Synthesis of new 5-phenyl[1,2,4]triazole derivatives as ligands for the 5-HT$_{1A}$ serotonin receptor

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Dedicated to Professor Vincenzo Tortorella on his “Fuori Ruolo” Status

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Abstract

A series of 4-amino-3-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]thio]-5-(substituted phenyl)[1,2,4]triazoles (5a-f) and the isomeric 4-amino-2-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-5-(substitutedphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (6a-f) and a series of 3-[[2-[4-(2-methoxy or 2-nitrophenyl)1-piperazinyl]ethyl]thio]-5-(substituted phenyl)[1,2,4]triazoles (8a-i) were synthesized with the aim of obtaining new selective 5-HT$_{1A}$ ligands, with reduced affinity for the $\alpha_1$-adrenoceptor subtypes. New compounds were tested in radioligand binding experiments where many of them showed a preferential affinity for the 5-HT$_{1A}$ receptor.

Keywords: 5-Phenyl[1,2,4]triazole, 5-HT$_{1A}$ serotonin receptor, $\alpha_1$-adrenoceptor subtypes, triazole ring alkylation

Introduction

5-HT$_{1A}$ serotonin receptors belong to the seven-transmembrane-domain (7-TM) receptor superfamily$^1$ and mediate many physiological effects of the serotonin. Increasing evidence for the role of 5-HT$_{1A}$ receptor in psychiatric disorders, such as depression and anxiety,$^2$-$^4$ has encouraged the search for selective ligands.$^5$-$^7$ To date several potent 5-HT$_{1A}$ receptor ligands are known, however their potential use as drugs or pharmacological tools is often limited by their undesired affinity for other receptor types such as an example the dopaminergic D$_2$ receptor and the $\alpha_1$-adrenoceptor ($\alpha_1$-AR). In particular, over the years many efforts have been made to discover 5-HT$_{1A}$ receptor ligands with reduced affinity for $\alpha_1$-AR.$^5$-$^7$
In a previous paper we have described the synthesis of new 5-phenyl[1,2,4]triazole derivatives (1-2, n=2, Figure 1) as 5-HT$_{1A}$ receptor ligands. These molecules present a phenylpiperazinyl (PP) residue that forms an essential part of the molecule for 5-HT$_{1A}$ affinity and a 5-aryl[1,2,4]triazole moiety connected by a propyl chain. Some of them had shown high affinity and selectivity for 5-HT$_{1A}$ receptor respect to the $\alpha_1$-AR. We now report a further development of the structure-activity relationships (SAR) on these classes of compounds. In particular, in this paper we synthesized and tested for affinity and selectivity for 5HT$_{1A}$ over $\alpha_1$-AR, a new series of 5-aryl[1,2,4]triazoles in which the main modification is the length of the chain connecting PP and triazole moieties, that here is constituted by two methylene groups instead of three (1,2, n = 1, Figure 1). In an effort to optimize the 5-position of the triazole ring, a phenyl group substituted with a number of electronically dissimilar residue was chosen.

Figure 1. R = H, 2-Cl, 4-OCH$_3$, 3-OCH$_3$, 4-CH$_3$, 4-Cl, 4-Br, 4-$n$-C$_3$H$_7$O, 4-C$_6$H$_5$SO$_2$, 4-C$_6$H$_5$; X = H, NH$_2$; n = 1, 2; R$_1$ = OCH$_3$, NO$_2$.

Results and Discussion

The general strategy for the synthesis of compounds 5a-f, 6a-f and 8a-i is summarized in schemes 1 and 2. A number of 4-amino-5-aryl-2,4-dihydro-3H[1,2,4]triazole-3-thiones (3a-f) and 5-aryl-2,4-dihydro-3H[1,2,4]triazole-3-thiones (7a-g) were chosen as starting materials. Attempt to conduct the reaction described in scheme 1 in the same experimental conditions reported in a previous paper, (ethanol in the presence of potassium hydroxide and a catalytic amount of potassium iodide), failed. In fact, in these experimental conditions, 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine 4a, gave the undesired 1-(2-ethoxyethyl)-4-(2-methoxyphenyl)piperazine, thus reducing the yield in the desired compounds. A similar reactivity had been shown by other chloroethylamines in ethanol in presence of potassium hydroxide. In order to improve yields of both S- and N-alkylated isomers, we carried out the reaction using refluxing acetone and potassium carbonate, experimental conditions often used when 1-(chloroalkyl)-4-(2-substitutedphenyl)piperazines where employed as alkylating agents. In these experimental conditions, using 3a-f as starting materials, both the S-alkylated
and the N-alkylated isomers were obtained (scheme 1). Flash chromatography of the crude material allowed the recovery of both isomers with an average yield of 45-50% for the S-alkylated isomers and 12-18% for the N-alkylated (ratio 3:1). As reported for triazoles previously described, analysis of the $^1$H NMR spectra of the two isomeric series is diagnostic for the assignment of structure. Both spectra present in the range from 5.7 to 6.2 $\delta$, a broad singlet signal which integrates for two hydrogens and disappears in the presence of D$_2$O; it is analogous to the signal seen in the $^1$H NMR spectra of 4-amino-5-aryl-2,4-dihydro-3H[1,2,4]triazole-3-thiones 3a–f and it has to be attributed to the NH$_2$ group. This implies that alkylation of compounds 3a–f does not take place at the NH$_2$ substituent. Furthermore, in both spectra, a triplet for the methylene group of the ethyl chain that connects the PP moiety and the triazole part of the molecule, is observed. For derivatives 5a–f, this triplet has a chemical shift of 3.3 $\delta$, which is typical for S–CH$_2$ connectivity, and for isomers 6a–f of 4.3 $\delta$, for NCSN–CH$_2$ connectivity. These results were confirmed by $^{13}$C-NMR spectra where a signal at 29.66 $\delta$ was observed for the S-alkylated derivative 5d, whereas the same signal is shifted to 46.37 $\delta$ for the N-alkylated derivative 6d.

(i) K$_2$CO$_3$, 1 eq.; KI, cat.; acetone; reflux, 12 hrs.

**Scheme 1**

The preparation of derivatives 8a–i (scheme 2), which lack the amino substituent in the 4-position of the triazole, was accomplished using similar reaction conditions as for the synthesis of compounds 5a–f and 6a–f. Derivatives (7a–g) and the appropriate 1-(2-chloroethyl)-4-(2-substitutedphenyl)piperazine (4a-b) were reacted in alkaline medium. In this case, only the S-alkylated isomers 8a–i were isolated from the reaction mixture.
The starting 4-amino-5-aryl-2,4-dihydro-3H[1,2,4]triazole-3-thione (3a-f) and 5-aryl-2,4-dihydro-3H[1,2,4]triazole-3-thione (7a-g) were prepared as previously described.\textsuperscript{8,14-17} All the synthesized compounds were tested in binding experiments to evaluate their affinity and selectivity for the 5-HT\textsubscript{1A} receptor over the \(\alpha_{1}\)-AR. The results are presented in tables 1 and 2 and are expressed as \(K_i\) (nM).

Previously we described a new class of 5-aryl[1,2,4]triazole derivatives as ligands for the 5-HT\textsubscript{1A} serotonin receptor selective over the \(\alpha_{1}\)-AR.\textsuperscript{8} In this paper we analyzed three series of molecules: compounds 5a-f and 8a-i, characterized by a thioethyl chain between the 5-aryl[1,2,4]triazole and the PP portions, and compounds 6a-f, in which an ethyl chain is present. As a general trend, most compounds 5a-f and 8a-i showed good and preferential affinity for the 5-HT\textsubscript{1A} receptor over the \(\alpha_{1}\)-AR. On the other hand most of compounds 6a-f, displayed lower affinity and selectivity. Such results indicated that the point of the attachment of the phenylpiperazinethyl moiety (3 or 2-position of the phenyl triazole ring) is critical for affinity and selectivity. Moreover, a comparison of binding data of new synthesized compounds with those of previously reported analogues,\textsuperscript{8} shows that the shortening of connecting alkyl chain generally led to a slight decrease of both affinity and selectivity. Substituents on the phenyl ring in the 5-position of the triazole seem to have little effect on 5-HT\textsubscript{1A} receptor affinity with the exception of bulkier substituents that reduce the affinity. The presence of an amino group in the 4-position of the triazole ring generally reduced the affinity for both class of receptors, but the \(\alpha_{1}\)-AR are more sensitive. As described in the previous paper,\textsuperscript{8} the 2-methoxyphenyl-piperazinyl derivatives showed higher affinity at both receptors than their respective 2-nitrophenyl analogues (compare 8a and 8h; 8e and 8i). Among tested molecules, compound 5a resulted the most interesting derivative of this series showing good affinity and selectivity for the 5-HT\textsubscript{1A} receptors.
Table 1. Structure and binding properties of compounds 5a-f and 6a-f

![Structure 5a-f](image1)

![Structure 6a-f](image2)

<table>
<thead>
<tr>
<th>Starting materials</th>
<th>Compound</th>
<th>R</th>
<th>5-HT\textsubscript{1A}R\textsuperscript{a} (K\textsubscript{i}(nM±sd))</th>
<th>(\alpha\textsubscript{1}AR\textsuperscript{b} (K\textsubscript{i}(nM±sd))</th>
<th>Selectivity\textsuperscript{c}</th>
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<tbody>
<tr>
<td>3a</td>
<td>5a</td>
<td>H</td>
<td>10.53±0.58</td>
<td>206.93±17.56</td>
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<tr>
<td>3b</td>
<td>5b</td>
<td>2-Cl</td>
<td>25.36±1.56</td>
<td>143.35±15.27</td>
<td>5.6</td>
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<tr>
<td>3c</td>
<td>5c</td>
<td>4-CH\textsubscript{3}</td>
<td>24.97±1.17</td>
<td>117.59±10.59</td>
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<tr>
<td>3d</td>
<td>5d</td>
<td>4-Cl</td>
<td>15.21±1.20</td>
<td>101.55±14.50</td>
<td>6.7</td>
</tr>
<tr>
<td>3e</td>
<td>5e\textsuperscript{d}</td>
<td>4-OC\textsubscript{3}H\textsubscript{7}</td>
<td>48.78±3.02</td>
<td>274.39±44.28</td>
<td>5.6</td>
</tr>
<tr>
<td>3f</td>
<td>5f</td>
<td>4-SO\textsubscript{2}C\textsubscript{6}H\textsubscript{5}</td>
<td>60.48±7.40</td>
<td>326.12±44.21</td>
<td>5.4</td>
</tr>
<tr>
<td>3a</td>
<td>6a</td>
<td>H</td>
<td>139.31±23.10</td>
<td>143.34±21.10</td>
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<tr>
<td>3b</td>
<td>6b</td>
<td>2-Cl</td>
<td>261.85±19.10</td>
<td>88.90±20.50</td>
<td>0.3</td>
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<tr>
<td>3c</td>
<td>6c</td>
<td>4-CH\textsubscript{3}</td>
<td>147.51±7.02</td>
<td>127.59±16.53</td>
<td>0.9</td>
</tr>
<tr>
<td>3d</td>
<td>6d</td>
<td>4-Cl</td>
<td>120.97±6.63</td>
<td>187.44±17.32</td>
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<tr>
<td>3e</td>
<td>6e\textsuperscript{d}</td>
<td>4-OC\textsubscript{3}H\textsubscript{7}</td>
<td>460.98±38.24</td>
<td>147.28±16.6</td>
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<tr>
<td>3f</td>
<td>6f</td>
<td>4-SO\textsubscript{2}C\textsubscript{6}H\textsubscript{5}</td>
<td>N.T.</td>
<td>N.T.</td>
<td></td>
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\textsuperscript{a}Affinity at \[^3\text{H}\]-8-OH-DPAT-labelled 5-HT\textsubscript{1A} sites.

\textsuperscript{b}Affinity at \[^3\text{H}\]prazosin-labelled \(\alpha\textsubscript{1}\)-adrenergic sites.

\textsuperscript{c}Selectivity for 5-HT\textsubscript{1A} over \(\alpha\textsubscript{1}\) receptors is expressed as the ratio \(K_i\alpha_i/K_i\text{ 5-HT}\textsubscript{1A}\).

\textsuperscript{d}Physical and spectroscopic data are reported in reference 8.
### Table 2. Structure and binding properties of compounds 8a-i

![Chemical Structure of 8a-i](image)

<table>
<thead>
<tr>
<th>Starting materials</th>
<th>Compound</th>
<th>R</th>
<th>R₁</th>
<th>5-HT₁AR&lt;sup&gt;a&lt;/sup&gt;</th>
<th>α₁AR&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Selectivity&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>7a</td>
<td>8a</td>
<td>H</td>
<td>OCH₃</td>
<td>6.37±0.4</td>
<td>20.05±1.0</td>
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<tr>
<td>7b</td>
<td>8b</td>
<td>2-Cl</td>
<td>OCH₃</td>
<td>7.32±0.4</td>
<td>13.9±1.0</td>
<td>1.9</td>
</tr>
<tr>
<td>7c</td>
<td>8c</td>
<td>4-CH₃</td>
<td>OCH₃</td>
<td>5.85±0.9</td>
<td>19.70±3.0</td>
<td>3.4</td>
</tr>
<tr>
<td>7d</td>
<td>8d</td>
<td>4-Cl</td>
<td>OCH₃</td>
<td>8.16±0.4</td>
<td>28.70±7.0</td>
<td>3.5</td>
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<tr>
<td>7e</td>
<td>8e</td>
<td>4-OC₃H₇</td>
<td>OCH₃</td>
<td>30.90±5.0</td>
<td>56.20±5.0</td>
<td>1.8</td>
</tr>
<tr>
<td>7f</td>
<td>8f</td>
<td>4-SO₂C₆H₅</td>
<td>OCH₃</td>
<td>54.10±8.0</td>
<td>21.10±2.0</td>
<td>0.4</td>
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<tr>
<td>7g</td>
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<td>4-NO₂</td>
<td>OCH₃</td>
<td>16.34±2.0</td>
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<td>7a</td>
<td>8h</td>
<td>H</td>
<td>NO₂</td>
<td>117.90±11.0</td>
<td>88.00±9.0</td>
<td>0.7</td>
</tr>
<tr>
<td>7e</td>
<td>8i</td>
<td>4-OC₃H₇</td>
<td>NO₂</td>
<td>735.00±93.53</td>
<td>425.00±106.79</td>
<td>0.6</td>
</tr>
</tbody>
</table>

<sup>a</sup>Affinity at [³H]-8-OH-DPAT-labelled 5-HT₁A sites.

<sup>b</sup>Affinity at [³H]prazosin-labelled α₁-adrenergic sites.

<sup>c</sup>Selectivity for 5-HT₁A over α₁ receptors is expressed as the ratio Kᵢα₁/Kᵢ 5-HT₁A.

In conclusion, a new series of 4-amino-3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]thio]-5-(substitutedphenyl)[1,2,4]triazoles (5a-f) and the isomeric 4-amino-2-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-5-(substitutedphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (6a-f) and a new series of 3-[2-[4-(2-substitutedphenyl)1-piperazinyl]ethyl]thio]-5-aryl[1,2,4]triazoles (8a-i) were synthesized and tested in radioligand binding assays to evaluate their affinity and selectivity for the 5-HT₁A over α₁-adrenergic receptor. Generally this compounds showed Kᵢ values in the nanomolar range and selectivity for the 5-HT₁A receptor.

### Experimental Section

**General Procedures.** Melting points were determined using a Buchi 510 apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer in KBr disk. ¹H NMR spectra were recorded on a Varian 200 MHz instrument (200 MHz for ¹H NMR
and 50 MHz for $^{13}$C NMR) in DMSO-$d_6$ solution. Chemical shifts are given in ppm ($\delta$) relative to tetramethylsilane as internal standard; coupling constants ($J$) are given in Hz. Signals were characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet) and br (broad signals). Elemental analyses were performed on a Carlo Erba Elemental Analyzer Mod. 1108 apparatus and the data of C, H, N are within $\pm$ 0.4% of calculated values. All the compounds synthesized were tested for purity on TLC (aluminum sheets coated with silica gel 60 F$_{254}$, Merck) and visualized by UV ($\lambda$ =254 and 366 nm). Preparative chromatographic separations were conducted by means of flash chromatography using Merck Silica gel 60 0.040-0.063 mm. Compounds 3a-f and 7a-g were synthesized as previously reported.$^{8,14-17}$

**General procedure for 4-amino-3-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]thio]-5-(substitutedphenyl)[1,2,4]triazoles (5a-f) and 4-amino-2-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-5-(substitutedphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (6a-f)**

A mixture of the appropriate 4-amino-5-substituted-2,4-dihydro-3H[1,2,4]triazole-3-thione (1 mmol) (3a-f), potassium carbonate (1 mmol) and potassium iodide (10 mg) in 25 mL of acetone was refluxed for 15 min. A solution of 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperezine (4a) (1 mmol) in 10 mL of acetone was then added dropwise and the reaction mixture was refluxed for 12 h. After cooling at room temperature, the mixture was concentrated in vacuo, diluted with 40 mL of water and extracted with ether ($4 \times 20$ mL). The extracts were combined, washed with brine, dried and evaporated under vacuum to give a residue containing a mixture of both the desired products 5a-f and 6a-f. Column chromatography of the residue on silica gel, using ethyl acetate as eluent, gave the fast moving isomer (6). The corresponding isomer (5) was obtained using a mixture of ethyl acetate/methanol (9:1 v/v) as eluent. The following compounds were obtained:

4-Amino-3-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]thio]-5-phenyl[1,2,4]triazole (5a). The desired compound was obtained as a white solid. Yield 50%; mp 164-165°C; IR (KBr) v 3283, 3171, 3062, 2936, 2820, 1498, 1449, 1240, 751 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.45-2.65 (m, 4H, N-CH$_2$ piperazine), 2.71 (t, $J = 6.6$ Hz, 2H, S-CH$_2$-CH$_2$-N), 2.82-2.97 (m, 4H, N-CH$_2$ piperazine), 3.32 (t, $J = 6.6$ Hz, 2H, S-CH$_2$-CH$_2$-N), 3.75 (s, 3H, OCH$_3$), 6.14 (br s, 2H, NH$_2$), 6.80-6.94 (m, 4H, aromatic), 7.47-7.53 (m, 3H, aromatic), 7.98-8.03 (m, 2H, aromatic). Calculated for C$_{21}$H$_{26}$N$_6$OS: C, 61.44; H, 6.39; N, 20.48; S, 7.79; found: C, 61.30; H, 6.40; N, 20.22; S, 7.76.

4-Amino-5-(2-chlorophenyl)-3-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]thio][1,2,4]triazole (5b). The desired compound was obtained as a white solid. Yield 48%; mp 106-107°C; IR (KBr) v 3281, 3150, 2938, 2815, 1593, 1499, 1449, 1240, 749 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.48-2.62 (m, 4H, N-CH$_2$ piperazine), 2.72 (t, $J = 6.6$ Hz, 2H, S-CH$_2$-CH$_2$-N), 2.85-3.10 (m, 4H, N-CH$_2$ piperazine), 3.35 (t, $J = 6.6$ Hz, 2H, S-CH$_2$-CH$_2$-N), 3.75 (s, 3H, OCH$_3$), 6.14 (br s, 2H, NH$_2$), 6.85-6.91 (m, 4H, aromatic), 7.46-7.53 (m, 3H, aromatic), 7.98-8.03 (m, 2H, aromatic). Calculated for C$_{21}$H$_{25}$ClN$_6$OS: C, 56.68; H, 5.66; N, 18.88; S, 7.20; found: C, 56.60; H, 5.60; N, 18.88; S, 7.20.
4-Amino-3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethylthio]-5-(4-methylphenyl)[1,2,4]triazole (5c). The desired compound was obtained as a white solid. Yield 50%; mp 146-147°C; IR (KBr) ν 3268, 2931, 2821, 1496, 1457, 1309, 1236, 1018, 1003, 761 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.37 (s, 3H, CH₃), 2.48-2.62 (m, 4H, N-CH₂ piperazine), 2.70 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 2.83-2.99 (m, 4H, N-CH₂ piperazine), 3.31 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 3.75 (s, 3H, OCH₃), 6.11 (br s, 2H, NH₂), 6.80-6.94 (m, 4H, aromatic), 7.29-7.33 (m, 2H, aromatic), 7.88-7.92 (m, 2H, aromatic). Calculated for C₂₂H₂₈N₆O₃S: C, 62.23; H, 6.64; N, 19.79; S, 7.55; found: C, 62.23; H, 6.60; N, 19.76; S, 7.50.

4-Amino-5-(4-chlorophenyl)-3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethylthio][1,2,4]triazole (5d). The desired compound was obtained as a white solid. Yield 52%; mp 148-149°C; IR (KBr) ν 3448, 3265, 2931, 2818, 1496, 1447, 1240, 1003, 763 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.42-2.60 (m, 4H, N-CH₂ piperazine), 2.70 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 2.85-2.97 (m, 4H, N-CH₂ piperazine), 3.33 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 3.75 (s, 3H, OCH₃), 6.17 (br s, 2H, NH₂), 6.79-6.94 (m, 4H, aromatic), 7.57-7.61 (m, 2H, aromatic), 8.04-8.08 (m, 2H, aromatic). ¹³C-NMR (DMSO-d₆) δ 29.66, 49.89, 52.65, 55.27, 57.17, 111.86, 117.87, 120.75, 122.42, 125.88, 128.60, 134.39, 141.14, 151.96, 152.83, 153.96. Calculated for C₂₁H₂₅ClN₆O₃S: C, 56.68; H, 5.66; N, 18.88; S, 7.20; found: C, 56.70; H, 5.66; N, 18.88; S, 7.20.

4-Amino-3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethylthio]-5-(4-phenylsulphonylphenyl)-4H[1,2,4]triazole (5f). The desired compound was obtained as a white solid. Yield 43%; mp 201-202°C; IR (KBr) ν 3328, 3055, 2937, 2825, 1592, 1499, 1448, 1241, 1241, 1155, 1107, 750 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.47-2.52 (m, 4H, N-CH₂ piperazine), 2.70 (t, J = 6.5 Hz, 2H, S-CH₂-CH₂-N), 2.80-2.95 (m, 4H, N-CH₂ piperazine), 3.32 (t, J = 6.5 Hz, 2H, S-CH₂-CH₂-N), 3.74 (s, 3H, OCH₃), 6.20 (br s, NH₂), 6.74-6.77 (m, 2H, aromatic), 6.98-6.92 (m, 2H, aromatic), 7.60-7.73 (m, 3H, aromatic), 7.99-8.12 (m, 2H, aromatic), 8.26-8.30 (m, 2H, aromatic). Calculated for C₂₇H₃₀N₆O₃S₂: C, 58.88; H, 5.49; N, 15.26; S, 11.64; found: C, 58.90; H, 5.45; N, 15.20; S, 11.64.

4-Amino-2-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethylthio]-5-phenyl-2,4-dihydro-3H[1,2,4]triazole-3-thione (6a). The desired compound was obtained as a white solid. Yield 15%; mp 172-173°C; IR (KBr) ν 3286, 3142, 2950, 2838, 1500, 1463, 1230, 1139, 750 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.55-2.69 (m, 4H, N-CH₂ piperazine), 2.81 (t, J = 6.5 Hz, 2H, CSN-CH₂-CH₂-N), 2.85-2.90 (m, 4H, N-CH₂ piperazine), 3.75 (s, 3H, OCH₃), 5.90 (br s, 2H, NH₂), 6.85-6.90 (m, 4H, aromatic), 7.53-7.56 (m, 3H, aromatic), 8.00-8.05 (m, 2H, aromatic). Calculated for C₂₁H₂₆N₆O₃S: C, 61.44; H, 6.39; N, 20.48; S, 7.79; found: C, 61.47; H, 6.27; N, 20.50; S, 7.79.

4-Amino-5-(2-chlorophenyl)-2-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (6b). The desired compound was obtained as a white solid. Yield 17%; mp 158-159°C; IR (KBr) ν 3446, 3269, 2934, 2821, 1616, 1497, 1458, 1235, 1026, 752 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.52-2.72 (m, 4H, N-CH₂ piperazine), 2.82-2.86 (t, J = 6.2 Hz, CSN-CH₂-CH₂-N), 2.90-3.18 (m, 4H, N-CH₂ piperazine), 3.76 (s, 3H, OCH₃), 4.35 (t, J = 6.2 Hz, 2H, CSN-CH₂-CH₂-N), 5.67 (br s, 2H, NH₂), 6.84-6.93 (m, 4H, aromatic), 7.47-7.69 (m,
4H, aromatic). Calculated for C$_{21}$H$_{25}$ClN$_6$OS: C, 56.68; H, 5.66; N, 18.88; S, 7.20; found: C, 56.68; H, 5.60; N, 18.80; S, 7.27.

4-Amino-2-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-5-(4-methylphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (6c). The desired compound was obtained as a white solid. Yield 13%; mp 169-170°C; IR (KBr) $\nu$ 3261, 2933, 2830, 1497, 1436, 1379, 1338, 1235, 1030, 749 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.37 (s, 3H, CH$_3$), 2.53-2.69 (m, 4H, N-CH$_2$ piperazine), 2.81 (t, $J$ = 6.5 Hz, 2H, CN-CH$_2$CH$_2$-N), 2.87-2.99 (m, 4H, N-CH$_2$ piperazine), 3.76 (s, 3H, OCH$_3$), 4.33 (t, $J$ = 6.5 Hz, 2H, CN-CH$_2$CH$_2$-N), 5.88 (br s, 2H, NH$_2$), 6.84-6.92 (m, 4H, aromatic), 7.32-7.37 (m, 2H, aromatic), 7.91-7.95 (m, 2H, aromatic). Calculated for C$_{22}$H$_{28}$N$_6$OS: C, 62.23; H, 6.64; N, 19.79; S, 7.55; found: C, 62.20; H, 6.64; N, 19.70; S, 7.55.

4-Amino-5-(4-chlorophenyl)-2-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (6d). The desired compound was obtained as a white solid. Yield 16%; mp 196-197°C; IR (KBr) $\nu$ 3263, 3043, 2988, 2826, 1496, 1452, 1238, 1091, 1034, 1011, 848, 747 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.52-2.65 (m, 4H, N-CH$_2$ piperazine), 2.81 (t, $J$ = 6.5 Hz, 2H, CN-CH$_2$CH$_2$-N), 2.84-3.00 (m, 4H, N-CH$_2$ piperazine), 3.75 (s, 3H, OCH$_3$), 4.33 (t, $J$ = 6.5 Hz, 2H, CN-CH$_2$CH$_2$-N), 5.91 (br s, 2H, NH$_2$), 6.85-6.90 (m, 4H, aromatic), 7.61-7.65 (m, 2H, aromatic), 8.05-8.09 (m, 2H, aromatic). $^{13}$C-NMR (DMDO-$d_6$) $\delta$ 46.37, 49.95, 52.87, 55.10, 55.30, 111.88, 117.90, 120.83, 122.33, 124.19, 128.76, 129.89, 135.43, 141.18, 147.57, 151.95, 166.69. Calculated for C$_{21}$H$_{25}$ClN$_6$OS: C, 56.72; H, 5.70; N, 18.80; S, 7.15.

4-Amino-2-[[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-5-(4-phenylsulphonylphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (6f). The desired compound was obtained as a white solid. Yield 15%; mp 208-209°C; IR (KBr) $\nu$ 3451, 3273, 3141, 2934, 2815, 1497, 1448, 1320, 1160, 1105, 745 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$) $\delta$ 2.57-2.65 (m, 4H, N-CH$_2$ piperazine), 2.83 (t, $J$ = 6.4 Hz, 2H, CN-CH$_2$CH$_2$-N), 2.85-2.98 (m, 4H, N-CH$_2$ piperazine), 3.78 (s, 3H, OCH$_3$), 4.34 (t, $J$ = 6.4 Hz, 2H, CN-CH$_2$CH$_2$-N), 5.93 (br s, 2H, NH$_2$), 6.84-6.90 (m, 4H, aromatic), 7.60-7.72 (m, 3H, aromatic), 7.98-8.31 (m, 6H aromatic). Calculated for C$_{27}$H$_{30}$N$_6$O$_3$S$_2$: C, 58.88; H, 5.49; N, 15.26; S, 11.64; found: C, 58.90; H, 5.45; N, 15.26; S, 11.64.

**General procedure for 3-[[2-[4-(2-substitutedphenyl)1-piperazinyl][ethyl][thio]-5-aryl[1,2,4]triazole derivatives (8a-i)**

A solution of 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine (4a) or 1-(2-chloroethyl)-4-(2-nitrophenyl)piperazine (4b) (1 mmol) in 10 mL of acetone was added dropwise during 45 min to a mixture of the appropriate 5-aryl-2,4-dihydro-3H[1,2,4]triazole-3-thione (7a-g) (1 mmol), potassium carbonate (1 mmol) and potassium iodide (10 mg) dissolved in 25 mL of acetone. The reaction was refluxed with stirring for 6 h. After cooling, the mixture was concentrated in vacuo, diluted with water (50 mL) and extracted with ethyl acetate (3 x 50 mL). The extracts were combined, washed with brine and dried over anhydrous sodium sulphate. After evaporation of the solvent, the residual oil was purified by column chromatography (ethyl acetate/methanol 9:1 v/v).
The following compounds were obtained:

**3-[[2-[4-(2-Methoxyphenyl)1-piperazinyl]ethyl]thio]-5-phenyl[1,2,4]triazole (8a).** The desired compound was obtained as a white solid. Yield 72%; mp 102-103°C; IR (KBr) ν 3065, 2916, 2824, 1659, 1589, 1500, 1461, 1331, 1023, 922, 724 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.59-2.68 (m, 4H, N-CH₂ piperazine), 2.72 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 2.90-2.95 (m, 4H N-CH₂ piperazine), 3.33 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 3.76 (s, 3H, OCH₃), 6.84-6.99 (m, 4H, aromatic), 7.40-7.56 (m, 3H, aromatic), 7.94-7.99 (m, 2H, aromatic), 14.34 (br s, 1H, NH). Calculated for C₂₁H₂₅N₅OS: C, 63.77; H, 6.37; N, 17.70; S, 8.10; found: C, 63.70; H, 6.35; N, 17.80; S, 8.00.

**3-[[5-(2-Chlorophenyl)]-[[2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl]thio][1,2,4]triazole (8b).** The desired compound was obtained as a white solid. Yield 69%; mp 95-97°C; IR (KBr) ν 3060, 2935, 2818, 1733, 1666, 1592, 1499, 1451, 1307, 1241, 1122, 1025, 922, 744 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.51-2.60 (m, 4H, N-CH₂ piperazine), 2.72 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 2.93-2.99 (m, 4H, N-CH₂ piperazine), 3.31 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 3.76 (s, 3H, OCH₃), 6.85-6.92 (m, 4H, aromatic), 7.45-7.51 (m, 2H, aromatic), 7.76-7.82 (m, 2H, aromatic). Calculated for C₂₁H₂₄ClON₅S: C, 58.66; H, 5.62; N, 16.28; S, 7.45; found: C, 58.60; H, 5.65; N, 16.20; S, 7.45.

**3-[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]thio]-5-(4-methylphenyl)[1,2,4]triazole (8c).** The desired compound was obtained as a white solid. Yield 75%; mp 110-112°C; IR (KBr) ν 3062, 2937, 2818, 2361, 2337, 1591, 1500, 1451, 1327, 1241, 1122, 826, 744 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.35 (s, 3H, CH₃), 2.55-2.65 (m, 4H, N-CH₂ piperazine), 2.71 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 2.87-2.96 (m, 4H, N-CH₂ piperazine), 3.35 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 3.76 (s, 3H, OCH₃), 6.85-6.93 (m, 4H, aromatic), 7.29-7.34 (m, 2H, aromatic), 7.83-7.88 (m, 2H, aromatic), 14.26 (br s, 1H, NH). Calculated for C₂₂H₂₇N₅OS: C, 64.51; H, 6.64; N, 17.10; S, 7.82; found: C, 64.41; H, 6.60; N, 17.00; S, 7.82.

**3-[[5-(4-Chlorophenyl)]-[[2-[4-(2-methoxyphenyl)1-piperazinyl]ethyl]thio][1,2,4]triazole (8d).** The desired compound was obtained as a white solid. Yield 70%; mp 122-124°C; IR (KBr) ν 3424, 3062, 2937, 2931, 2902, 2876, 2361, 2337, 1735, 1588, 1500, 1451, 1413, 1318, 1247, 791 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.55-2.61 (m, 4H, N-CH₂ piperazine), 2.72 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 2.87-2.96 (m, 4H, N-CH₂ piperazine), 3.35 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 3.76 (s, 3H, OCH₃), 6.85-6.93 (m, 4H, aromatic), 7.55-7.59 (m, 2H, aromatic), 7.95-8.00 (m, 2H, aromatic). Calculated for C₂₁H₂₄ClON₅S: C, 58.66; H, 5.62; N, 16.28; S, 7.45; found: C, 58.63; H, 5.58; N, 16.20; S, 7.45.

**3-[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethyl]thio]-5-(4-propyloxyphenyl)[1,2,4]triazole (8e).** The desired compound was obtained as a white solid. Yield 65%; mp 113-115°C; IR (KBr) ν 3244, 3062, 2938, 2831, 2708, 2361, 1735, 1588, 1500, 1451, 1413, 1318, 1247, 791 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.55-2.61 (m, 4H, N-CH₂ piperazine), 2.72 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 2.87-2.96 (m, 4H, N-CH₂ piperazine), 3.35 (t, J = 6.8 Hz, 2H, S-CH₂-CH₂-N), 3.76 (s, 3H, OCH₃), 6.85-6.93 (m, 4H, aromatic), 7.55-7.59 (m, 2H, aromatic), 7.95-8.00 (m, 2H, aromatic). Calculated for C₂₂H₂₇N₅OS: C, 64.51; H, 6.64; N, 17.10; S, 7.82; found: C, 64.41; H, 6.60; N, 17.00; S, 7.82.
6.6 Hz, 2H, O-CH₂-CH₂-CH₃), 6.85-6.98 (m, 4H, aromatic), 7.02-7.08 (m, 2H, aromatic), 7.85-7.91 (m, 2H, aromatic). Calculated for C₂₄H₃₁N₅O₂S: C, 63.54; H, 6.88; N, 15.43; S, 7.06; found: C, 63.50; H, 6.80; N, 15.45; S, 15.40.

3-[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethythio]-5-(4-phenylsulphonylphenyl)][1,2,4]triazole (8f). The desired compound was obtained as a white solid. Yield 79%; mp 185-186°C; IR (KBr) ν 3061, 2916, 2829, 2361, 1732, 1667, 1595, 1499, 1449, 1156, 1104, 1022, 780 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.57-2.60 (m, 4H, N-CH₂ piperazine), 2.68 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 2.81-2.92 (m, 4H, N-CH₂ piperazine), 3.36 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 3.75 (s, 3H, OCH₃), 6.81-6.93 (m, 4H, aromatic), 7.55-7.73 (m, 3H, aromatic), 7.93-8.30 (m, 6H, aromatic). Calculated for C₂₇H₂₉N₅O₃S₂: C, 60.53; H, 5.45; N, 13.07; S, 11.96; found: C, 60.38; H, 5.30; N, 13.00; S, 11.90.

3-[[2-[4-(2-Methoxyphenyl)-1-piperazinyl]ethythio]-5-(4-nitrophenyl)][1,2,4]triazole (8g). The desired compound was obtained as a yellow solid. Yield 65%; mp 131-132°C; IR (KBr) ν 3083, 2924, 2830, 1934, 1601, 1510, 1456, 1342, 1109, 1024, 856, 751 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.58-2.63 (m, 4H, N-CH₂ piperazine), 2.74 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 2.89-2.98 (m, 4H, N-CH₂ piperazine), 3.40 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 3.76 (s, 3H, OCH₃), 6.83-6.94 (m, 4H, aromatic), 8.19-8.26 (m, 2H, aromatic), 8.34-8.41 (m, 2H, aromatic). Calculated for C₂₁H₂₄N₆O₃S: C, 57.25; H, 5.49; N, 19.07; S, 7.27; found: C, 57.30; H, 5.45; N, 19.19; S, 7.30.

3-[[2-[4-(2-Nitrophenyl)-1-piperazinyl]ethythio]-5-phenyl][1,2,4]triazole (8h). The desired compound was obtained as a yellow solid. Yield 73%; mp 147-148°C; IR (KBr) ν 3111, 2816, 1733, 1603, 1566, 1518, 1332, 1261, 1231, 1121, 1000, 725 cm⁻¹; ¹H NMR (DMSO-d₆) δ 2.55-2.66 (m, 4H, N-CH₂ piperazine), 2.72 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 2.89-3.12 (m, 4H, N-CH₂ piperazine), 3.34 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 7.08-7.16 (m, 1H, aromatic), 7.28-7.33 (m, 1H, aromatic), 7.46-7.62 (m, 1H, aromatic), 7.65-7.85 (m, 1H, aromatic), 7.90-8.01 (m, 2H aromatic). Calculated for C₂₀H₂₂N₆O₂S: C, 58.51; H, 5.40; N, 20.47; found: C, 58.55; H, 5.40; N, 20.40; S, 7.83.

3-[[2-[4-(2-Nitrophenyl)-1-piperazinyl]ethythio]-5-(4-propyloxyphenyl)][1,2,4]triazole (8i). The desired compound was obtained as a yellow solid. Yield 76%; mp 153-154°C; IR (KBr) ν 3093, 2935, 2875, 2819, 1735, 1607, 1519, 1452, 1331, 1237, 1173, 1003, 839, 751 cm⁻¹; ¹H NMR (DMSO-d₆) δ 0.98 (t, J = 7.6 Hz, 3H, O-CH₂-CH₂-CH₃), 1.65-1.85 (m, 2H, O-CH₂-CH₂-CH₃), 2.53-2.65 (m, 4H, N-CH₂ piperazine), 2.71 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 2.89-2.99 (m, 4H, N-CH₂ piperazine), 3.30 (t, J = 6.6 Hz, 2H, S-CH₂-CH₂-N), 3.98 (t, J = 6.6 Hz, 2H, O-CH₂-CH₂-CH₃), 7.03-7.17 (m, 3H, aromatic), 7.27-7.33 (m, 1H, aromatic), 7.50-7.63 (m, 1H, aromatic), 7.75-7.98 (m, 3H, aromatic), 14.15 (br s, 1H, NH). Calculated for C₂₃H₂₈N₆O₃S: C, 58.69; H, 6.06; N, 18.05; S, 6.88; found: C, 58.70; H, 6.10; N, 18.05; S, 6.90.

**Binding experiments.** Binding assays were performed on male CRL:CD(SD)BR-COBS rats weighing about 150 g. The animals were killed by decapitation, and their brains were rapidly dissected (hippocampus for 5-HT₁AR; cortex for α₁-AR), frozen and stored at -80 °C until the day of the assay.
Tissue were homogenized in about 50 volumes of ice-cold 50 mM Tris–HCl buffer (pH 7.4) using an Ultra Turrax TP-1810 (2×20 s) and centrifuged at 50,000 g for 10 min (Beckman model J-21B refrigerated centrifuge). The pellet was resuspended in the same volume of fresh buffer, incubated at 37 °C for 10 min and centrifuged again at 50,000 g for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before. The pellet was then resuspended in the appropriate incubation buffer [50 mM Tris–HCl (pH 7.7) containing 10 mM pargyline and, for the 5-HT$_1$A receptor, 4 mM CaCl$_2$ or, for the $\alpha_1$-adrenoceptor, 0.1% ascorbic acid] just before the binding assay.

Binding assays were performed as previously described.$^{18}$ Briefly, the following incubation conditions were used: 5-HT$_1$A receptor: [³H]-8-OH-DPAT (specific activity 157 Ci/mmol, NEN) final concentration 1 nM, 30 min at 25 °C (non-specific binding: 5-HT 10 mM); $\alpha_1$-adrenoceptor: [³H]prazosin (specific activity 71.8 Ci/mmol, NEN) final concentration 0.2 nM, 30 min at 25 °C (non-specific binding: prazosin 1 mM).

Incubation was stopped by rapid filtration under vacuum through GF/B filters which were then washed with 12 mL (4×3 times) of ice-cold 50 mM Tris–HCl buffer (pH 7.4) using a Brandel M-48R apparatus and counted in 4 mL of Ultima Gold MV (Packard) in a 1204 Betaplate BS (Wallac) liquid scintillation spectrometer with counting efficiency about 60%. Dose–inhibition curves were analysed by the `Allfit’ program to obtain the concentration of unlabelled drugs that inhibited ligand binding by the 50%.$^{19}$ The $K_i$ values were derived from the IC$_{50}$ values.$^{20}$

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References and Notes

9. Analytical and spectroscopic data for 1-(2-Ethoxyethyl)-4-(2-methoxy-phenyl)-piperazine: pale yellow oil. $^1$H NMR (CDCl$_3$) δ 1.22 (t, J = 7.1 Hz, 3H, O-CH$_2$-CH$_3$), 2.65-2.75 (m, 6 H, N-CH$_2$ and O-CH$_2$-CH$_2$-N), 3.10-3.12 (m, 4H, N-CH$_2$), 3.52 (q, J = 7.1 Hz, 2H, O-CH$_2$-CH$_3$), 3.61 (t, J = 6.0 Hz, 2H, O-CH$_2$-CH$_2$-N), 3.86 (s, 3H, O-CH$_3$), 6.88-7.00 (m, 4H, aromatic). Calculated for C$_{15}$H$_{24}$N$_2$O: C, 68.15; H, 9.15; N, 10.60; found: C, 67.99; H, 9.19; N, 10.68.