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Design and Synthesis of *S*-(−)-2-[[4-(napht-1-yl)piperazin-1-yl]-methyl]-1,4-dioxoperhydropyrrolo[1,2-*a*]pyrazine (CSP-2503) Using Computational Simulation. A 5-HT_{1A} Receptor Agonist

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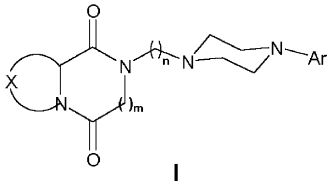
Abstract—Based on a computational model for 5-HT_{1A}R-ligand interaction and QSAR studies, we have designed and synthesized a new series of arylpiperazines **2**–**8** which exhibit high 5-HT_{1A}R affinity and selectivity over α_1-adrenergic receptors. Among them, compound CSP-2503 (**4**) has been pharmacologically characterized as a 5-HT_{1A}R agonist at somatodendritic and postsynaptic sites, endowed with anxiolytic properties.

Introduction

The identification of multiple serotonin (5-HT) receptor subtypes in recent years has been accompanied by a parallel explosion in the development of drugs that alter 5-HT neurotransmission.[1,2] Specially, the 5-HT_{1A} receptor (5-HT_{1A}R) is a major target for neurobiological research and drug development, due to its implication in many (patho)physiological processes.[3–5] Agonists and partial agonists have been proven to be effective in anxiety and depression.[6–9] In addition to therapeutic applications in the field of psychiatry, more recent preclinical studies have suggested that 5-HT_{1A}R agonists have also pronounced neuroprotective properties.[10–12]

In the course of a program aimed at the discovery of new 5-HT_{1A}R agents, we have synthesized a series of arylpiperazines of general structure **I** ($n=3$, 4),[13–19] which showed affinity for both 5-HT_{1A} and α_1-adrenergic receptors due to the high degree of homology in both their transmembrane amino acid sequence and structure. It is widely accepted that the rhodopsin family of G protein-coupled receptors (GPCRs), including receptors for biogenic amines, share a comparable transmembrane structure formed by a highly organized heptahelical transmembrane bundle.[20] In the present work, we have used a computational model between the 5-HT_{1A}R and arylpiperazines of formula **I** (X = –$(CH_2)_3$–, $m=0$, $n=4$, Ar = *m*-(ethylsulfonamido)phenyl;[21] X = –$(CH_2)_3$–, $m=0$, $n=4$, Ar = *m*-(acetylamino)phenyl)[22] and previous 3-D-QSAR studies[23] for the synthesis of a new series of arylpiperazines **I** ($n=1$) which exhibit high 5-HT_{1A}R affinity and selectivity over α_1-adrenoceptors. Among them, compound CSP-2503 (**4**) has been pharmacologically characterized as a 5-HT_{1A}R agonist endowed with anxiolytic properties.



I

Computational simulation

Figure 1a shows compound **1** (X = –$(CH_2)_3$–, $m=0$, $n=4$, Ar = naphth-1-yl) in the binding pocket of the 5-HT_{1A}R. This type of arylpiperazine, with a chain length of $n=4$ connecting both rings, was predicted[21,22]

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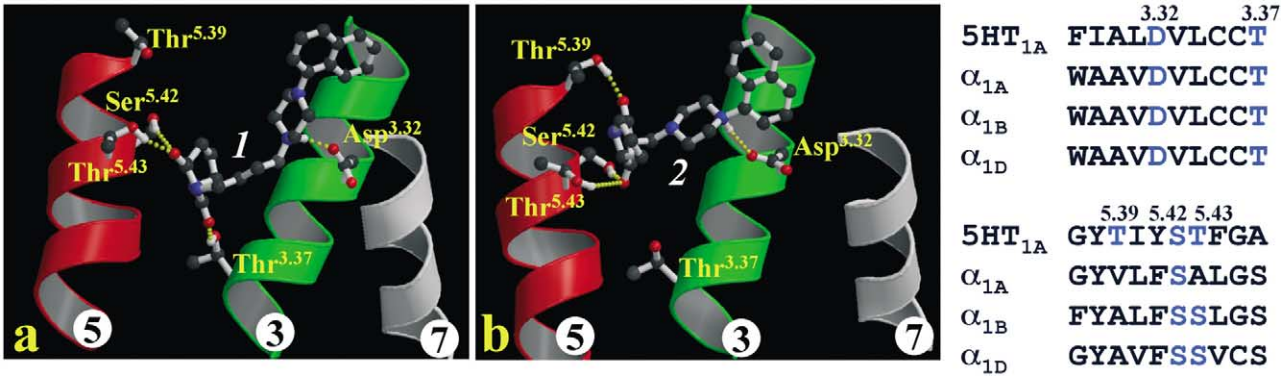
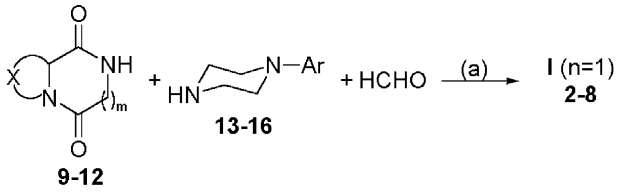


Figure 1. Interactions of compounds **1** and **2** with a model of the 5-HT_{1A}R. Hypothesis for 5-HT_{1A}/α_1 selectivity.

to interact with $Asp^{3.32}$ throughout the protonated NH group of the piperazine ring, and with $Thr^{3.37}$, $Ser^{5.42}$, and $Thr^{5.43}$ throughout the hydantoin moiety. This model reproduces the suggested interaction of Ser/Thr at positions 5.42 and 5.43 with the hydroxyl/carbonyl moiety of the ligand.[24] The presence of $Asp^{3.32}$, $Thr^{3.37}$, $Ser^{5.42}$, and Thr/$Ser^{5.43}$ in both 5-HT_{1A} and α_1-adrenergic receptors (Fig. 1) explains the lack of selectivity of arylpiperazine derivatives with $n=4$. Notably, $Thr^{5.39}$, located in helix 5, is pointing inside the bundle and is present only in the 5-HT_{1A}R (Fig. 1). Thus, the interaction of the ligand with the side chain of $Thr^{5.39}$ would provide the desired selectivity. This interaction is achieved by shortening the chain length to $n=1$ and avoiding the ligand to expand deep in the bundle as compound **1**. Figure 1b shows compound **2** (X = $-(CH_2)_3-$, m = 0, n = 1, Ar = naphth-1-yl) interacting with $Asp^{3.32}$ throughout the protonated NH group of the piperazine ring, and with $Thr^{5.39}$, $Ser^{5.42}$, and $Thr^{5.43}$ throughout the hydantoin moiety. Table 1 shows the binding affinity of these compounds. Clearly, compound **2** is selective versus the α_1-adrenoceptor while **1** binds both receptors, as predicted by the computer models.

Chemistry

Based on the proposed hypothesis for 5-HT_{1A}/α_1 selectivity, we have synthesized a new series of arylpiperazines **I** ($n=1$), in which the volume of the pharmacophore is increased in positions *ortho* and *meta* of the aromatic ring. Previous QSAR studies[23] showed that this is an additional structural feature that accounts for 5-HT_{1A}/α_1 selectivity. Compounds **2–8** were obtained by Mannich reaction of bicyclohydantoins **9**,**10**[25,26] or diketopiperazines **11**,**12**[27,28] with the appropriate bicycloarylpiperazines **13–16**[29–31] in the presence of formaldehyde (Scheme 1).



Scheme 1. (a) See Tables 1 and 2 for chemical structures of compounds **2–8**.

Table 1. Binding data of compounds I[a]

Compd	X	*m*	*n*	Ar	$K_i \pm$SEM (5-HT_{1A})	$K_i \pm$SEM (α_1)
1	$(CH_2)_3$	0	4	Naphth-1-yl	2.4 ± 0.1	64.9 ± 1.5
2	$(CH_2)_3$	0	1	Naphth-1-yl	10.4 ± 0.8	> 1000

[a]Values are means of 2–4 experiments performed in triplicate.

Pharmacology

Affinity data

Target compounds were assessed for in vitro binding affinity at serotoninergic 5-HT_{1A} and α_1-adrenergic receptors by radioligand binding assays, using [^{3}H]-8-OH-DPAT[32] and [^{3}H]prazosin,[33] respectively, in rat cerebral cortex membranes. All the synthesized compounds **2–8** exhibited high 5-HT_{1A}R affinity and selectivity over α_1-adrenergic receptors (Table 2), confirming our hypotheses for 5-HT_{1A}/α_1 selectivity in this class of arylpiperazine ligands. Compound **4** (CSP-2503) was also evaluated for affinity at serotonin 5-HT_{2A} ($K_i = 13.5 \pm 2.5$ nM), 5-HT_3 ($K_i = 8.9 \pm 1.4$ nM), 5-HT_4 ($K_i > 10{,}000$ nM) and 5-HT_7 ($K_i = 100.9 \pm 1.4$ nM) receptors, serotonin transporter ($K_i = 976.3 \pm 42.8$ nM), dopamine D_2 receptors ($K_i = 192.1 \pm 20.1$ nM), and benzodiazepine receptors ($K_i > 10{,}000$ nM). The following specific ligands and tissue sources were used: 5-HT_{2A}, [^{3}H]ketanserin, rat cerebral frontal cortex membranes;[34] 5-HT_3, [^{3}H]LY278584, rat cerebral cortex membranes;[35] 5-HT_4, [^{3}H]GR113808, rat striatum membranes;[36] 5-HT_7, [^{3}H]-5-CT, rat hypothalamus

Table 2. Binding data of compounds I[a]

Compd	X	*m*	Ar	$K_i \pm$SEM (5-HT_{1A})	$K_i \pm$SEM (α_1)
3	$(CH_2)_4$	0	Naphth-1-yl	5.6 ± 0.3	> 1000
4 (CSP-2503)	$(CH_2)_3$	1	Naphth-1-yl	4.1 ± 1.2	> 1000
5	$(CH_2)_4$	0	Benzodioxan-5-yl	9.3 ± 0.4	> 1000
6	$(CH_2)_4$	0	Benzodioxepin-6-yl	6.1 ± 0.4	> 1000
7	$(CH_2)_3$	1	Benzodioxepin-6-yl	12.3 ± 2.1	> 1000
8	$(CH_2)_3$	0	Benzimidazol-4-yl	4.1 ± 0.2	> 10,000

[a]Values are means of 2–4 experiments performed in triplicate.

membranes;[37] 5-HT transporter, [^{3}H]paroxetine, rat cerebral cortex membranes;[38] D_2, [^{3}H]raclopride, rat striatum membranes;[39] benzodiazepine, [^{3}H]flunitrazepam, rat cerebral cortex membranes.[40]

Pharmacological characterization of CSP-2503 (4)

Presynaptic 5-HT_{1A}R activity was assessed by measuring mouse rectal temperature.[41] The administration of CSP-2503 provoked a dose related decrease in mice rectal temperature (Fig. 2). This induced hypothermia suggests that CSP-2503 acts on 5-HT_{1A} somatodendritic autoreceptors.

The transduction mechanism of CSP-2503 was determined by using HeLa cells expressing human 5-HT_{1A}Rs.[42] CSP-2503 inhibited in a dose dependent manner the cAMP increase induced by forskolin. The half maximal effect (EC_{50}) observed was 0.15 μM and the maximal inhibitory effect was 90.3±1.3%. This negative control of CSP-2503 on adenylate cyclase activity indicates a transduction system coupled to 5-HT_{1A}R stimulation.

Functional activity of CSP-2503 on 5-HT_{1A}Rs was further assessed by evaluating its ability to decrease 5-HT neuronal activity.[43] The administration of CSP-2503 induced a decrease in 5-hydroxyindoleacetic acid (5-HIAA)/5-HT ratio in whole hypothalamus of mice (Fig. 3). These results further indicate that CSP-2503 behaves as a 5-HT_{1A}R agonist acting at the somatodendritic site.

Furthermore, we have evaluated the potential anxiolytic activity of CSP-2503 by using the light/dark box test.[44] Indeed, the administration of CSP-2503 (10 mg/kg) caused an increase in the time that mice spent in the lit area (155.4±9.3 vs 83±13 s, $P<0.05$). The 5-HT_{1A}R agonist 8-OH-DPAT was tested in the same test as reference compound, at a dose of 2.5 mg/kg (time spent in the lit area: 188.8±26 vs 105±14.7 s). These measurements were performed thirty min after the *sc* administration of the drug or vehicle and for the period of 5 min.

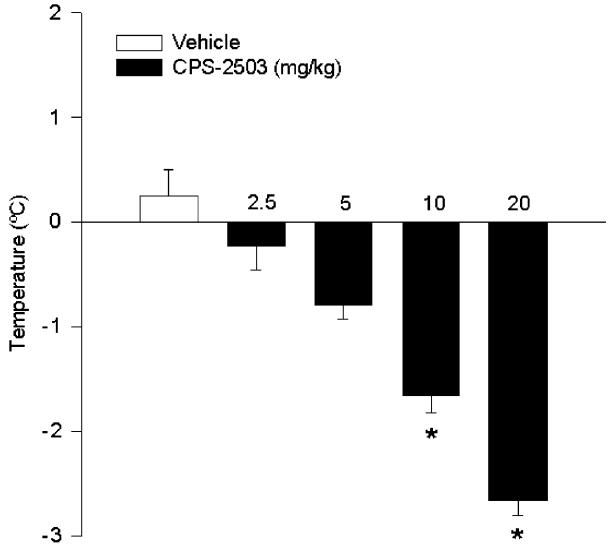


Figure 2. Dose–response effect of CSP-2503 on rectal temperature. *Values of CSP-2503 that decrease more than 1.1 °C and are significantly different ($P<0.05$) from their respective basal rectal temperature before *sc* drug administration.

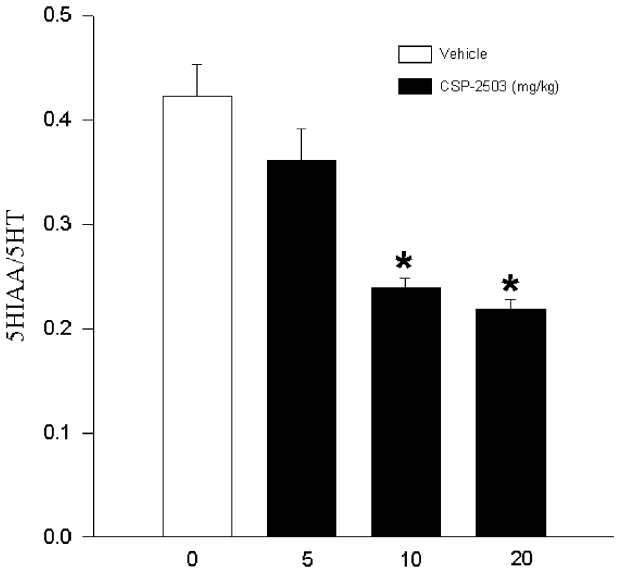


Figure 3. Dose–response effect of CSP-2503 on hypothalamic 5-HT activity. *Values of CSP-2503 treated mice that are significantly different ($P<0.05$) from vehicle group.

These results indicate that CSP-2503 is an agonist of the 5-HT_{1A}R at the somatodendritic and postsynaptic sites, with anxiolytic potential. In order to complete its pharmacological profile, further behavioural and neurochemical evaluation are currently in progress, though the present data suggest that CSP-2503 may be therapeutically useful in the treatment of anxiety-related disorders.

Conclusions

Based on our recently proposed computational model for 5-HT_{1A}R-ligand interaction, we have synthesized a new series of arylpiperazines **I** ($n=1$) which exhibit high 5-HT_{1A}R affinity and selectivity over α_1-adrenergic receptors. Among them, compound CSP-2503 (**4**) has been pharmacologically characterized as a 5-HT_{1A}R agonist at somatodendritic and postsynaptic sites, endowed with anxiolytic properties.

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