N-[ω-4-(2-Methoxyphenyl)-1-piperazinyl]alkyl]-2-quinolinamines as High-Affinity Fluorescent 5-HT1A Receptor Ligands

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We here report on the design, synthesis, binding affinities, and fluorescent properties of a series of serotonin 5-HT1A receptor ligands, with N-[ω-[4-(2-methoxyphenyl)-1-piperazinyl]alkyl]-2-quinolinamine structure. Several of the new ligands displayed nanomolar affinity at 5-HT1A receptor and good fluorescent properties. In particular, derivative 24 showed a favorable combination of 5-HT1A receptor affinity (Ki = 0.4 nM), Stokes shift (excitation wavelength = 381 nm, emission wavelength = 455 nm), and quantum yield in ethanol (Φ = 0.45).

Introduction

Fluorescence spectroscopy is becoming a valuable addition to the array of techniques available for real time direct visualization of fluorescent ligand–receptor interactions in biological systems. For instance, scanning confocal microscopy,1–3 fluorescence correlation spectroscopy,4,5 and two-photon fluorescence laser scanning microscopy6,7 allow the noninvasive imaging and quantification of these interactions in single living cells under a wide variety of experimental conditions. Moreover, fluorescence polarization techniques have been used in analytical and clinical chemistry as versatile screening alternatives with greatly reduced environmental and cost impacts compared with radioligand assays.8

The serotonin-1A (5-HT1A) receptor is an important member of the large family of serotonin receptors, and it is perhaps the most extensively studied for a number of reasons. One of the main reasons is the availability of the selective agonist 8-OH-DPAT9 that has allowed extensive biochemical, physiological, and pharmacological characterization of the receptor. The 5-HT1A receptor was the first among all the serotonin receptors to be cloned and sequenced. The human, rat, and mouse 5-HT1A receptors have been cloned and their amino acid sequences deduced. More importantly, the receptor has been stably expressed in a number of neural and non-neural cell lines. Furthermore, it was the first serotonin receptor for which polyclonal antibodies were obtained, allowing their visualization at the subcellular level in various regions of the brain.9 Starting in the mid-1980s, the 5-HT1A receptor has been the object of intense study which culminated in the introduction into the market of the 5-HT1A receptor partial agonist buspirone (1) as an anxiolytic (Chart 1).10 Other 5-HT1A agents have demonstrated a role for the 5-HT1A receptor in various pathologies. For instance, the mixed 5-HT1A/D2 agents bifeprunox (2) and SLV308 (3) have been studied for the treatment of schizophrenia and Parkinson’s disease, respectively; vilazodone (4) has been proposed as a fast-acting antidepressant, as well as the 5-HT1A antagonist robalzotan (5), xaliproden (6) and repinotan (7) have been investigated as neuroprotective agents. Very recently, the 5-HT1A receptor agonist F 13640 (8) underwent phase II clinical trials for the treatment of severe, chronic pain.11 Therefore, after two decades of research, the 5-HT1A receptor is still under active investigation.

Several structurally different compounds are known to bind 5-HT1A receptor.12 Among these, a large number of N1-substituted N4-arylpiperazines (the so-called “long-chain” arylpiperazines) have been extensively studied. The general formula of these compounds presents an 1-arylpiperazine linked through an alkyl chain of variable length to a terminal fragment, belonging to one of the following four structural classes: (i) imides, (ii) amides, (iii) alkyl, arylalkyl, or heteroarylalkyl derivatives, and (iv) tetralins.13 During the course of our studies we have published several papers dealing with the synthesis and structure-affinity relationships elucidation of “long-chain” arylpiperazines as 5-HT1A receptor ligands.14,15

Chart 1. Structures of 5-HT1A Receptor Agents

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Abbreviations: 8-OH-DPAT, 8-hydroxy-N,N-dipropylaminotetraline; GPCR, G-protein-coupled receptor; BODIPY, dipyrromethene boron difluoride.
Recently, we embarked on a research program to develop high affinity fluorescent ligands for certain GPCR, specifically the 5-HT1A receptor.

Fluorescent probes have been prepared by labeling a ligand with a fluorophore, such as fluorescein, BODIPY, coumarin, and dansyl, into an area of the structure that would have minimal influence on biological activity. However, this structural modification has been reported to cause variations of lipophilicity, affinity, selectivity, and intrinsic activity.

For our purpose, we have envisaged a different strategy by including the fluorescent core into a framework endowed with affinity for the target receptor. In this way, it is possible to overcome the above-mentioned shortcomings and allow access to an entire series of fluorescent ligands with the possibility to optimize the fluorescence properties and receptor affinity at the same time.

We have focused our attention on N-[2-[(2-methoxyphenyl)-1-piperazinyl]ethyl]-6-nitro-2-quinolinamine (9, Table 1) that we reported as mixed serotonin transporter/5-HT1A receptor ligand (5-HT1A $K_i = 14.2 \text{ nM}$). In particular, we wondered if the 9 was fluorescent because the 2-quinolinamine moiety closely resembles that of 2-aminopyridine, which is one of the fluorescent standards used for quantum yield determination (Table 1). Actually, 9 demonstrated to be fluorescent (Table 1), and therefore, it represented our starting point to develop fluorescent 5-HT1A receptor ligands. Subsequent structural modifications of 9 were performed with the twofold aim to increase both 5-HT1A receptor affinity and fluorescent emission.

### Table 1. Binding Affinities of Ligands 9–22, and 24 for Serotonin 5-HT1A Receptor and Their Fluorescence Properties in Ethanol

<table>
<thead>
<tr>
<th>compd</th>
<th>$R_1$</th>
<th>$R_2$</th>
<th>$n$</th>
<th>$K_i$ (nM ± SEM$^a$)</th>
<th>excitation $\lambda_{max}$ (nm)</th>
<th>emission $\Phi_{max}$ (nm)</th>
<th>$\Phi$</th>
</tr>
</thead>
<tbody>
<tr>
<td>9</td>
<td>NO$_2$</td>
<td>H</td>
<td>2</td>
<td>14.2 ± 2.7$^a$</td>
<td>271</td>
<td>450</td>
<td>0.09</td>
</tr>
<tr>
<td>10</td>
<td>H</td>
<td>H</td>
<td>2</td>
<td>33 ± 4</td>
<td>343</td>
<td>406</td>
<td>0.17</td>
</tr>
<tr>
<td>11</td>
<td>H</td>
<td>H</td>
<td>3</td>
<td>13 ± 1</td>
<td>342</td>
<td>405</td>
<td>0.30</td>
</tr>
<tr>
<td>12</td>
<td>H</td>
<td>H</td>
<td>4</td>
<td>9.7 ± 0.3</td>
<td>341</td>
<td>390</td>
<td>0.46</td>
</tr>
<tr>
<td>13</td>
<td>H</td>
<td>H</td>
<td>5</td>
<td>0.030 ± 0.008</td>
<td>347</td>
<td>392</td>
<td>0.35</td>
</tr>
<tr>
<td>14</td>
<td>OCH$_3$</td>
<td>H</td>
<td>2</td>
<td>28 ± 2</td>
<td>362</td>
<td>405</td>
<td>0.52</td>
</tr>
<tr>
<td>15</td>
<td>OCH$_3$</td>
<td>H</td>
<td>3</td>
<td>22.0 ± 0.8</td>
<td>360</td>
<td>408</td>
<td>0.52</td>
</tr>
<tr>
<td>16</td>
<td>OCH$_3$</td>
<td>H</td>
<td>4</td>
<td>0.20 ± 0.05</td>
<td>359</td>
<td>405</td>
<td>0.54</td>
</tr>
<tr>
<td>17</td>
<td>OCH$_3$</td>
<td>H</td>
<td>5</td>
<td>2.6 ± 0.8</td>
<td>360</td>
<td>410</td>
<td>0.53</td>
</tr>
<tr>
<td>18</td>
<td>H</td>
<td>OCH$_3$</td>
<td>2</td>
<td>2.1 ± 0.4</td>
<td>361</td>
<td>381</td>
<td>0.14</td>
</tr>
<tr>
<td>19</td>
<td>H</td>
<td>OCH$_3$</td>
<td>3</td>
<td>0.20 ± 0.06</td>
<td>358</td>
<td>380</td>
<td>0.47</td>
</tr>
<tr>
<td>20</td>
<td>H</td>
<td>OCH$_3$</td>
<td>4</td>
<td>4.6 ± 0.2</td>
<td>365</td>
<td>382</td>
<td>0.49</td>
</tr>
<tr>
<td>21</td>
<td>H</td>
<td>OCH$_3$</td>
<td>5</td>
<td>0.50 ± 0.05</td>
<td>362</td>
<td>380</td>
<td>0.45</td>
</tr>
<tr>
<td>22</td>
<td>CH$_3$</td>
<td>H</td>
<td>4</td>
<td>0.54 ± 0.01</td>
<td>346</td>
<td>400</td>
<td>0.20</td>
</tr>
<tr>
<td>24</td>
<td>NH$_2$</td>
<td>H</td>
<td>4</td>
<td>0.38 ± 0.02</td>
<td>381</td>
<td>455</td>
<td>0.45</td>
</tr>
<tr>
<td>2-aminopyridine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>290</td>
<td>353</td>
<td>0.37</td>
</tr>
</tbody>
</table>

$^a$ The values are the mean ± SEM from three independent experiments.

The target compounds were prepared according to Scheme 1. Final compounds 10–22 were prepared by condensing 2-chloroquinolines 25a–d with the appropriate amine 26a–d. Target compound 24 was obtained as follows: 2-chloro-6-nitroquinoline (25e) reacted with amine 26d to give nitro derivative 23, which was hydrogenated to afford 24. The starting materials were purchased from commercial sources or prepared according to literature, as detailed in the Experimental Section.

### Results and Discussion

The affinity values for serotonin 5-HT1A receptor and fluorescent properties of the target compounds are displayed in Table 1. Derivative 9 possessed good binding affinity for 5-HT1A receptor and quite low fluorescent emission. Therefore, we performed structural modifications with the aim to improve both properties. It is well documented that the fluorescence emission of aromatic substances containing a nitro group is generally weak, primarily as a result of large non radiative decay from the excited state.

On the other hand, it has been reported that an electron-donating group such as methoxy (having inductive +I and resonance +M effects) can increase fluorescence emission. On the basis of such considerations, the unsubstituted quinoline 10 was prepared as well as the 6-methoxy- and 7-methoxyquinolines 14 and 18, respectively. These latter compounds would allow us to evaluate the effect of conjugation on fluorescence emission. As far as the affinity for 5-HT1A receptor was concerned, we tested derivatives 10, 14, and 18 and their homologues 11–13, 15–17, and 19–21 bearing an alkyl chain from three to five methylene units because optimization of the intermediate alkyl chain length is a key step when studying “long-chain” arylpiperazines. Considering the fluorescent properties of 10, it can be observed that removal of nitro group from 9 resulted in an enhancement of fluorescent emission. The nitro group replacement in 9 by the methoxy substituent (compound 14) resulted in a considerable increase of $\Phi$ value (0.09 vs 0.52). Shifting of the methoxy from 6- to 7-position (compounds 14 and 18) diminished fluorescence emission ($\Phi = 0.52$ and 0.14, respectively). Elongation of the alkyl chain length of 10, 14, and 18 increased fluorescence emission, which was more evident for the unsubstituted quinolines 11–13 and for the 7-methoxyquinolines 19–21. These results confirmed that introduction of a methoxy group on the aromatic ring can increase fluorescence, especially when conjugation of $\pi$-system was extended. Moreover, compounds with a four methylene chain (12, 16, and 20) showed higher quantum yields than their respective homologues. Finally, compounds 10–21 showed high difference of excitation to emission maximal wavelengths (Stokes shift).

As far as the 5-HT1A receptor affinity is concerned, the analogues 10 and 14 displayed similar affinity as 9, whereas 18 was slightly more potent than the nitro derivative 9. Elongation of the alkyl chain of 10, 14, and 18 had a beneficial effect on 5-HT1A receptor affinity. In fact, compounds 13 ($n = 5$), 16 ($n = 4$), 19 ($n = 3$), and 21 ($n = 5$) displayed affinities in the subnanomolar range. However, a general trend did not emerge because maximum 5-HT1A receptor affinity was shown by compounds with different linker length.

Taken together, 5-HT1A receptor affinity data and fluorescence properties indicated that the pursued optimization strategy was successful, especially for derivative 16 which showed the highest $\Phi$ value within this series and very high 5-HT1A receptor affinity ($K_i = 0.20 \text{ nM}$).

Finally, we wanted to explore if other electron donating substituents in 6-position (i.e., methyl or amino) would have beneficial effect on fluorescence. Methyl derivative 22 showed worst fluorescence properties than 16 because of lower excitation and emission wavelengths and lower quantum yield, whereas the amino derivative 24 (Figure 1) retained acceptable quantum yield and displayed excitation and emission maximum at higher wavelengths than 16. Moreover, compounds 22 and 24 demonstrated to be as potent as 16 at 5-HT1A receptor.
Next, we studied the intrinsic activity of compounds 16 and 24 because fluorescent agonists or antagonists provide different information on receptor trafficking. The intrinsic activity at 5-HT<sub>1A</sub> receptor of 16 and 24 was assessed in an isolated guinea pig ileum assay. Both compounds displayed agonistic properties at 5-HT<sub>1A</sub> receptor. In fact, the derivative 16 acted as a full agonist (EC<sub>50</sub> = 34.4 ± 7.0 µM), and its activity was reverted by the 5-HT<sub>1A</sub> receptor antagonist WAY-100635 in a dose-dependent manner (p<sub>A2</sub> = 7.94 ± 0.79), whereas 24 behaved as a partial agonist (81% of the maximal response, EC<sub>50</sub> = 62.1 ± 3.5 µM).

Conclusions

We have identified a series of fluorescent ligands for 5-HT<sub>1A</sub> receptors from the structural modifications of the weakly fluorescent 5-HT<sub>1A</sub> ligand N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]butyl]-6-nitro-2-quinolinamine (9). Several of the newly prepared ligands displayed nanomolar affinity at 5-HT<sub>1A</sub> receptor and fluorescent properties suitable for use in two-photon microscopy, being their excitation wavelengths within the excitation range of lasers currently available (720–880 nm). In particular, the fluorescent ligand 24 showed a favorable combination of 5-HT<sub>1A</sub> receptor affinity (K<sub>i</sub> = 0.4 nm), Stokes shift (excitation wavelength = 381 nm, emission wavelength = 455 nm), and quantum yield in ethanol (Φ = 0.45). Fluorescence visualization experiments in cell lines expressing 5-HT<sub>1A</sub> by two photon laser microscopy are in due course.

Experimental Section

General Procedure for the Preparation of Derivatives 10–23. A mixture of chloro derivative 25a–e (3.6 mmol) and amine 26a–d (5.4 mmol) was heated in a closed glass tube at 150 °C for 5 h (24 h when 25b was used). After cooling, the reaction mixture was diluted with CH<sub>2</sub>Cl<sub>2</sub> and washed with a 20% aqueous solution of Na<sub>2</sub>CO<sub>3</sub>. The separated organic layer was dried (Na<sub>2</sub>SO<sub>4</sub>) and concentrated under reduced pressure. The crude residue was purified by chromatography (CHCl<sub>3</sub>/CH<sub>3</sub>OH, 19:1 as eluent) giving the expected compound as pale yellow oil.

6-Amino-N-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]butyl]-2-quinolinamine (24). Nitro derivative 23 (0.32 g, 0.73 mmol) was dissolved in ethanol and hydrogenated at normal pressure and room temperature in the presence of 10% Pd/C (0.1 g) until the uptake ceased. The catalyst was removed by filtration through Celite, and the solvent was evaporated in vacuo to give a crude residue that was chromatographed (CHCl<sub>3</sub>/CH<sub>3</sub>OH, 19:1 as eluent) giving the final compound as a yellow solid (0.140 g, 47% yield):

**Figure 1.** Excitation and emission spectra of compound 24 in ethanol.

**References**


