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 α_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, α_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 α_1-adrenoceptor (α_1-AR). In particular, over the years many efforts have been made to discover 5-HT_{1A} receptor ligands with reduced affinity for α_1-AR.[5-7]

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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.[8] 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 α_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 α_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.

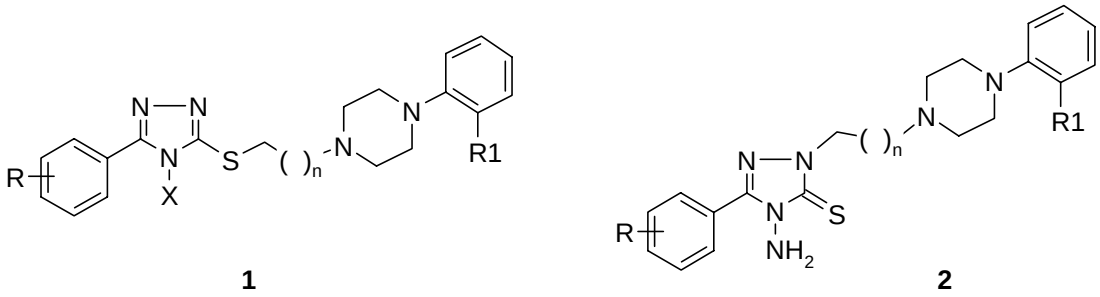
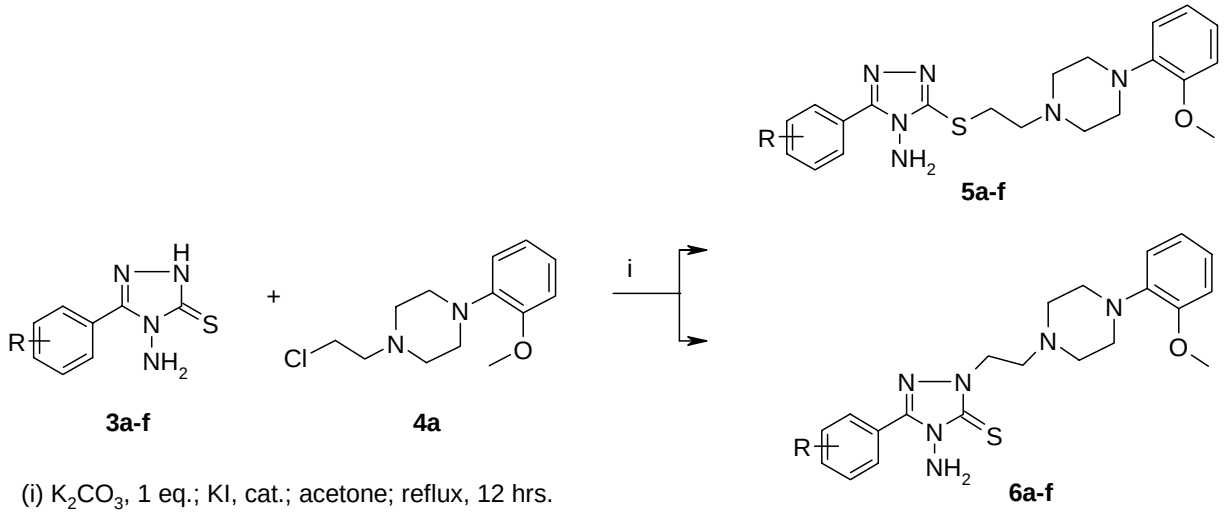


Figure 1. R = H, 2-Cl, 4-OCH_3, 3-OCH_3, 4-CH_3, 4-Cl, 4-Br, 4-*n*-C_3H_7O, 4-$C_6H_5SO_2$, 4-C_6H_5; X = H, NH_2; n = 1, 2; R_1 = OCH_3, NO_2.

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),[8] 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,[9] thus reducing the yield in the desired compounds. A similar reactivity had been shown by other chloroethylamines in ethanol in presence of potassium hydroxide.[10] 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.[11,12] In these experimental conditions, using **3a-f** as starting materials, both the S-alkylated

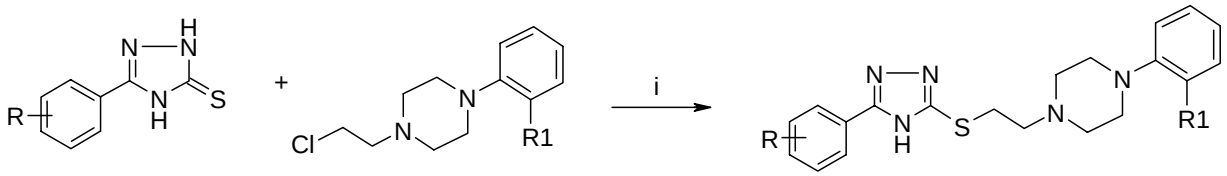
and the N-alkylated isomers were obtained (scheme 1). Flash chromatography of the crude material allowed the recovery of both isomers with an average yield of 45-50% for the S-alkylated isomers and 12-18% for the N-alkylated (ratio 3:1). As reported for triazoles previously described, [8] analysis of 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 δ, a broad singlet signal which integrates for two hydrogens and disappears in the presence of D_2O; 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 δ, which is typical for S–CH_2 connectivity, and for isomers **6a**–**f** of 4.3 δ, for NCSN–CH_2 connectivity.[8-13] These results were confirmed by ^{13}C-NMR spectra where a signal at 29.66 d was observed for the S-alkylated derivative **5d**, whereas the same signal is shifted to 46.37 δ for the N-alkylated derivative **6d**.



(i) K_2CO_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.



7a-g **4a-b** **8a-i**

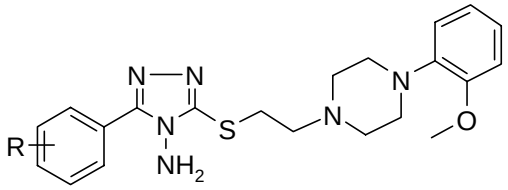
(i) K_2CO_3, 1 eq.; KI, cat.; acetone; reflux, 6 hrs.

Scheme 2

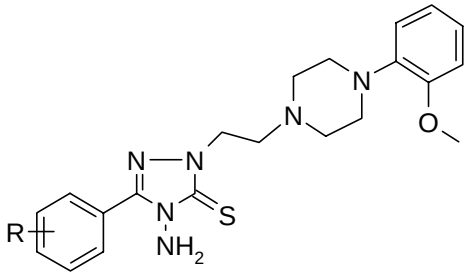
The starting 4-amino-5-aryl-2,4-dihydro-3H[1,2,4]triazole-3-thione (**3a-f**) and 5-aryl-2,4-dihydro-3H[1,2,4]triazole-3-thione (**7a-g**) were prepared as previously described.[8,14-17]
All the synthesized compounds were tested in binding experiments to evaluate their affinity and selectivity for the 5-HT_{1A} receptor over the α_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_{1A} serotonin receptor selective over the α_1AR.[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_{1A} receptor over the α_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, [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_{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 α_1-AR are more sensitive. As described in the previous paper, [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_{1A} receptors.

Table 1. Structure and binding properties of compounds **5a-f** and **6a-f**



5a-f



6a-f

Starting materials	Compound	R	5-HT_{1A}R[a] K_i(nM±sd)	α_1AR[b] K_i(nM±sd)	Selectivity[c]
3a	**5a**	H	10.53±0.58	206.93±17.56	19.6
3b	**5b**	2-Cl	25.36±1.56	143.35±15.27	5.6
3c	**5c**	4-CH_3	24.97±1.17	117.59±10.59	4.7
3d	**5d**	4-Cl	15.21±1.20	101.55±14.50	6.7
3e	**5e**[d]	4-OC_3H_7	48.78±3.02	274.39±44.28	5.6
3f	**5f**	4-$SO_2C_6H_5$	60.48±7.40	326.12±44.21	5.4
3a	**6a**	H	139.31±23.10	143.34±21.10	1
3b	**6b**	2-Cl	261.85±19.10	88.90±20.50	0.3
3c	**6c**	4-CH_3	147.51±7.02	127.59±16.53	0.9
3d	**6d**	4-Cl	120.97±6.63	187.44±17.32	1.5
3e	**6e**[d]	4-OC_3H_7	460.98±38.24	147.28±16.6	0.3
3f	**6f**	4-$SO_2C_6H_5$	N.T.	N.T.	

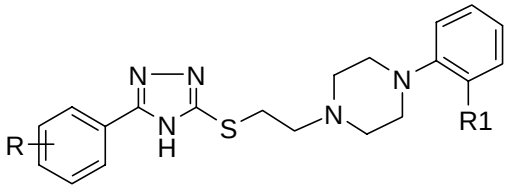
[a]Affinity at [^{3}H]-8-OH-DPAT-labelled 5-HT_{1A} sites.

[b]Affinity at [^{3}H]prazosin-labelled α_1-adrenergic sites.

[c]selectivity for 5-HT_{1A} over α_1 receptors is expressed as the ratio $K_i\,\alpha_1/K_i$ 5-$HT_{1A.}$

[d]physical and spectroscopic data are reported in reference 8.

Table 2. Structure and binding properties of compounds **8a-i**



8a-i

Starting materials	Compound	R	R_1	5-HT_{1A}R[a] K_i(nM±sd)	α_1AR[b] K_i(nM±sd)	Selectivity[c]
7a	**8a**	H	OCH_3	6.37±0.4	20.05±1.0	3.1
7b	**8b**	2-Cl	OCH_3	7.32±0.4	13.9±1.0	1.9
7c	**8c**	4-CH_3	OCH_3	5.85±0.9	19.70±3.0	3.4
7d	**8d**	4-Cl	OCH_3	8.16±0.4	28.70±7.0	3.5
7e	**8e**	4-OC_3H_7	OCH_3	30.90±5.0	56.20±5.0	1.8
7f	**8f**	4-$SO_2C_6H_5$	OCH_3	54.10±8.0	21.10±2.0	0.4
7g	**8g**	4-NO_2	OCH_3	16.34±2.0	42.20±5.0	2.6
7a	**8h**	H	NO_2	117.90±11.0	88.00±9.0	0.7
7e	**8i**	4-OC_3H_7	NO_2	735.00±93.53	425.00±106.79	0.6

[a]Affinity at [^{3}H]-8-OH-DPAT-labelled 5-HT_{1A} sites.
[b]Affinity at [^{3}H]prazosin-labelled α_1-adrenergic sites.
[c]selectivity for 5-HT_{1A} over α_1 receptors is expressed as the ratio K_i α_1/K_i 5-HT_{1A}.

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-HT_{1A} over α_1-adrenergic receptor. Generally this compounds showed K_i values in the nanomolar range and selectivity for the 5-HT_{1A} 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. ^{1}H NMR spectra were recorded on a Varian 200 MHz instrument (200 MHz for ^{1}H NMR

and 50 MHz for ^{13}C NMR) in DMSO-d_6 solution. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard; coupling costants (*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 ± 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 (λ =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)piperazine (**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 × 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) ν 3283, 3171, 3062, 2936, 2820, 1498, 1449, 1240, 751 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 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 $C_{21}H_{26}N_6OS$: 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) ν 3281, 3150, 2938, 2815, 1593, 1499, 1449, 1240, 749 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 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, S-*CH_2*-CH_2-N), 3.76 (s, 3H, OCH_3), 5.91 (br s, 2H, NH_2), 6.85-6.91 (m, 4H, aromatic), 7.46-7.66 (m, 4H, aromatic). Calculated for $C_{21}H_{25}ClN_6OS$: 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]ethyl]thio]-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-d_6) δ 2.37 (s, 3H, CH_3), 2.48-2.62 (m, 4H, N-CH_2 piperazine), 2.70 (t, J = 6.6 Hz, 2H, S-CH_2-CH_2-N), 2.83-2.99 (m, 4H, N-CH_2 piperazine), 3.31 (t, J = 6.6 Hz, 2H, S-CH_2-CH_2-N), 3.75 (s, 3H, OCH_3), 6.11 (br s, 2H, NH_2), 6.80-6.94 (m, 4H, aromatic), 7.29-7.33 (m, 2H, aromatic), 7.88-7.92 (m, 2H, aromatic). Calculated for $C_{22}H_{28}N_6OS$: 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]ethyl]thio][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 (DMDO-d_6) δ 2.42-2.60 (m, 4H, N-CH_2 piperazine), 2.70 (t, J = 6.6 Hz, 2H, S-CH_2-CH_2-N), 2.85-2.97 (m, 4H, N-CH_2 piperazine), 3.33 (t, J = 6.6 Hz, 2H, S-CH_2-CH_2-N), 3.75 (s, 3H, OCH_3), 6.17 (br s, 2H, NH_2), 6.79-6.94 (m, 4H, aromatic), 7.57-7.61 (m, 2H, aromatic), 8.04-8.08 (m, 2H, aromatic). ^{13}C-NMR (DMDO-d_6) δ 29.66, 49.89, 52.65, 55.27, 57.17, 111.86, 117.87, 120.75, 122.42, 125.88, 128.60, 129.37, 134.39, 141.14, 151.96, 152.83, 153.96. Calculated for $C_{21}H_{25}ClN_6OS$: 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]ethyl]thio]-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-d_6) δ 2.47-2.52 (m, 4H, N-CH_2 piperazine), 2.70 (t, J = 6.5 Hz, 2H, S-CH_2-CH_2-N), 2.80-2.95 (m, 4H, N-CH_2 piperazine), 3.32 (t, J = 6.5 Hz, 2H, S-CH_2-CH_2-N), 3.74 (s, 3H, OCH_3), 6.20 (br s, NH_2), 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, 4H, aromatic), 8.26-8.30 (m, 2H, aromatic). Calculated for $C_{27}H_{30}N_6O_3S_2$: 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]ethyl]-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-d_6) δ 2.55-2.69 (m, 4H, N-CH_2 piperazine), 2.81 (t, J = 6.5 Hz, 2H, CSN-CH_2-CH_2-N), 2.85-2.90 (m, 4H, N-CH_2 piperazine), 3.75 (s, 3H, OCH_3), 4.34 (t, J = 6.5 Hz, 2H, CSN-CH_2-CH_2-N), 5.90 (br s, 2H, NH_2 which exchange with D_2O), 6.85-6.90 (m, 4H, aromatic), 7.53-7.56 (m, 3H, aromatic), 8.00-8.05 (m, 2H, aromatic). Calculated for $C_{21}H_{26}N_6OS$: 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-d_6) δ 2.52-2.72 (m, 4H, N-CH_2 piperazine), 2.82-2.86 (t, J = 6.2 2H, CSN-CH_2-CH_2-N), 2.90-3.18 (m, 4H, N-CH_2 piperazine), 3.76 (s, 3H, OCH_3), 4.35 (t, J = 6.2 Hz, 2H, CSN-CH_2-CH_2-N), 5.67 (br s, 2H, NH_2), 6.84-6.93 (m, 4H, aromatic), 7.47-7.69 (m,

4H, aromatic). Calculated for $C_{21}H_{25}ClN_6OS$: 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) ν 3261, 2933, 2830, 1497, 1436, 1379, 1338, 1235, 1030, 749 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.37 (s, 3H, CH_3), 2.53-2.69 (m, 4H, N-CH_2 piperazine), 2.81 (t, J = 6.5 Hz, 2H, CSN-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, CSN-*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 $C_{22}H_{28}N_6OS$: 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) ν 3263, 3043, 2988, 2940, 2826, 1496, 1452, 1238, 1091, 1034, 1011, 848, 747 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.52-2.65 (m, 4H, N-CH_2 piperazine), 2.81 (t, J = 6.5 Hz, 2H, CSN-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, CSN-*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) δ 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 $C_{21}H_{25}ClN_6OS$: C, 56.68; H, 5.66; N, 18.88; S, 7.20; found: 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) ν 3451, 3273, 3141, 2934, 2815, 1497, 1448, 1320, 1236, 1160, 1105, 745 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.57-2.65 (m, 4H, N-CH_2 piperazine), 2.83 (t, J = 6.4 Hz, 2H, CSN-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, CSN-*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 $C_{27}H_{30}N_6O_3S_2$: 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 × 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-d_6) δ 2.59-2.68 (m, 4H, N-CH_2 piperazine), 2.72 (t, J = 6.8 Hz, 2H, S-CH_2-*CH_2*-N), 2.90-2.95 (m, 4H N-CH_2 piperazine), 3.33 (t, J = 6.8 Hz, 2H, S-*CH_2*-CH_2-N), 3.76 (s, 3H, OCH_3), 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 $C_{21}H_{25}N_5OS$: 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-d_6) δ 2.51-2.60 (m, 4H, N-CH_2 piperazine), 2.72 (t, J = 6.8 Hz, 2H, S-CH_2-*CH_2*-N), 2.93-2.99 (m, 4H, N-CH_2 piperazine), 3.31 (t, J = 6.8 Hz, 2H, S-*CH_2*-CH_2-N), 3.76 (s, 3H, OCH_3), 6.85-6.92 (m, 4H, aromatic), 7.45-7.51 (m, 2H, aromatic), 7.76-7.82 (m, 2H, aromatic). Calculated for $C_{21}H_{24}ClON_5S$: 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, 746 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.35 (s, 3H, CH_3), 2.55-2.65 (m, 4H, N-CH_2 piperazine), 2.71 (t, J = 6.8 Hz, 2H, S-CH_2-*CH_2*-N), 2.89-3.01 (m, 4H, N-CH_2 piperazine), 3.33 (t, J = 6.8 Hz, 2H, S-*CH_2*-CH_2-N), 3.76 (s, 3H, OCH_3), 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 $C_{22}H_{27}N_5OS$: 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-[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, 2939, 2831, 2708, 2361, 1735, 1588, 1500, 1451, 1413, 1318, 1247, 791 cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.55-2.61 (m, 4H, N-CH_2 piperazine), 2.72 (t, J = 6.8 Hz, 2H, S-CH_2-*CH_2*-N), 2.87-2.96 (m, 4H, N-CH_2 piperazine), 3.35 (t, J = 6.8 Hz, 2H, S-*CH_2*-CH_2-N), 3.76 (s, 3H, OCH_3), 6.85-6.93 (m, 4H, aromatic), 7.55-7.59 (m, 2H, aromatic), 7.95-8.00 (m, 2H, aromatic). ^{13}C NMR (DMSO-d_6) δ 29.02, 49.88, 52.65, 55.27, 57.22, 111.87, 117.90, 120.80, 122.40, 127.57, 127.91, 129.00, 134.23, 141.12, 151.95, 153.50, 155.28. Calculated for $C_{21}H_{24}ClON_5S$: 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 3067, 2936, 2876, 2816, 2364, 2337, 1612, 1501, 1455, 1241, 1175, 837, 749 cm^{-1}; ^1H NMR (DMSO-d_6) δ 0.98 (t, J = 7.6 Hz, 3H, O-CH_2-CH_2-*CH_3*), 1.65-1.84 (m, 2H, O-CH_2-*CH_2*-CH_3), 2.58-2.67 (m, 4H, N-CH_2 piperazine), 2.73 (t, J = 6.6 Hz, 2H, S-CH_2-*CH_2*-N), 2.89-2.96 (m, 4H, N-CH_2 piperazine), 3.30 (t, J = 6.6 Hz, 2H, S-*CH_2*-CH_2-N), 3.76 (s, 3H, OCH_3), 3.98 (t, J =

6.6 Hz, 2H, O-*CH*$_2$-CH$_2$-CH$_3$), 6.85-6.98 (m, 4H, aromatic), 7.02-7.08 (m, 2H, aromatic), 7.85-7.91 (m, 2H, aromatic). Calculated for $C_{24}H_{31}N_5O_2S$: 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]ethyl]thio]-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}; ^{1}H NMR (DMSO-d_6) δ 2.57-2.60 (m, 4H, N-CH$_2$ piperazine), 2.68 (t, *J* = 6.6 Hz, 2H, S-CH$_2$-*CH*$_2$-N), 2.81-2.92 (m, 4H, N-CH$_2$ piperazine), 3.36 (t, *J* = 6.6 Hz, 2H, S-*CH*$_2$-CH$_2$-N), 3.75 (s, 3H, OCH$_3$), 6.81-6.93 (m, 4H, aromatic), 7.55-7.73 (m, 3H, aromatic), 7.93-8.30 (m, 6H, aromatic). Calculated for $C_{27}H_{29}N_5O_3S_2$: 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]ethyl]thio]-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}; ^{1}H NMR (DMSO-d_6) δ 2.58-2.63 (m, 4H, N-CH$_2$ piperazine), 2.74 (t, *J* = 6.6 Hz, 2H, S-CH$_2$-*CH*$_2$-N), 2.89-2.98 (m, 4H, N-CH$_2$ piperazine), 3.40 (t, *J* = 6.6 Hz, 2H, S-*CH*$_2$-CH$_2$-N), 3.76 (s, 3H, OCH$_3$), 6.83-6.94 (m, 4H, aromatic), 8.19-8.26 (m, 2H, aromatic), 8.34-8.41 (m, 2H, aromatic). Calculated for $C_{21}H_{24}N_6O_3S$: 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]ethyl]thio]-5-phenyl[1,2,4]triazole (8h). The desired compound was obtained as a yellow solid. Yield 73%; mp 147-148; IR (KBr) ν 3111, 2816, 1733, 1603, 1566, 1518, 1332, 1261,1231, 1121, 1000, 725 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 2.55-2.66 (m, 4H, N-CH$_2$ piperazine), 2.72 (t, *J* = 6.6 Hz, 2H, S-CH$_2$-*CH*$_2$-N), 2.89-3.12 (m, 4H, N-CH$_2$ piperazine), 3.34 (t, *J* = 6.6 Hz, 2H, S-*CH*$_2$-CH$_2$-N), 7.08-7.16 (m, 1H, aromatic), 7.28-7.33 (m, 1H, aromatic), 7.46-7.62 (m, 4H aromatic), 7.65-7.85 (m, 1H, aromatic), 7.90-8.01 (m, 2H aromatic). Calculated for $C_{20}H_{22}N_6O_2S$: C, 58.51; H, 5.40; N, 20.47; S, 7.81; found: C, 58.55; H, 5.40; N, 20.40; S, 7.83.

3[[2-[4-(2-Nitrophenyl)-1-piperazinyl]ethyl]thio]-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, 973, 839, 751 cm^{-1}; ^{1}H NMR (DMSO-d_6) δ 0.98 (t, *J* = 7.6 Hz, 3H, O-CH$_2$-CH$_2$-*CH*$_3$), 1.65-1.85 (m, 2H, O-CH$_2$-*CH*$_2$-CH$_3$), 2.53-2.65 (m, 4H, N-CH$_2$ piperazine), 2.71 (t, *J* = 6.6 Hz, 2H, S-CH$_2$-*CH*$_2$-N), 2.89-2.99 (m, 4H, N-CH$_2$ piperazine), 3.30 (t, *J* = 6.6 Hz, 2H, S-*CH*$_2$-CH$_2$-N), 3.98 (t, *J* = 6.6 Hz, 2H, O-*CH*$_2$-CH$_2$-CH$_3$), 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 $C_{23}H_{28}N_6O_3S$: 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-HT$_{1A}$R; cortex for α_1-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_{1A} receptor, 4 mM $CaCl_2$ or, for the α_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_{1A} receptor: [^{3}H]-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); α_1-adrenoceptor: [^{3}H]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

1. Fargin, A.; Raymond, J. R.; Lohse, M. J.; Kobilka, B. K.; Caron, M. G.; Lefkowitz, R. J. *Nature* **1988**, *335*, 358.
2. Broekkamp, C. L. E.; Leysen, D.; Peeters, B. W. M. M.; Pinder, R. M. *J. Med. Chem.* **1995**, *38*, 4615.
3. Blier, P.; de Montigny, C. *Trends Pharmacol. Sci.* **1994**, *15*, 220.
4. Hamon, M. *Trends Pharmacol. Sci.* **1994**, *15*, 36.
5. Raghupathi, R. K.; Rydelek-Fitzgerald, L.; Teitler, M.; Glennon R. A. *J. Med. Chem.* **1991**, *34*, 2633.
6. Orjales, A.; Alonso-Cires, L.; Labeaga, L.; Corcostegui, R. *J. Med. Chem.* **1995**, *38*, 1273.
7. Perrone, R.; Berardi, F.; Colabufo, N. A.; Leopoldo, M.; Tortorella, V. *Med. Chem. Res.* **1999**, *9*, 340.

8. Sarvà, M. C.; Romeo, G.; Guerrera, F.; Siracusa, M.; Salerno, L.; Russo, F.; Cagnotto, A.; Goegan, M.; Mennini, T. *Bioorg. Med. Chem.* **2002**, *10*, 313.
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 $C_{15}H_{24}N_2O$: C, 68.15; H, 9.15; N, 10.60; found: C, 67.99; H, 9.19; N, 10.68.
10. Block, H. W.; Mason, J. P. *J. Am. Chem. Soc.* **1941**, *63,* 298.
11. Betti, L.; Botta, M.; Corelli, F.; Floridi, M.; Giannaccini, G.; Maccari, L.; Manetti, F.; Strappaghetti, G.; Tafi, A.; Corsano, S. *J. Med. Chem.* **2002**, *45*, 3603.
12. Montesano, F.; Barlocco, D.; Dal Piaz, V.; Leonardi, A.; Poggesi, E.; Fanelli, F.; De Benedetti, P. G. *Bioorg. Med. Chem.* **1998**, 6, 925.
13. Modica, M.; Santagati, M.; Russo, F.; Selvaggini, C.; Cagnotto, A.; Pennini, T. *Eur. J. Med. Chem.* **2000**, *35*, 677.
14. Mndzhoyan, A. L.; Afrikyan, V. G.; Dokhikyan, A. A. *Izvest. Akad. Nauk Armyan. S.S.R., Ser. Khim. Nauk.* **1957**, *10*, 357.
15. Mndzhoyan, A. L.; Afrikyan, V. G.; Dokhikyan, A. A. *Izvest. Akad. Nauk Armyan. S.S.R., Ser. Khim. Nauk.* **1957**, *10*, 363.
16. Saramet, I.; Banciu, M. D.; Draghici, C. *Revue Roumaine de Chimie* **1991**, *36*, 135.
17. Dieter, H-R.; Engel, J.; Klingler, K.; Kutscher, B.; Szelenyi, S.; Achterrath-Tuckermann, U.; Schmidt, J.; Metzenauer, P. *Eur. Pat. Appl.* 1994, EP 584487 A2 19940302.
18. Caccia, S.; Confalonieri, S.; Guiso, G.; Bernasconi, P.; Cagnotto, A.; Skorupska, M.; Mennini, T. *Psychopharmacol.* **1994**, *115*, 502.
19. De Lean, A.; Munson, P.J.; Rodbard, D. *Am. J. Physiol.* **1978**, *235*, E97.
20. Cheng, Y.; Prusoff, W. H. *Biochem. Pharmacol.* **1973**, *22*, 3099.