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[1,2,4]Triazole Derivatives as 5-HT_{1A} Serotonin Receptor Ligands

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Abstract—A series of new 4-amino-3-[3-[4-(2-methoxy or nitro phenyl)-1-piperazinyl] propyl]thio]-5-(substitutedphenyl)[1,2,4]triazoles **11a**–**t** was synthesized in order to obtain compounds with high affinity and selectivity for 5-HT_{1A} receptor over the α_1-adrenoceptor. A series of isomeric 4-amino-2-[3-[4-(2-methoxy or nitro phenyl)-1-piperazinyl]propyl]-5-(substitutedphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thiones **12a**–**r** was also isolated and characterized. New compounds were tested to evaluate their affinity for 5-HT_{1A} receptor and α_1-adrenoceptor in radioligand binding experiments. As a general trend, triazoles **11a**–**t** showed a preferential affinity for the 5-HT_{1A} receptor whereas isomeric 2,4-dihydro-3H[1,2,4]triazole-3-thiones **12a**–**r** preferentially bind to the α_1-adrenoceptor site. Several molecules showed affinities in the nanomolar range and 4-amino-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]-5-(4-propyloxy-phenyl)[1,2,4]triazole (**11o**) was the most selective derivative for the 5-HT_{1A} receptor (K_i α_1/K_i 5-HT_{1A} = 55). The decrease in 5-HT_{1A} receptor selectivity in 3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]-5-(substitutedphenyl)[1,2,4] triazole **14a**–**b**, lacking in the amino group in 4-position of the triazole ring, in comparison with their analogues in the series **11a**–**t**, suggest that the amino function represents a critical structural feature in determining 5-HT_{1A} receptor selectivity in this class of compounds.

Introduction

Serotonin (5-HT) is an important neurotransmitter that mediates a wide variety of physiological responses in both the peripheral and central nervous systems.

The receptors activated by 5-HT have been divided into at least seven classes (5-HT_{1-7}) and each class has been further subdivided into different subtypes.[1] Among them, the 5-HT_{1A} receptor is one of the best studied, as a result of the early availability of selective ligands such as 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT) **1** (Chart 1) which in the tritiated form is used to label the 5-HT_{1A} receptor and whose *R* enantiomer acts as a potent 5-HT_{1A} agonist.[2] The 5-HT_{1A} receptor has been cloned and belongs to the G-protein coupled receptors superfamily.[3] It is generally accepted that it is involved in psychiatric disorders such as depression and anxiety.[4–6] In a recent paper, 5-HT_{1A}-knockout mice were shown to behave with an anxiety-related disorder similarly to humans.[7]

Several potent 5-HT_{1A} receptor ligands are already known and, from a chemical point of view, they can be subdivided into different classes.[8,9] Some effective 5-HT_{1A} receptor partial agonists such as buspirone **2** and ipsapirone **3** belong to the arylpiperazine class and they are currently used in therapy as anxiolitic and antidepressant drugs. However, a limitation in the potential use of many 5-HT_{1A} receptor ligands as drugs or pharmacological tools, is their undesired high affinity for other receptor subtypes. The dopaminergic D_2 receptor and the α_1-adrenoceptor are two examples of receptor sites to which several 5-HT_{1A} ligands bind with high affinity. For example, possibly as a reflection of the high degree of similarity (45%) in the amino acid sequence between the 5-HT_{1A} and the α_1-adrenoceptor, some 5-HT_{1A} receptor ligands such as NAN190 **4** show good affinity for the α_1-adrenoceptor sites.[10,11] On the other hand, some well-known α_1-adrenoceptor ligands, such as WB4101 **5** or BMY 7378 **6**, bind to 5-HT_{1A} receptor.[12–14]

In the last years, many efforts have been made to discover 5-HT_{1A} receptor ligands devoid of affinity for α_1-adrenoceptor.[11,15,16] In previous papers we have reported the synthesis of new thieno[2,3-*d*]pyrimidines as 5-HT_{1A} receptor ligands.[17,18] Some of them, such as compound **7** and its nitro derivative **8** had shown high affinity at the 5-HT_{1A} receptor sites coupled to a reduced α_1-adre-

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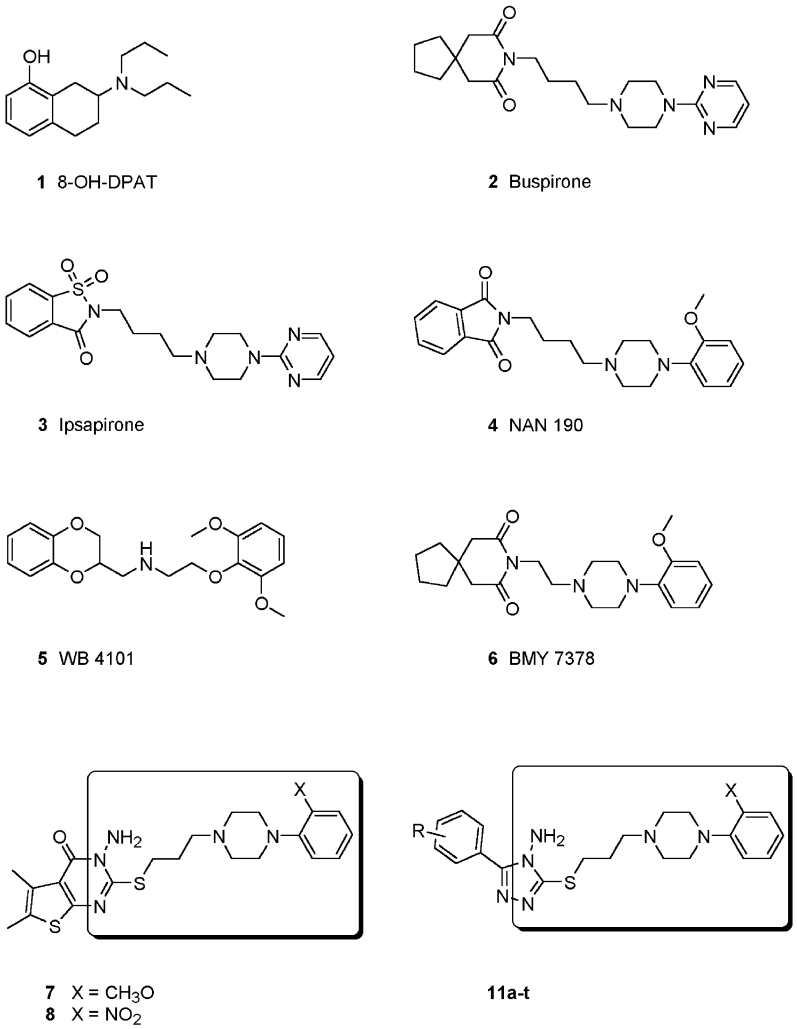


Chart 1.

nergic affinity. As in many arylpiperazine ligands, the structures of **7** and **8** can be subdivided in three principal parts. One is represented by the phenylpiperazinyl (PP) moiety, which forms an essential part of the pharmacophore of the molecule for 5-HT_{1A} affinity. The second is constituted by a thioalkyl chain (whose length highly influences affinity and selectivity for the 5-HT_{1A} receptor) which connects the PP moiety with the third part, the 3-amino-5,6-dimethylthieno[2,3-*d*]pyrimidine-4(3H)-one heterocycle. Several structural modifications have already been made in this heterocyclic moiety by changing the nature of substituents or by substitution of the thiophene ring for other heterocyclic rings.[17,18] From these studies, the presence of an amino group in 3-position of the thieno[2,3-*d*]pirimidine nucleus emerged as one of the most important structural features which, in this class of molecules, induces high affinity and selectivity for the 5-HT_{1A} receptor.

With the aim to obtain new 5-HT_{1A} receptor ligands with improved selectivity over the α_1-adrenoceptor, we now report a new series of derivatives **11a**–**t**, structurally related to compounds **7** and **8**, in which the 3-amino-5,6-dimethylthieno[2,3-*d*]pyrimidine-4(3H)-one part was exchanged for a 4-amino-5-aryl[1,2,4] triazole. In this new series, an *N*-amino substituent is mantained in the *ortho* position respect to the thioalkyl chain. Substituents on the phenyl ring in the 5-position of triazole ring were chosen with the aim of varying the physicochemical properties. They were selected according to the principal properties approach, in which the original variables (π, MR, σ_m, σ_p, Sterimol parameters: L and B_i–B_{iv}) for each substituent are described by means of fewer new variables which are linear combinations of the original ones and are mutually uncorrelated.[19] Thus, we selected the following eight substituents: CH_3O, CH_3, Cl, Br, *n*-C_3H_7O, $C_6H_5SO_2$, $C_6H_5OCH_2$ and C_6H_5, in the *para* position of the phenyl ring. We also decided to enlarge the series with the preparation of some *meta* or *ortho* substituted and unsubstituted derivatives.

During the synthesis of the new compounds, we noticed that, together with the desired triazole (**11a**–**t**), isomeric 2,4-dihydro-3H[1,2,4]triazole-3-thione derivatives (**12a**–**r**) (Table 1) were also formed. In many cases, we also accomplished their isolation and structural characterization.

We also synthesized compound **11u**, along with its isomer **12s**, bearing a shorter connecting chain between the PP moiety and the triazole ring.

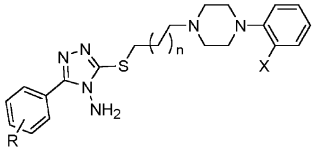
To test the importance of the *N*-amino group for the interaction of new compounds with the 5-HT_{1A} receptor, we prepared analogues **14a–b** (Table 1) lacking in the amino function in 4-position of triazole ring.

Compounds **11a–u** and **14a–b** along with some 2,4-dihydro-3H[1,2,4]triazole-3-thione derivatives (**12a–e**, **12g**, **12s**) were tested in binding experiments to evaluate their affinity and selectivity for the 5-HT_{1A} receptor with respect to the α_1-adrenoceptor.

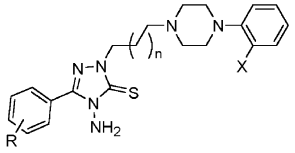
Chemistry

The general strategy for the synthesis of compounds **11a–u** and **12a–s** is summarized in Scheme 1. A number of 4-amino - 5 - (substitutedphenyl) - 2,4 - dihydro - 3H[1,2,4]triazole-3-thiones (**9a–k**) were chosen as starting materials. They were reacted in ethanol, in the presence of sodium hydroxide and a catalytic amount of potassium iodide, with 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine (**10a**), 1-(3-chloropropyl)-4-(2-nitrophenyl)piperazine (**10b**) or 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine (**10c**), to give desired products **11a–u** (Table 1) in moderate to good yields. In these experimental conditions, isomeric *N*-alkylated derivatives **12a–s** were also formed in lower yields and, in many cases, we were able to isolate these side products. 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 5.8 to 6.2 δ, a broad singlet signal which integrates for two hydrogens and disappeares in presence of D_2O; it is analogous to the signal seen in the ^{1}H NMR spectra of 4-amino-5-(substitutedphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thiones (**9a–k**) and it has to be attributed to the NH_2 group. This implies that alkylation of compounds **9a–k** does not take place at the NH_2 substituent. In both spectra, a triplet for the methylene group of the propyl chain that connects the PP moiety and the triazole part of the molecule, is observed. For derivatives **11a–u**, this triplet has a chemical shift of 3.15 δ, which is typical for S–CH_2 connectivity, and for isomers **12a–s** of 4.2 δ, which is typical for N–CH_2 connectivity.[18]

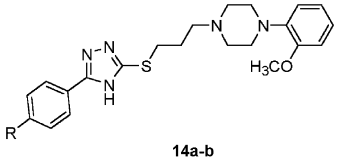
Table 1. Structure of compounds **11a–u**, **12a–s** and **14a–b**



11a-u



12a-s



14a-b

Compound	*n*	R	X
11a, **12a**, **14a**	1	H	CH_3O
11b, **12b**	1	H	NO_2
11c, **12c**	1	2-Cl	CH_3O
11d, **12d**	1	2-Cl	NO_2
11e, **12e**, **14b**	1	4-CH_3O	CH_3O
11f, **12f**	1	4-CH_3O	NO_2
11g, **12g**	1	3-CH_3O	CH_3O
11h, **12h**	1	3-CH_3O	NO_2
11i, **12i**	1	4-CH_3	CH_3O
11j, **12j**	1	4-CH_3	NO_2
11k, **12k**	1	4-Cl	CH_3O
11l, **12l**	1	4-Cl	NO_2
11m, **12m**	1	4-Br	CH_3O
11n, **12n**	1	4-Br	NO_2
11o, **12o**	1	4-*n*-C_3H_7O	CH_3O
11p, **12p**	1	4-*n*-C_3H_7O	NO_2
11q, **12q**	1	4-$C_6H_5SO_2$	CH_3O
11r, **12r**	1	4-$C_6H_5SO_2$	NO_2
11s	1	4-$C_6H_5OCH_2$	CH_3O
11t	1	4-C_6H_5	CH_3O
11u, **12s**	0	4-*n*-C_3H_7O	CH_3O

Many of the starting 4-amino-5-(substitutedphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione were already known (compounds **9a–c**, **9f–g**) and were prepared as previous described.[20–22] The synthesis of starting materials **9d** and **9h–k** was accomplished following the procedure described in Scheme 2. The appropriate benzoylhydrazide was reacted with carbon disulfide in presence of potassium hydroxide to give the corresponding potassium dithiocarbazinate which in presence of hydrazine hydrate afforded 4-amino-5-(substitutedphenyl)-2,4-dihydro-3H[1,2,4] triazole-3-thiones (**9d**, **9h–k**) in good yields.

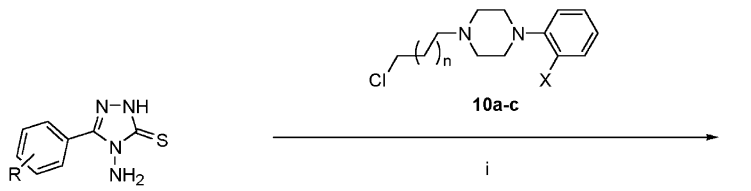
The preparation of derivatives **14a** and **14b** (Table 1), which lack the amino substituent in the 4-position of the triazole, was accomplished using similar reaction conditions as for the synthesis of compounds **11a–u**. 5-Phenyl-2,4-dihydro-3H[1,2,4]triazole-3-thione (**13a**) or 5-(4-methoxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (**13b**) and 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine (**10a**) are reacted in alcaline medium (Scheme 3). In this case, only the *S*-alkylated isomers **14a–b** were isolated from the reaction mixture.

Pharmacology

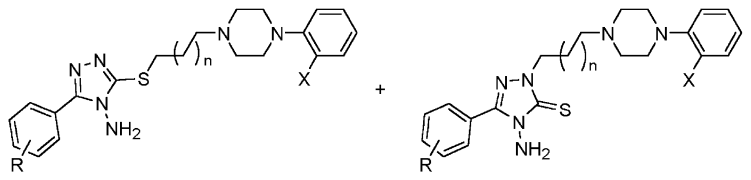
The affinity of test compounds **11a–s**, **12a–e**, **12g**, **12s**, **6a** and **6b** for 5-HT_{1A} and α_1 receptors was established by measuring their ability to displace in vitro the radioligands [^{3}H]-8-OH-DPAT (rat hippocampus) or [^{3}H]-prazosin (rat cortex), respectively. Compounds **11h**, **11i** and **11o** were also tested in binding assays to evaluate their affinity for the dopaminergic D_2 receptor using [^{3}H]spiperone (rat striatum) as radioligand. The results are summarized in Table 2 and expressed as K_i (nM) values along with the affinities of buspirone **2** and ipsapirone **3** for comparison.[23]

Results and Discussion

As a general trend, compounds **11a–t**, with the phenylpiperazinylpropyl chain bonded to the exocyclic sulfur atom, showed good and preferential affinity for the 5-HT_{1A} receptor over the α_1-adrenoceptor whereas most



9a-k



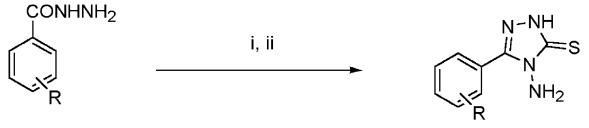
11a-u 12a-s

R = H; 2-Cl; 4-CH_3O; 3-CH_3O; 4-CH_3;4-Cl; 4-Br;
4-*n*-C_3H_7O; 4-$C_6H_5SO_2$; 4-$C_6H_5OCH_2$; 4-C_6H_5;

X = CH_3O; NO_2;

n = 0, 1;

Scheme 1. Synthesis of compounds **11a**–**u** and **12a**–**s**. Reagent and conditions: (i) EtOH, KOH, reflux.



9d R = 3-CH_3O
9h R = 4-*n*-C_3H_7O;
9i R = 4-$C_6H_5SO_2$;
9j R = 4-$C_6H_5OCH_2$;
9k R = 4-C_6H_5;

Scheme 2. Synthesis of compounds **9d**, **9h**–**k**. Reagent and conditions: (i) CS_2, KOH, EtOH, reflux; (ii) N_2H_4, reflux.

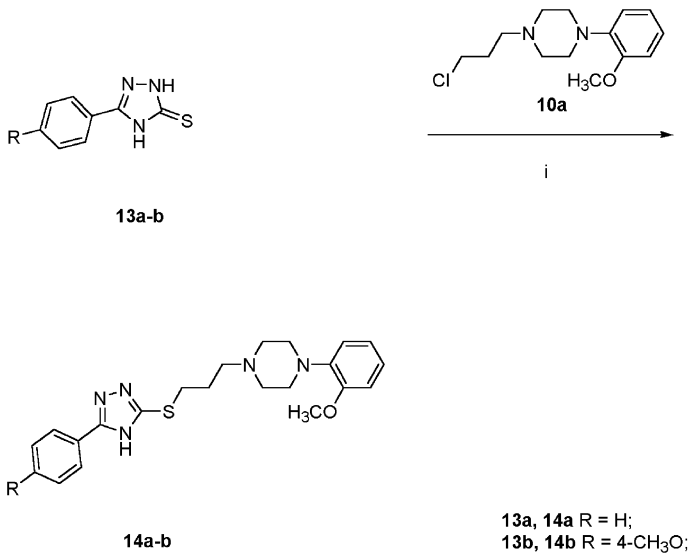
of compounds **12a**–**e**, **12g**, bearing the phenylpiperazinylpropyl chain in 2-position of the 2,4-dihydro-3H[1,2,4]triazole-3-thione ring, displayed a preferential affinity for the α_1-adrenoceptor. Besides the obvious differences in connectivity, the two series of molecules differ in the length of the connecting chain between the triazole and the piperazine ring. In compounds **11a**–**t**, the spacer is four atoms long whereas in **12a**–**e** and **12g** it is one atom shorter. In previous series of arylpiperazine 5-HT_{1A} receptor ligands, the optimum chain length for 5-HT_{1A} receptor affinity appeared to be four atoms.[17,24,25] A decrease in length of the chain often increase α_1-adrenoceptor affinity. This might explain the observed differences in affinity/selectivity between compounds **11a**–**t**, **12a**–**e** and **12g**.

In general, the 2-methoxyphenylpiperazinyl derivatives showed higher affinity at both receptor sites than their respective 2-nitrophenylpiperazinyl analogues. In the **11**-series, they also showed a higher 5-HT_{1A} selectivity as compared to the nitro analogues. It should be noted, however, that the nitro analogue **11h** (K_i 5-HT_{1A} = 3.69 nM) shows a higher selectivity than its methoxy analogue **11g** (ratio 43 vs 21).

In general, in the **11**-series, substituents on the phenyl ring in the 5-position of the triazole seem to have little effect on 5-HT_{1A} receptor affinity. All the 2-methoxyphenylpiperazinyl compounds showed good affinity for the receptor with variations in K_i values not greater than one order of magnitude ($1.71 \leq K_i \leq 12.86$), with **11c** having the highest affinity.

Compounds such as **11i**, bearing a 4-methyl group, and **11s** with a bulkier 4-phenoxymethyl substituent display similar affinity values for the 5-HT_{1A} receptor (**11i**, $K_i = 1.77$ nM; **11s**, $K_i = 1.71$ nM). Since the initial selection of substituents had been done trying to maximize variations in their physical–chemical features, the observed trend suggests that the receptor binding site is able to accomodate substituents of different nature and shape. On the other hand, introduction of a substituent on the phenyl ring generally leads to an increase in 5-HT_{1A} receptor selectivity, with the exceptions of compounds **11q** and **11r** (R = 4-$C_6H_5SO_2$). Compound **11o** (R = 4-C_3H_7O) shows the highest selectivity in this series (ratio of 55).

The 5-HT_{1A} receptor affinity of 2,4-dihydro-3H[1,2,4]triazole-3-thione derivatives (**12a**–**e**, **12g**), having a shorter connecting chain, was notably lower than the affinity



Scheme 3. Synthesis of compounds **14a**–**b**. Reagent and conditions: (i) EtOH, KOH, reflux.

Table 2. In vitro affinities of test compounds **11a**–**u**, **12a**–**e**, **12g**, **12s** and **14a**–**b** at the 5-HT_{1A} α_1 and D_2 receptors sites (K_i in nM ± SD)[a]

Compound	5-HT_{1A}	α_1	D_2	Selectivity[b]
11a	2.68±0.13	16.30±2.92	NT[c]	6
11b	23.05±2.99	95.25±18	NT	4
11c	1.03±0.1	15.51±2.18	NT	15
11d	7.66±0.99	78.70±17	NT	10
11e	3.84±0.32	69.33±1.39	NT	18
11f	17.80±3.18	258.3±19	NT	14
11g	2.3±0.79	47.84±5.10	NT	21
11h	3.69±0.83	167.34±32	290±40	43 (78)[d]
11i	1.77±0.43	70.96±13	127±15	40 (72)[d]
11j	29.56±7	262.59±9.43	NT	9
11k	2.84±0.27	77.38±5.87	NT	27
11l	21.91±3.28	211.34±14.71	NT	10
11m	2.96±0.27	91.21±5.48	NT	31
11n	27.35±2.19	132.21±14.15	NT	5
11o	2.85±0.18	157.49±67.40	136±27	55 (48)[d]
11p	26.74±0.76	276.11±58.54	NT	10
11q	12.86±0.82	43.76±5.69	NT	3
11r	114.37±12.81	168.24±21.76	NT	1.4
11s	1.71±0.11	24.9±4	NT	15
11t	4.18±0.29	134.3±27	NT	32
11u	48.8±3.0	274.4±44.3	NT	5.6
12a	54±5	17±1	NT	0.3
12b	314±54	43±5	NT	0.1
12c	77±5	39±5	NT	0.5
12d	188±15	40±3	NT	0.2
12e	10±1	18±4	NT	1.8
12g	14±1	12±1	NT	0.8
12s	461±38.2	147.3±16.6	NT	0.3
14a	1.85±0.30	1.71±0.42	NT	0.9
14b	2.50±0.20	6.13±0.25	NT	2.4
2	15[e]	600[e]	42[e]	40 (2.8)[d]
3	5.5[e]	200[e]	400[e]	36 (72)[d]

[a]K_i values were calculated as described in Experimental and are means (±SD) of three separate experiments.
[b]Selectivity for 5-HT_{1A} over α_1 receptor is expressed as the ratio K_i α_1/K_i 5-HT_{1A}.
[c]NT, not tested.
[d]Selectivity for 5-HT_{1A} over D_2 receptor is expressed as the ratio K_i D_2/K_i 5-HT_{1A}.
[e]Data taken from ref 23.

showed by their structural isomers **11a**–**e** and **11g**. However, with the exception of compound **12c**, they showed increased affinity for the α_1-adrenoceptor sites. As a result of these two trends, 5-HT_{1A} selectivity is lost and, with the exception of compound **12e**, a modest α_1-adrenoceptor selectivity is obtained.

In compound **11u**, the connecting thioalkyl chain is one methylene shorter than in **11o**. Although **11u** showed lower affinity for both 5-HT_{1A} and α_1 receptors, the decrease was more evident at the 5-HT_{1A} sites. This resulted in a 10-fold loss of selectivity with respect to **11o**. Compound **12s**, the regioisomer of **11u**, displayed the lower 5-HT_{1A} receptor affinity among test compounds and, as the other derivatives in the **12**-series, a modest α_1-adrenoceptor selectivity.

Compounds **14a** and **14b**, analogues of **11a** and **11e**, respectively, lack the NH_2 group in 4-position on the triazole ring and were synthesized and tested to evaluate the importance of the amino function in the formation of the receptor-ligand complex. As previously reported, the amino group present in the structure of thieno[2,3-*d*]pyrimidines **7** and **8** improves their 5-HT_{1A} receptor selectivity by enhancing the affinity of the molecules for this receptor over the α_1-adrenoceptor.[18]

Compounds **14a** and **14b** were equipotent in 5-HT_{1A} affinity to compounds **11a** and **11e**. However, the affinity for the α_1-adrenoceptor site has increased 10-fold. As a consequence, compounds **14a** and **14b** do not show any 5-HT_{1A}/α_1 receptor selectivity. Thus, in this 1,2,4-triazole class, the NH_2 substituent enhances selectivity via its detrimental effect on α_1-adrenoceptor affinity.

Compounds **11h**, **11i** and **11o**, the most selective derivatives for the 5-HT_{1A} over the α_1 receptor, were also tested in binding assays to evaluate their affinity for the dopaminergic D_2 receptor. They proved to be poor D_2

ligands; their D_2/5-HT_{1A} selectivity ratios are comparable to the ratio showed by ipsapirone and notably higher than the one of buspirone.

In conclusion, a new series of 4-amino-3-[3-[4-(2-methoxy or nitro phenyl)-1-piperazinyl]propyl]thio]-5-(substitutedphenyl)[1,2,4]triazoles **11a**–**t** was synthesized and tested in radioligand binding assays to evaluate their affinity and selectivity for the 5-HT_{1A} receptor with respect to the α_1-adrenergic receptor. Generally, these compounds showed K_i values in the nanomolar range and selectivity for the 5-HT_{1A} receptor. 4-Amino-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]-5-(4-propyloxyphenyl)[1,2,4]triazole (**11o**) showed good selectivity for the 5-HT_{1A} receptor over both the α_1-adrenoceptor (ratio of 55) and the D_2 receptor (ratio of 48). Synthetic and pharmacological work is in progress to further evaluate the structure-affinity relationships in this class of 5-HT_{1A} receptor ligands.

Experimental

Chemistry

Melting points were determined in a Gallemkamp apparatus with a digital thermometer MFB-595 in glass capillary tubes and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer FTIR 1600 spectrometer in KBr disks. Elemental analyses for C, H, N and S were within $\pm 0.4\%$ of theoretical values and were performed on a Carlo Erba Elemental Analyzer Mod. 1108 apparatus. ^{1}H NMR spectra were recorded at 200 MHz on a Varian Inova Unity 200 spectrometer in DMSO-d_6 solution. Chemical shifts are given in δ values (ppm), using tetramethylsilane as the internal standard; coupling constants (*J*) are given in Hz. Signal multiplicities are characterized as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), br (broad signal).

4-Amino-5-phenyl-2,4-dihydro-3H[1,2,4]triazole-3-thione (**9a**), 4-amino-5-(2-chlorophenyl)-2,4-dihydro-3H[1,2,4]-triazole-3-thione (**9b**), 4-amino-5-(4-methoxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (**9c**), 4-amino-5-(4-methylphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (**9e**), 4-amino-5-(4-chlorophenyl)-2,4-dihydro-3H[1,2,4]-triazole-3-thione (**9f**), 4-amino-5-(4-bromophenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (**9g**), 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine (**10a**), 1-(3-chloropropyl)-4-(2-nitrophenyl)piperazine (**10b**), 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine (**10c**), 5-phenyl-2,4-dihydro-3H[1,2,4]triazole-3-thione (**13a**) and 5-(4-methoxyphenyl-2,4-dihydro-3H[1,2,4]triazole-3-thione (**13b**) were prepared as previously described.[18,20–22,26–29]

4-Amino-5-(1,1′-biphenyl-4-yl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (9k). A solution of 1,1′-biphenyl-4-carbohydrazide (1.652 g, 7.7 mmol) in absolute ethanol (50 mL) was slowly added dropwise to a suspension of potassium hydroxide (0.690 g, 10.5 mmol) in ethanol (30 mL).[30] After stirring at room temperature for 1 h, carbon disulfide (0.799 g, 10.5 mmol) was added dropwise and the resulting mixture was stirred overnight. The solid potassium dithiocarbazinate was filtered off, washed with ether and dried (2.38 g). It was then dissolved in water (5 mL) and hydrazine monohydrate (0.771 g, 15.4 mmol) was added; the reaction mixture was refluxed until the evolution of hydrogen sulfide ceased. After cooling, water (20 mL) was added and the mixture was acidified with hydrochloric acid. The thick solid mass which separated out was collected by filtration, washed with water, dried and recrystallized from ethanol to give 0.62 g (32%) of **9k** as a white solid: mp 190 °C; IR (KBr) 3107, 1620, 1565, 1511, 1473, 1317, 1233, 1031, 951, 837, 729 (cm^{-1}); ^{1}H NMR (DMSO-d_6) δ 8.15–8.11 (m, 2H), 7.87–7.71 (m, 4H), 7.55–7.37 (m, 3H), 5.84[31] (br s, 2H).

Compounds **9d**, **9h**, **9i** and **9j** were prepared as **9k** starting from the suitable benzoic acid hydrazide.

4-Amino-5-(3-methoxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (9d). Recrystallization from ethanol gave 0.97 g (57%) of **9d** as a white solid: mp 199 °C; IR (KBr) 3298, 3121,1623, 1579, 1529, 1477, 1316, 1212, 1177, 954, 754 (cm^{-1}); ^{1}H NMR (DMSO-d_6) δ 13.95[31] (br s, 1H), 7.68–7.35 (m, 3H), 7.21–7.00 (m, 1H), 5.80[31] (s, 2H), 3.80 (s, 3H).

4-Amino-5-(4-propyloxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (9h). Recrystallization from ethanol gave 1.04 g (54%) of **9h** as a white solid: mp 200 °C; IR (KBr) 3294, 3115, 3017, 2939, 1611, 1512, 1177, 973, 840, 579 (cm^{-1}); ^{1}H NMR (DMSO-d_6) δ 13.81[31] (br s, 1H), 8.04–7.90 (m, 2H), 7.14–6.99 (m, 2H), 5.76[31] (br s, 2H), 4.00 (t, $J = 6.6$, 2H), 1.89–1.64 (m, 2H), 0.99 (t, $J = 7.2$, 3H).

4-Amino-5-(4-phenylsulphonylphenyl)-2,4-dihydro-3H-[1,2,4]triazole-3-thione (9i). Recrystallization from ethanol gave 1.02 g (40%) of **9i** as a white solid: mp 190 °C; IR (KBr) 3305, 3200, 3101, 1623, 1465, 1308, 1156, 1106, 1031, 752 (cm^{-1}); ^{1}H NMR (DMSO-d_6) δ 13.78[31] (br s, 1H), 8.34–8.23 (m, 2H), 8.17–8.07 (m, 2H), 8.05–7.95 (m, 2H), 7.76–7.58 (m, 3H), 5.80[31] (s, 2H).

4-Amino-5-(4-phenoxymethylphenyl)-2,4-dihydro-3H-[1,2,4]triazole-3-thione (9j). Recrystallization from ethanol gave 0.80 g (35%) of **9j** as a white solid: mp 220 °C; IR (KBr) 3456, 3140, 2361, 1683, 1599, 1500, 1309, 1241, 1042, 753 (cm^{-1}); ^{1}H NMR (DMSO-d_6) δ 13.77[31] (br s, 1H), 8.11–7.96 (m, 2H), 7.65–7.50 (m, 2H), 7.40–7.25 (m, 2H), 7.14–6.95 (m, 3H), 5.73[31] (br s, 2H).

General procedure for the synthesis of [1,2,4]triazole derivatives (11a–u) and 2,4-dihydro-3H[1,2,4]triazole-3-thione derivatives (12a–s). To a solution of the appropriate 4-amino-5-substituted-2,4-dihydro-3H[1,2,4]triazole-3-thione (**9a**–**k**) (2.0 mmol) in hot absolute ethanol (10 mL), sodium hydroxide (0.08 g, 2.0 mmol) and potassium iodide (0.01 g) were added. A solution of 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine (**10a**), 1-(3-chloropropyl)-4-(2-nitrophenyl)piperazine (**10b**) or 1-(2-chloroethyl)-4-(2-methoxyphenyl)piperazine (**10c**) (2.0 mmol) in absolute ethanol (5 mL) was added dropwise during 15 min. Then, the reaction mixture was refluxed for 12 h. After cooling, it was diluted with

water (30 mL), extracted with ether (4×20 mL). The combined ethereal phases were washed with brine, dried and removed in vacuo. The oily residue, which contained both *S*- and *N*-substituted isomers, was purified by flash-chromatography. Elution with pure ethyl acetate yielded compounds **12a**–**s**. Subsequent elution with ethyl acetate containing 10% (v/v) methanol yielded compounds **11a**–**u**.

4-Amino-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]-5-phenyl[1,2,4]triazole (11a). Flash chromatography purification gave 0.42 g (50%) of **11a** as a white solid: mp 135 °C; IR (KBr) 3331, 3213, 3061, 2941, 2821, 1590, 1498, 1461, 1240, 743 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.98–7.90 (m, 2H), 7.52–7.40 (m, 3H), 6.95–6.76 (m, 4H), 6.07[31] (br s, 2H), 3.72 (s, 3H), 3.16 (t, $J=7.4$, 2H), 3.04–2.84 (m, 4H), 2.60–2.35 (m, 6H), 1.95–1.75 (m, 2H).

4-Amino-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]thio]-5-phenyl[1,2,4]triazole (11b). Flash chromatography purification gave 0.47 g (54%) of **11b** as a yellow solid: mp 147 °C; IR (KBr) 3332, 3075, 2940, 2842, 1605, 1567, 1521, 1445, 1337, 700 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.03–7.93 (m, 2H), 7.82–7.75 (m, 1H), 7.63–7.46 (m, 4H), 7.33–7.27 (m, 1H), 7.17–7.05 (m, 1H), 6.12[31] (br s, 2H), 3.20 (t, $J=7.4$, 2H), 3.09–2.92 (m, 4H), 2.60–2. 35 (m, 6H), 2.02–1.78 (m, 2H).

4-Amino-5-(2-chlorophenyl)-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11c). Flash chromatography purification gave 0.38 g (42%) of **11c** as a white solid: mp 137 °C; IR (KBr) 3242, 3064, 2935, 2816, 1591, 1497, 1451, 1239, 744 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.63–7.38 (m, 4H), 6.96–6.74 (m, 4H), 5.80[31] (br s, 2H), 3.72 (s, 3H), 3.18 (t, $J=7.4$, 2H), 3.05–2.75 (m, 4H), 2.65–2.28 (m, 6H), 2.00–1.78 (m, 2H).

4-Amino-5-(2-chlorophenyl)-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11d). Flash chromatography purification gave 0.31 g (33%) of **11d** as a yellowish oil; IR (KBr) 3342, 3184, 2946, 2824, 1604, 1517, 1449, 1343, 767 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.81–7.70 (m, 1H), 7.68–7.38 (m, 5H), 7.35–7 21 (m, 1H), 7.16–7.00 (m, 1H), 5.79[31] (br s, 2H), 3.17 (t, $J=7.4$, 2H), 3.08–2.85 (m, 4H), 2.63–2.30 (m, 6H), 1.98–1.79 (m, 2H).

4-Amino-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-thio]-5-(4-methoxyphenyl)[1,2,4]triazole (11e). Flash chromatography purification gave 0.34 g (41%) of **11e** as a white solid: mp 138 °C; IR (KBr) 3419, 2934, 2817, 1612, 1499, 1453,1242, 1024, 746 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.02–7.88 (m, 2H), 7.15–7.01 (m, 2H), 6.99–6.77 (m, 4H), 6.05[31] (br s, 2H), 3.82 (s, 3H), 3.77 (s, 3H), 3.18 (t, $J=7.2$, 2H), 3.04–2.80 (m, 4H), 2.65–2.35 (m, 6H), 2.03–1.80 (m, 2H).

4-Amino-5-(4-methoxyphenyl)-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11f). Flash chromatography purification gave 0.42 g (45%) of **11f** as a yellow solid: mp 172 °C; IR (KBr) 3455, 3322, 2938, 2837, 1605, 1527, 1437, 1382, 1258, 747 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.01–7.88 (m, 2H), 7.85–7.76 (m, 1H), 7.64–7.50 (m, 1H), 7.35–7.24 (m, 1H), 7.17–6.98 (m, 3H), 6.06[31] (br s, 2H), 3.82 (s, 3H), 3.18 (t, $J=7.4$, 2H), 3.08–2.90 (m, 4H), 2.64–2.38 (m, 6H), 2.02–1.80 (m, 2H).

4-Amino-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-thio]-5-(3-methoxyphenyl)[1,2,4]triazole (11g). Flash chromatography purification gave 0.34 g (37%) of **11g** as a white solid: mp 132 °C; IR (KBr) 3450, 3067, 2934, 2822, 1589, 1497, 1460, 1239, 1025, 745 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.62–7.50 (m, 2H), 7.48–7.38 (m, 1H), 7.13–7.02 (m, 1H), 6.99–6.79 (m, 4H), 6.12[31] (br s, 2H), 3.81 (s, 3H), 3.77 (s, 3H), 3.20 (t, $J=7.2$, 2H), 3.08–2.85 (m, 4H), 2.67–2.37 (m, 6H), 2.02–1.81 (m, 2H).

4-Amino-5-(3-methoxyphenyl)-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11h). Flash chromatography purification gave 0.28 g (30%) of **11h** as a yellowish solid: mp 120 °C; IR (KBr) 3334, 3209, 3059, 2943, 2812, 1600, 1524, 1478, 1368, 1231, 737 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.82–7.72 (m, 1H), 7.63–7.48 (m, 3H), 7.45–7.23 (m, 2H), 7.18–7.00 (m, 2H), 6.11[31] (br s, 2H), 3.80 (s, 3H), 3.19 (t, $J=7.2$, 2H), 3.08–2.90 (m, 4H), 2.60–2.37 (m, 6H), 2.01–1.80 (m, 2H).

4-Amino-35-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio]-5-(4-methylphenyl)[1,2,4]triazole (11i). Flash chromatography purification gave 0.45 g (51%) of **11i** as a white solid: mp 170 °C; IR (KBr) 3324, 3130, 2936, 2830, 1592, 1498, 1444, 1237, 1117, 741 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.95–7.83 (m, 2H), 7.39–7.28 (m, 2H), 7.01–6.80 (m, 4H), 6.08[31] (br s, 2H), 3.77 (s, 3H), 3.20 (t, $J=7.4$, 2H), 3.07–2.88 (m, 4H), 2.63–2.40 (m, 6H), 2.37 (s, 3H), 2.01–1.80 (m, 2H).

4-Amino-5-(4-methylphenyl)-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11j). Flash chromatography purification gave 0.50 g (55%) of **11j** as a yellow solid: mp 172 °C; IR (KBr) 3324, 3134, 2945, 2829, 1604, 1524, 1445, 1359, 1234, 1120, 744 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.94–7.83 (m, 2H), 7.82–7.72 (m, 1H), 7.62–7.48 (m, 1H), 7.37–7.23 (m, 3H), 7.18–7.04 (m, 1H), 6.07[31] (br s, 2H), 3.18 (t, $J=7.2$, 2H), 2.89–3.04 (m, 4H), 2.58–2.40 (m, 6H), 2.36 (s, 3H), 2.01–1.80 (m, 2H).

4-Amino-5-(4-chlorophenyl)-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11k). Flash chromatography purification gave 0.22 g (24%) of **11k** as a white solid: mp 167 °C; IR (KBr) 3321, 3125, 2934, 2832, 1592, 1499, 1439, 1238, 1117, 745 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.09–7.96 (m, 2H), 7.65–7.54 (m, 2H), 7.01–6.79 (m, 4H), 6.13[31] (br s, 2H), 3.77 (s, 3H), 3.21 (t, $J=7.2$, 2H), 3.04–2.79 (m, 4H), 2.63–2.36 (m, 6H), 2.03–1.80 (m, 2H).

4-Amino-5-(4-chlorophenyl)-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11l). Flash chromatography purification gave 0.33 g (35%) of **11l** as a yellow solid: mp 146 °C; IR (KBr) 3319, 3111, 2948, 2818, 1604, 1563, 1512, 1436, 1300, 1232, 1088, 830, 740

(cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.11–7.96 (m, 2H), 7.84–7.73 (m, 1H), 7.67–7.47 (m, 3H), 7.36–7.23 (m, 1H), 7.16–7.04 (m, 1H), 6.14[31] (br s, 2H), 3.21 (t, $J=7.4$, 2H), 3.08–2.90 (m, 4H), 2.59–2.35 (m, 6H), 2.01–1.80 (m, 2H).

4-Amino-5-(4-bromophenyl)-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11m). Flash chromatography purification gave 0.34 g (34%) of **11m** as a white solid: mp 167 °C; IR (KBr) 3320, 3120, 2933, 2831, 1590, 1499, 1437, 1237, 1116, 744 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.06–7.90 (m, 2H), 7.78–7.67 (m, 2H), 7.02–6.78 (m, 4H), 6.14[31] (br s, 2H), 3.77 (s, 3H), 3.21 (t, $J=7.0$, 2H), 3.08–2.77 (m, 4H), 2.64–2.35 (m, 6H), 2.02–1.80 (m, 2H).

4-Amino-5-(4-bromophenyl)-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11n). Flash chromatography purification gave 0.30 g (29%) of **11n** as a yellow solid: mp 141 °C; IR (KBr) 3321, 3109, 2946, 2829, 1601, 1522, 1438, 1363, 1231, 1118, 829, 742 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.04–7.92 (m, 2H), 7.84–7.66 (m, 3H), 7.64–7.50 (m, 1H), 7.37–7.25 (m, 1H), 7.17–7.05 (m, 1H), 6.14[31] (br s, 2H), 3.21 (t, $J=7.4$, 2H), 3.07–2.90 (m, 4H), 2.65–2.35 (m, 6H), 2.02–1.80 (m, 2H).

4-Amino-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-thio]-5-(4-propyloxyphenyl)[1,2,4]triazole (11o). Flash chromatography purification gave 0.34 g (35%) of **11o** as a white solid: mp 141 °C; IR (KBr) 3330, 3058, 2936, 2824, 1610, 1499, 1447, 1243, 1119, 747 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.98–7.86 (m, 2H), 7.13–7.01 (m, 2H), 6.98–6.80 (m, 4H), 6.06[31] (br s, 2H), 3.99 (t, $J=6.4$, 2H), 3.76 (s, 3H), 3.18 (t, $J=7.4$, 2H), 3.04–2.89 (m, 4H), 2.63–2.39 (m, 6H), 2.01–1.82 (m, 2H), 1.80–1.65 (m, 2H), 0.99 (t, $J=7.4$, 3H).

4-Amino-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]thio]-5-(4-propyloxyphenyl)[1,2,4]triazole (11p). Flash chromatography purification gave 0.43 g (43%) of **11p** as a yellow solid: mp 149 °C; IR (KBr) 3329, 2949, 2839, 1606, 1520, 1422, 1348, 1240, 1121, 841, 742 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.98–7.87 (m, 2H), 7.84–7.73 (m, 1H), 7.63–7.50 (m, 1H), 7.36–7.23 (m, 1H), 7.17–6.98 (m, 3H), 6.06[31] (br s, 2H), 3.99 (t, $J=6.2$, 2H), 3.18 (t, $J=7.4$, 2H), 3.08–2.83 (m, 4H), 2.60–2.37 (m, 6H), 2.02–1.82 (m, 2H), 1.80–1.63 (m, 2H), 0.99 (t, $J=7.2$, 3H).

4-Amino-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-thio]-5-(4-phenylsulphonylphenyl)[1,2,4]triazole (11q). Flash chromatography purification gave 0.28 g (25%) of **11q** as a white solid: mp 199 °C; IR (KBr) 3453, 3330, 2933, 2814, 1596, 1497, 1445, 1237, 1152, 1116, 750 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.33–8.20 (m, 2H), 8.18–7.94 (m, 4H), 7.77–7.58 (m, 3H), 7.00–6.78 (m, 4H), 6.20[31] (br s, 2H), 3.76 (s, 3H), 3.22 (t, $J=7.0$, 2H), 3.07–2.85 (m, 4H), 2.63–2.37 (m, 6H), 2.01–1.80 (m, 2H).

4-Amino-3-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-thio]-5-(4-phenylsulphonylphenyl)[1,2,4]triazole (11r). Flash chromatography purification gave 0.30 g (26%) of **11r** as a yellow solid: mp 178 °C; IR (KBr) 3450, 3327, 2938, 2817, 1602, 1528, 1446, 1314, 1154, 1115, 752 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.31–8.19 (m, 2H), 8.16–7.96 (m, 4H), 7.83–7.50 (m, 5H), 7.36–7.25 (m, 1H), 7.16–7.05 (m, 1H), 6.17[31] (br s, 2H), 3.21 (t, $J=7.2$, 2H), 3.05–2.89 (m, 4H), 2.58–2.37 (m, 6H), 2.01–1.80 (m, 2H).

4-Amino-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-thio]-5-(4-phenoxymethylphenyl)[1,2,4]triazole (11s). Flash chromatography purification gave 0.39 g (37%) of **11s** as a white solid: mp 151 °C; IR (KBr) 3249, 3114, 2934, 2816, 1593, 1500, 1450, 1243, 1022, 752 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.03–7.99 (m, 2H), 7.60–7.56 (m, 2H), 7.34–7.26 (m, 2H), 7.05–6.86 (m, 7H), 6.12[31] (br s, 2H), 5.18 (s, 2H), 3.76 (s, 3H), 3.20 (t, $J=7.00$, 2H), 2.95 (br s, 4H), 2.50–2.43 (m, 6H), 1.99–1.88 (m, 2H).

4-Amino-5-(1,1′-biphenyl-4-yl)-3-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]thio][1,2,4]triazole (11t). Flash chromatography purification gave 0.32 g (32%) of **11t** as a white solid: mp 145–147 °C; ^1H NMR (DMSO-d_6) δ 8.20–8.03 (m, 2H), 7.91–7.63 (m, 4H), 7.88–7.60 (m, 3H), 7.01–6.78 (m, 4H), 6.18[31] (br s, 2H), 3.77 (s, 3H), 3.22 (t, $J=7.2$, 2H), 3.08–2.77 (m, 4H), 2.69–2.32 (m, 6H), 2.03–1.81 (m, 2H).

4-Amino-3-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-thio]-5-(4-propyloxyphenyl)[1,2,4]triazole (11u). Flash chromatography purification gave 0.54 g (58%) of **11u** as a white solid: mp 151 °C; IR (KBr) 3241, 3114, 2957, 2818, 1611, 1503, 1452, 1242, 1024, 747 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.97–7.92 (m, 2H), 7.10–7.02 (m, 2H), 6.94–6.79 (m, 4H), 6.09[31] (br s, 2H), 3.99 (t, $J=6.6$, 2H), 3.75 (s, 3H), 3.30 (t, $J=6.6$, 2H), 2.99–2.83 (m, 4H), 2.70 (t, $J=6.6$, 2H) 2.61–2.45 (m, 4H), 1.85–1.66 (m, 2H), 0.99 (t, $J=7.2$, 3H).

4-Amino-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-5-phenyl-2,4-dihydro-3H[1,2,4]triazole-3-thione (12a). Flash chromatography purification gave 0.13 g (15%) of **12a** as a white solid: mp 112–114 °C; IR (KBr) 3238, 3126, 2938, 2817, 1635, 1586, 1497, 1451, 1245, 1027, 744 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.10–7.95 (m, 2H), 7.63–7.43 (m, 3H), 6.98–6.75 (m, 4H), 5.85[31] (br s, 2H), 4.21 (t, $J=7.0$, 2H), 3.72 (s, 3H), 3.03–2.70 (m, 4H), 2.60–2.25 (m, 6H), 2.05–1.80 (m, 2H).

4-Amino-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-5-phenyl-2,4-dihydro-3H[1,2,4]triazole-3-thione (12b). Flash chromatography purification gave 0.18 g (20%) of **12b** as a yellow solid: mp 95–98 °C; IR (KBr) 3281, 3060, 2930, 2819, 1646, 1604, 1519, 1456, 1332, 1228, 694 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.10–7.91 (m, 2H), 7.85–7.70 (m, 1H), 7.69–7.42 (m, 3H), 7.35–7.20 (m, 1H), 7.17–7.00 (m, 1H), 5.85[31] (br s, 2H), 4.21 (t, $J=7.0$, 2H), 3.07–2.70 (m, 4H), 2.65–2.25 (m, 6H), 2.06–1.80 (m, 2H).

4-Amino-5-(2-chlorophenyl)-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (12c). Flash chromatography purification gave 0.11 g (12%) of **12c** as a yellow solid: mp 178 °C; IR (KBr)

3277, 3059, 2930, 2816, 1635, 1500, 1450, 1238, 1026, 744 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.72–7.45 (m, 4H), 7.00–6.78 (m, 4H), 5.64[31] (br s, 2H), 4.24 (t, $J=7.0$, 2H), 3.76 (s, 3H), 3.10–2.85 (m, 4H), 2.65–2.35 (m, 6H), 2.10–1.87 (m, 2H).

4-Amino-5-(2-chlorophenyl)-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (12d). Flash chromatography purification gave 0.16 g (17%) of **12d** as a yellow solid: mp 126 °C; IR (KBr) 3263, 3069, 2930, 2819, 1641, 1601, 1519, 1448, 1336, 1220, 1034, 769 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.88–7.78 (m, 1H), 7.75–7.45 (m, 5H), 7.42–7.25 (m, 1H) 7.20–7.03 (m, 1H), 5.65[31] (br s, 2H), 4.24 (t, $J=6.8$, 2H), 2.79–3.18 (m, 4H), 2.70–2.30 (m, 6H), 2.18–1.87 (m, 2H).

4-Amino-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-5-(4-methoxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (12e). Flash chromatography purification gave 0.11 g (12%) of **12e** as a white solid: mp 98 °C; IR (KBr) 3280, 2933, 2815, 1611, 1499, 1453, 1243, 1022, 833, 744 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.10–7.95 (m, 2H), 7.18–7.03 (m, 2H), 7.01–6.78 (m, 4H), 5.85[31] (br s, 2H), 4.22 (t, $J=7.0$, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 3.08–2.75 (m, 4H), 2.72–2.32 (m, 6H), 2.10–1.85 (m, 2H).

4-Amino-5-(4-methoxyphenyl)-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (12f). Flash chromatography purification gave 0.17 g (18%) of **12f** as a yellow solid: mp 112 °C; IR (KBr): 3277, 2934, 2826, 1583, 1498, 1458, 1231, 1025, 751 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.07–7.90 (m, 2H), 7.85–7.73 (m, 1H), 7.64–7.48 (m, 1H), 7.36–7.22 (m, 1H), 7.18–6.98 (m, 3H), 5.86[31] (br s, 2H), 4.22 (t, $J=6.8$, 2H), 3.83 (s, 3H), 3.76 (s, 3H), 3.12–2.89 (m, 4H), 2.65–2.30 (m, 6H), 2.10–1.85 (m, 2H).

4-Amino-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-5-(3-methoxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (12g). Flash chromatography purification gave 0.16 g (18%) of **12g** as a white solid: mp 145 °C; IR (KBr): 3265, 3118, 2989, 2935, 2826, 1583, 1497, 1458, 1231, 1026, 751 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.65–7.55 (m, 2H), 7.52–7.38 (m, 1H), 7.18–7.07 (m, 1H), 7.00–6.78 (m, 4H), 5.89[31] (br s, 2H), 4.24 (t, $J=7.0$, 2H), 3.80 (s, 3H), 3.75 (s, 3H), 3.10–2.73 (m, 4H), 2.65–2.28 (m, 6H), 2.10–1.85 (m, 2H).

4-Amino-5-(3-methoxyphenyl)-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (12h). Flash chromatography purification gave 0.19 g (20%) of **12h** as a yellow solid: mp 145–147 °C; IR (KBr) 3318, 2934, 2825, 1609, 1522, 1456, 1339, 1229, 1040, 747 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.79–7.68 (m, 1H), 7.62–7.35 (m, 4H), 7.33–7.20 (m, 1H), 7.15–7.00 (m, 2H), 5.85[31] (br s, 2H), 4.20 (t, $J=7.0$, 2H), 3.77 (s, 3H), 3.08–2.85 (m, 4H), 2.65–2.30 (m, 6H), 2.06–1.84 (m, 2H).

4-Amino-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-5-(4-methylphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (12i). Flash chromatography purification gave 0.15 g (17%) of **12i** as a white solid: mp 114 °C; IR (KBr) 3299, 3164, 2931, 2817, 1611, 1497, 1444, 1335, 1238, 1028, 750 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.98–7.85 (m, 2H), 7.42–7.30 (m, 2H), 7.00–6.78 (m, 4H), 5.85[31] (br s, 2H), 4.23 (t, $J=6.8$, 2H), 3.76 (s, 3H), 3.08–2.78 (m, 4H), 2.68–2.24 (m + s, 6H + 3H), 2.10–1.85 (m, 2H).

4-Amino-5-(4-methylphenyl)-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (12j). Flash chromatography purification gave 0.17 g (19%) of **12j** as a yellow solid: mp 128 °C; IR (KBr) 3294, 3070, 2928, 2827, 1604, 1513, 1457, 1341, 1231, 1125, 746 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.06–7.90 (m, 2H), 7.86–7.70 (m, 1H), 7.64–7.46 (m, 1H), 7.43–7.22 (m, 3H), 7.18–7.03 (m, 1H), 5.86[31] (br s, 2H), 4.23 (t, $J=6.8$, 2H), 3.10–2.85 (m, 4H), 2.65–2.30 (m + s, 6H + 3H), 2.10–1.85 (m, 2H).

4-Amino-5-(4-chlorophenyl)-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (12k). Flash chromatography purification gave 0.15 g (16%) of **12k** as a white solid: mp 106 °C; IR (KBr): 3244, 3132, 2943, 2818, 1592, 1494, 1448, 1242, 1093, 1030, 746 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.16–8.00 (m, 2H), 7.70–7.56 (m, 2H), 7.00–6.78 (m, 4H), 5.89[31] (br s, 2H), 4.24 (t, $J=7.0$, 2H), 3.76 (s, 3H), 3.05–2.80 (m, 4H), 2.65–2.30 (m, 6H), 2.10–1.87 (m, 2H).

4-Amino-5-(4-chlorophenyl)-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-2,4-dihyro-3H[1,2,4]triazole-3-thione (12l). Flash chromatography purification gave 0.16 g (17%) of **12l** as a yellow solid: mp 112–114 °C; IR (KBr) 3310, 2945, 2824, 1604, 1516, 1454, 1347, 1233, 1092, 838, 748 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.24–7.95 (m, 2H), 7.88–7.75 (m, 1H), 7.72–7.45 (m, 3H), 7.38–7.22 (m, 1H), 7.20–7.03 (m, 1H), 5.90[31] (br s, 2H), 4.25 (t, $J=6.8$, 2H), 3.15–2.78 (m, 4H), 2.67–2.28 (m, 6H), 2.13–1.83 (m, 2H).

4-Amino-5-(4-bromophenyl)-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-2,4-dihydro-3H[1,2,4]triazole-3-thione (12m). Flash chromatography purification gave 0.20 g (20%) of **12m** as a colourless oil; IR (KBr) 3273, 2930, 2814, 1593, 1497, 1451, 1238, 1010, 746 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.06–7.90 (m, 2H), 7.85–7.70 (m, 2H), 7.01–6.77 (m, 4H), 5.89[31] (br s, 2H), 4.24 (t, $J=6.8$ Hz, 2H), 3.76 (s, 3H), 3.10–2.75 (m, 4H), 2.65–2.32 (m, 6H), 2.13–1.85 (m, 2H).

4-Amino-5-(4-bromophenyl)-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-2,4-dihydro-3H[1,2,4]triazole-5-thione (12n). Flash chromatography purification gave 0.21 g (20%) of **12n** as a yellow solid: mp 146 °C; IR (KBr) 3268, 3170, 2954, 2823, 1604, 1514, 1443, 1343, 1233, 1012, 831, 746 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 7.90–8.01 (m, 2H), 7.65–7.79 (m, 3H), 7.45–7.58 (m, 1H), 7.20–7.32 (m, 1H), 7.01–7.16 (m, 1H), 5.85[31] (br s, 2H), 4.20 (t, $J=7.0$, 2H), 2.85–3.06 (m, 4H), 2.30–2.63 (m, 6H), 1.85–2.08 (m, 2H).

4-Amino-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-5-(4-propyloxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (12o). Flash chromatography purification gave 0.11 g (12%) of **12o** as a white solid: mp 94 °C; IR (KBr)

3281, 3130, 2933, 2823, 1646, 1612, 1499, 1458, 1245, 1026, 746 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.04–7.90 (m, 2H), 7.16–7.03 (m, 2H), 7.00–6.78 (m, 4H), 5.85[31] (br s, 2H), 4.22 (t, $J=7.0$, 2H), 4.00 (t, $J=6.6$, 2H), 3.75 (s, 3H), 3.03–2.82 (m, 4H), 2.63–2.33 (m, 6H), 2.09–1.85 (m, 2H), 1.84–1.65 (m, 2H), 0.99 (t, $J=7.6$, 3H).

4-Amino-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-5-(4-propyloxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (12p). Flash chromatography purification gave 0.15 g (15%) of **12p** as a yellowish oil; IR (KBr) 3282, 2933, 2816, 1607, 1498, 1450, 1250, 968, 835, 745 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.14–7.90 (m, 2H), 7.86–7.73 (m, 1H), 7.64–7.50 (m, 1H), 7.38–7.22 (m, 1H), 7.20–7.01 (m, 3H), 5.85[31] (br s, 2H), 4.22 (t, $J=7.0$, 2H), 4.00 (t, $J=6.6$, 2H), 3.08–2.84 (m, 4H), 2.64–2.33 (m, 6H), 2.08–1.85 (m, 2H), 1.82–1.60 (m, 2H), 0.99 (t, $J=7.4$, 3H).

4-Amino-2-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propyl]-5-(4-phenylsulphonylphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (12q). Flash chromatography purification gave 0.11 g (10%) of **12q** as a colourless oil; IR (KBr) 3290, 2929, 1669, 1499, 1454, 1284, 1154, 745 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.36–8.22 (m, 2H), 8.20–8.07 (m, 2H), 8.06–7.92 (m, 2H), 7.80–7.58 (m, 3H), 7.00–6.78 (m, 4H), 5.89[31] (br s, 2H), 4.25 (t, $J=7.0$, 2H), 3.75 (s, 3H), 3.05–2.72 (m, 4H), 2.65–2.30 (m, 6H), 2.10–1.85 (m, 2H).

4-Amino-2-[3-[4-(2-nitrophenyl)-1-piperazinyl]propyl]-5-(4-phenylsulphonylphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (12r). Flash chromatography purification gave 0.15 g (13%) of **12r** as a yellow solid: mp 184 °C; IR (KBr) 3285, 2920, 2817, 1602, 1516, 1448, 1286, 1154, 841, 743 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.36–8.22 (m, 2H), 8.20–8.07 (m, 2H), 8.06–7.92 (m, 2H), 7.84–7.45 (m, 5H), 7.35–7.21 (m, 1H), 7.17–7.03 (m, 1H), 5.90[31] (br s, 2H), 4.24 (t, $J=7.0$, 2H), 3.06–2.85 (m, 4H), 2.63–2.30 (m, 6H), 2.07–1.84 (m, 2H).

4-Amino-2-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-5-(4-propyloxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (12s). Flash chromatography purification gave 0.26 g (28%) of **12s** as a white solid: mp 122 °C; IR (KBr) 3268, 3155, 2938, 2876, 2815, 1613, 1501, 1452, 1258, 1240, 1177, 976, 745 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.00–7.96 (m, 2H), 7.10–7.03 (m, 2H), 6.92–6.85 (m, 4H), 5.87[31] (br s, 2H), 4.32 (t, $J=6.8$, 2H), 4.00 (t, $J=6.6$, 2H), 3.76 (s, 3H), 3.10–2.87 (m, 4H), 2.80 (t, $J=6.8$, 2H), 2.67–2.55 (m, 4H), 1.84–1.66 (m, 2H), 0.98 (t, $J=7.4$, 3H).

3-[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]thio]-5-phenyl[1,2,4]triazole (14a). To a solution of 5-phenyl-2,4-dihydro-3H[1,2,4]triazole-3-thione (**13a**) (0.50 g, 2.82 mmol) in hot absolute ethanol (20 mL), potassium hydroxide (0.16 g, 2.82 mmol) and potassium iodide (0.01 g) were added. A solution of 1-(3-chloropropyl)-4-(2-methoxyphenyl)piperazine (**10a**) (0.76 g, 2.82 mmol) in absolute ethanol (8 mL) was added dropwise during 45 min. Then, the reaction mixture was refluxed for 6 h. After cooling, the solvent was removed under vacuum and water (50 mL) was added. The mixture was then extracted with ethyl acetate (4×50 mL) and the combined organic phases were washed with brine and dried over anhydrous sodium sulphate. Distillation of the solvent under vacuum gave an oily residue which was flash-chromatographed on silica gel 60 (Merck, Darmstadt, Germany) using ethyl acetate as eluent. Fractions which were homogeneous on TLC were combined and dried over anhydrous sodium sulphate. Distillation of the solvent under vacuum gave 0.60 g (52%) of an oily residue of pure **6a** which slowly crystallized in a white solid: mp 100 °C; IR (KBr) 3447, 3062, 2937, 2815, 1589, 1499, 1458, 1241, 1125, 1021, 749, 728 (cm^{-1}); ^1H NMR (DMSO-d_6) δ 8.03–7.90 (m, 2H), 7.57–7.42 (m, 3H), 7.00–6.79 (m, 4H), 3.76 (s, 3H), 3.19 (t, $J=7.2$, 2H), 3.04–2.87 (m, 4H), 2.63–2.36 (m, 6H), 2.01–1.79 (m, 2H).

3-[3-[4-(2-Methoxyphenyl)-1-piperazinyl]propyl]thio]-5-(4-methoxyphenyl)[1,2,4] triazole (14b). The synthesis of this compound was accomplished following the method used for **14a**, starting from 5-(4-methoxyphenyl)-2,4-dihydro-3H[1,2,4]triazole-3-thione (**13b**). Flash chromatography purification on silica gel 60 using ethyl acetate as eluent afforded 0.82 g (66%) of **14b** as a white solid: mp 83 °C; IR (KBr) 3100, 2938, 2816, 1614, 1502, 1453, 1245, 1179, 1027, 751 (cm^{-1}). ^1H NMR (DMSO-d_6) δ 7.97–7.83 (m, 2H), 7.15–7.02 (m, 2H), 7.00–6.77 (m, 4H), 3.81 (s, 3H), 3.76 (s, 3H), 3.17 (t, $J=7.4$, 2H), 3.04–2.75 (m, 4H), 2.65–2.34 (m, 6H), 2.00–1.77 (m, 2H).

Pharmacology

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_1AR; striatum for D_2), 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 μM pargyline and, for the 5-HT_{1A} receptor, 4 mM $CaCl_2$ or, for the α_1-adrenoceptor, 0.1% ascorbic acid; 50 mM Tris–HCl (pH 7.1) containing 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% ascorbic acid for D_2 receptor] just before the binding assay.

Binding assays were performed as previously described.[32] Briefly, the following incubation conditions were used: 5-HT_{1A} 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 μM); 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 μM); D_2 receptor: [^{3}H]spiperone (specific activity 19.0 Ci/mmol, NEN) final concentration 0.2 nM, 15 min at 37 °C (non-specific binding: (−)-sulpiride 100 μM).

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%.[33] The K_i values were derived from the IC_{50} values.[34]

Procedures involving animals and their care were conducted in conformity with the institutional guidelines that are in compliance with national (D.L. n. 116, G.U., suppl. 40, Feb. 18, 1992) and international laws and policies (EEC Council Directive 86/609, OJ L 358, 1, Dec. 12, 1987; Guide for the Care and Use of Laboratory Animals, US National Research Council, 1996).

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