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

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Received 12 November 2002; revised 20 January 2003; accepted 10 February 2003

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 \( \alpha_{1} \)-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 1 (n = 3, 4), 13–19 which showed affinity for both 5-HT\(_{1A}\) and \( \alpha_{1} \)-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 1 (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 1 (n = 1) which exhibit high 5-HT\(_{1A}\)R affinity and selectivity over \( \alpha_{1} \)-adrenoceptors. Among them, compound CSP-2503 (4) has been pharmacologically characterized as a 5-HT\(_{1A}\)R agonist endowed with anxiolytic properties.

Computational simulation

Figure 1a shows compound 1 (X = –(CH\(_{2}\))\(_{3}\), m = 0, n = 4, Ar = napht-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...
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 group of the piperazine ring, and with Thr$^{3,37}$, Ser$^{5,42}$, and Thr/Ser$^{5,43}$ in both 5-HT$_{1A}$ and $\alpha_1$-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 I. 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 $\alpha_1$-adrenoceptor while 1 binds both receptors, as predicted by the computer models.

**Chemistry**

Based on the proposed hypothesis for 5-HT$_{1A}$/\(\alpha_1\) selectivity, we have synthesized a new series of arylpiperazines 1 ($n=1$), in which the volume of the pharmacophore is increased in positions ortho, meta of the aromatic ring. Previous QSAR studies showed that this is an additional structural feature that accounts for 5-HT$_{1A}$/\(\alpha_1\) selectivity. Compounds 2–8 were obtained by Mannich reaction of bicyclohydantoins 9,10,25,26 or diketopiperazines 11,12,27,28 with the appropriate bicycloarylpyrazines 13–16,29–31 in the presence of formaldehyde (Scheme 1).

**Table 1.** Binding data of compounds I

<table>
<thead>
<tr>
<th>Compd</th>
<th>X</th>
<th>m</th>
<th>n</th>
<th>Ar</th>
<th>$K_i$ ± SEM (5-HT$_{1A}$)</th>
<th>$K_i$ ± SEM ((\alpha_1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(CH$_2$)$_3$</td>
<td>0</td>
<td>4</td>
<td>Naphth-1-yl</td>
<td>2.4 ± 0.1</td>
<td>64.9 ± 1.5</td>
</tr>
<tr>
<td>2</td>
<td>(CH$_2$)$_3$</td>
<td>0</td>
<td>1</td>
<td>Naphth-1-yl</td>
<td>10.4 ± 0.8</td>
<td>&gt; 1000</td>
</tr>
</tbody>
</table>

*Values are means of 2–4 experiments performed in triplicate.

**Pharmacology**

**Affinity data**

Target compounds were assayed for in vitro binding affinity at serotoninergic 5-HT$_{1A}$ and $\alpha_1$-adrenergic receptors by radioligand binding assays, using [$^3H$]-8-OH-DPAT and [$^3H$]prazosin, respectively, in rat cerebral cortex membranes. All the synthesized compounds 2–8 exhibited high 5-HT$_{1A}$R affinity and selectivity over $\alpha_1$-adrenergic receptors (Table 2), confirming our hypotheses for 5-HT$_{1A}$/\(\alpha_1\) selectivity in this class of arylpiperazine ligands. Compound 4 (CSP-2503) was also evaluated for affinity at serotonin 5-HT$_{2A}$ ($K_i=13.5 ± 2.5$ nM), 5-HT$_{3}$ ($K_i=8.9 ± 1.4$ nM), 5-HT$_{4}$ ($K_i>10,000$ nM) and 5-HT$_{7}$ ($K_i=100.9 ± 1.4$ nM) receptors, serotonin transporter ($K_i=976.3 ± 42.8$ nM), dopamine D$_2$ receptors ($K_i=192.1 ± 20.1$ nM), and benzodiazepine receptors ($K_i>10,000$ nM). The following specific ligands and tissue sources were used: 5-HT$_{2A}$, [$^3H$]ketanserin, rat cerebral frontal cortex membranes; 5-HT$_{3}$, [$^3H$]LY278584, rat cerebral cortex membranes; 5-HT$_{4}$, [$^3H$]GR113808, rat striatum membranes; 5-HT$_{7}$, [$^3H$]-5-CT, rat hypothalamus.

**Table 2.** Binding data of compounds I

<table>
<thead>
<tr>
<th>Compd</th>
<th>X</th>
<th>m</th>
<th>n</th>
<th>Ar</th>
<th>$K_i$ ± SEM (5-HT$_{1A}$)</th>
<th>$K_i$ ± SEM ((\alpha_1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>(CH$_2$)$_3$</td>
<td>0</td>
<td>4</td>
<td>Naphth-1-yl</td>
<td>5.6 ± 0.3</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>4</td>
<td>(CH$_2$)$_3$</td>
<td>1</td>
<td>1</td>
<td>Naphth-1-yl</td>
<td>4.1 ± 1.2</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>(CSP-2503)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>(CH$_2$)$_3$</td>
<td>0</td>
<td>4</td>
<td>Benzodioxan-5-yl</td>
<td>9.3 ± 0.4</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>6</td>
<td>(CH$_2$)$_3$</td>
<td>0</td>
<td>4</td>
<td>Benzodioxepin-6-yl</td>
<td>6.1 ± 0.4</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>7</td>
<td>(CH$_2$)$_3$</td>
<td>1</td>
<td>1</td>
<td>Benzodioxepin-6-yl</td>
<td>12.3 ± 2.1</td>
<td>&gt; 1000</td>
</tr>
<tr>
<td>8</td>
<td>(CH$_2$)$_3$</td>
<td>0</td>
<td>4</td>
<td>Benzimidazol-4-yl</td>
<td>4.1 ± 0.2</td>
<td>&gt; 1000</td>
</tr>
</tbody>
</table>

*Values are means of 2–4 experiments performed in triplicate.
membranes; 5-HT transporter, [3H]paroxetine, rat cerebral cortex membranes; D₂, [3H]raclopride, rat striatum membranes; benzodiazepine, [3H]flunitrazepam, rat cerebral cortex membranes.

Pharmacological characterization of CSP-2503 (4)

Presynaptic 5-HT₁₅R activity was assessed by measuring mouse rectal temperature. 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-HT₁₅ somatodendritic autoreceptors.

The transduction mechanism of CSP-2503 was determined by using HeLa cells expressing human 5-HT₁₅Rs. CSP-2503 inhibited in a dose dependent manner the cAMP increase induced by forskolin. The half maximal effect (EC₅₀) 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-HT₁₅ stimulation.

Functional activity of CSP-2503 on 5-HT₁₅Rs was further assessed by evaluating its ability to decrease 5-HT neuronal activity. 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-HT₁₅R agonist acting at the somatodendritic site.

Furthermore, we have evaluated the potential anxiolytic activity of CSP-2503 by using the light/dark box test. 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-HT₁₅R 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.

These results indicate that CSP-2503 is an agonist of the 5-HT₁₅R 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-HT₁₅R-ligand interaction, we have synthesized a new series of arylpiperazines (n = 1) which exhibit high 5-HT₁₅R affinity and selectivity over α₂-adrenergic receptors. Among them, compound CSP-2503 (4) has been pharmacologically characterized as a 5-HT₁₅R agonist at somatodendritic and postsynaptic sites, endowed with anxiolytic properties.

Acknowledgements

This work was supported by Ministerio de Ciencia y Tecnología (BQU2001-1459), Comunidad Autónoma de Madrid (08.5/0079/2000), and CEPA-SCHWARZ-PHARMA. E. Fernández and I. Tejada are also grateful to U.N.E.D. for a predoctoral grant. Computer facilities were provided by the Centre de Computació i Comunicacions de Catalunya.