.0 ± 5.0	> 1000	> 1000
3b	1	*o*-OCH_3	93.8 ± 4.4	> 1000	> 1000
3c	1	*m*-Cl	49.4 ± 8.0	392 ± 44	> 1000
3d	1	*m*-CF_3	59.6 ± 4.3	> 1000	> 1000
3e	1	*p*-F	> 1000	746 ± 13	> 1000
3f	2	H	> 1000	214 ± 24	> 1000
3g	2	*o*-OCH_3	286 ± 5	71.5 ± 0 3	167[b]
3h	2	*m*-Cl	> 1000	> 1000	> 1000
3i	2	*m*-CF_3	> 1000	> 1000	> 1000
3j	2	*p*-F	> 1000	529 ± 19	> 1000
3k	3	H	> 1000	5.7 ± 0.3	> 1000
3l	3	*o*-OCH_3	665 ± 21	8.1 ± 1.2	156[b]
3m	3	*m*-Cl	127 ± 12	39.2 ± 7.5	801[b]
3n	3	*m*-CF_3	> 1000	62.7 ± 0.1	> 1000
3o	3	*p*-F	> 1000	8.3 ± 1.1	> 1000
3p	4	H	125 ± 36	6.4 ± 0.9	> 1000
3q	4	*o*-OCH_3	13.4 ± 3.3	14.4 ± 1.4	> 1000
3r	4	*m*-Cl	39.3 ± 8.9	16.4 ± 1.8	367[b]
3s	4	*m*-CF_3	15.8 ± 2.6	60.5 ± 1.8	689[b]
3t	4	*p*-F	3.3 ± 0.3	51.8 ± 1.1	351[b]

[a]All values are the mean ± SEM of two to four experiments performed in triplicate.

[b]Values taken from only one experiment.

An increase in the size of the alkyl chain to $n = 2$ leads to a marked decrease in the affinity for 5-HT_{1A} receptors and remains the inactivity for α_1 receptors. Only compound **3g** displays a moderate affinity for α_1-adrenergic sites.

The influence on affinity at both receptors of the size of the imide moiety, in compounds with three to four methylenes in the spacer, has a great importance since this structural feature could represent another selectivity modulator between 5-HT_{1A} and α_1-adrenergic receptors. So, ligands with a three carbon chain in the spacer display very low affinity at 5-HT_{1A} receptors, in contrast to the results we previously described,[8b] since the bicyclohydantoins **1** displayed high affinity with $n = 3$. Thus, the activity of compound **3n** (R = *m*-CF_3) is approximately 200-fold less than the activity of the corresponding bicyclohydantoin derivatives **1** (X = -$(CH_2)_3$-: K_i (5-HT_{1A}) = 3.8 nM; X = -$(CH_2)_4$-: K_i (5-HT_{1A}) = 5.7 nM). By contrast, compounds **3** have similar α_1-adrenergic K_i values to the bicyclohydantoins **1**. The loss of affinity for 5-HT_{1A} receptor binding sites in derivatives with $n = 3$ leads to highly selective compounds for α_1-adrenergic receptors. So, analogs **3k** and **3o** display high affinity for α_1 receptors and are inactive at 5-HT_{1A} binding sites.

Finally, an increase in the length spacer to four carbons leads to compounds with the highest affinity at 5-HT_{1A} receptors. Regarding the α_1-adrenergic receptor, an increase in the length spacer to $n = 4$ remains the high affinity at α_1 receptors for compounds **3**.

The study of the length of the alkyl chain and the imide substructure allows us to suggest some important differences between the no-pharmacophoric sites of both 5-HT_{1A} and α_1-adrenergic receptors, which could be of great importance in order to design new selective ligands. Regarding the 5-HT_{1A} receptor, these data define an optimum length of the spacer of four carbons, since compounds **3** with $n = 3$ are inactive. Ligands with $n = 3$ and with a no-pharmacophoric part of approximately 200 $Å^3$ would not reach the active site of the pharmacophore, while a decrease of this volume to approximately 100 $Å^3$ in the bicyclohydantoin derivatives **1** allows the ligand-receptor interaction, probably because a part of the hydantoin portion acts as a spacer. With respect to the α_1-adrenergic receptor, the optimum spacer length is three to four methylene units, so several compounds with $n = 3$ have shown high selectivity for this receptor. On the other hand, the abnormal affinity of derivatives **3** ($n = 1$) for 5-HT_{1A} receptors and the non influence of the imide portion on the affinity suggest an alternative manner of interaction with the receptor of this kind of ligand. This structural feature could be of great importance in order to design new selective ligands.

Compounds **3k** and **3o**, which are highly selective for α_1-adrenergic receptors *vs* 5-HT_{1A} and D_2 receptors, were evaluated for agonistic/antagonistic activity on ring segments of rat thoracic aorta contracted by phenylephrine. Compound **3o** ($pA_2 = 8.63$) displays high potency as antagonist at α_1-adrenergic receptors, while **3k** ($pA_2 = 7.79$) shows a moderate potency.

The agonistic/antagonistic character at the 5-HT_{1A} receptor of compounds **3d**, which is the most selective member of these series, and **3t**, with the highest affinity at this receptor, was evaluated by their ability to affect adenylyl cyclase activity in a rat hippocampus slice preparation. The results indicate that both compounds retain agonist properties at postsynaptic 5-HT_{1A} receptors. The same 5-HT_{1A} agonistic profile found in compounds **3d** ($n = 1$) and **3t** ($n = 4$) might suggest the existence of two different no-pharmacophoric steric pockets in this receptor. The synthesis of new compounds to further verify and exploit this working hypothesis is ongoing.

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