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Design, synthesis and biological evaluation of new 3-[(4-aryl)piperazin-1-yl]-1-arylpropane derivatives as potential antidepressants with a dual mode of action: serotonin reuptake inhibition and 5-HT_{1A} receptor antagonism

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Abstract

It has been suggested that the combination of a selective serotonin reuptake inhibitor (SSRI) and a 5-HT_{1A} receptor antagonist may facilitate the onset of the SSRIs antidepressant action. Accordingly, we describe the synthesis of a series of new 3-[(4-aryl)piperazin-1-yl]-1-arylpropane derivatives with structural modifications performed in Ar_1, Ar_2 and Z (Z is different functional groups) to obtain the sought dual activity. Compounds were evaluated for in vitro affinity at 5-HT_{1A} receptors and 5-HT transporter. The antidepressant-like activity of derivatives with the higher affinity was assessed initially using the forced swimming test (FST). Compound 1-(2,4-dimethylphenyl)-3-[(2-methoxyphenyl)piperazin-1-il]-1-propanone (**III.1.a**) showed the best antidepressant-like activity which was further confirmed in the learned helplessness test.

Keywords: Antidepressant; 5-HT_{1A} antagonist; 5-HT reuptake inhibitor; Arylpiperazine

1. Introduction

Abundant data support the contention of a frequently reduced central serotoninergic neurotransmission in depression. In particular, this pathological case involves suboptimal activity of the post-synaptic 5-HT_{1A} receptor subtype [1], in fact, 5-HT_{1A} postsynaptic agonists demonstrated efficacy in the treatment of depression disorders [2]. In addition, drugs which selectively inhibit the reuptake of serotonin (SSRI) are effective antidepressants with a low incidence of side effects; however, their therapeutic effect is achieved only after repeated administration [3,4]. No-selective serotoninergic activation following SSRI treatment might explain the slow onset of their antidepressant effects in humans and could be involved in some of the undesired side effects, notable loss of libido [5]. Therefore, one of the still unmet therapeutic needs is the availability of antidepressants with a more rapid onset of action. Extensive studies on the mechanism of action of the SSRIs led to the discovery of the key role of somatodendritic 5-HT_{1A} autoreceptors in a negative control of serotoninergic neurotransmission [6,7]. Microdialysis studies have shown that 5-HT_{1A} autoreceptor antagonists enhance the 5-HT reuptake blocking properties of SSRIs in terminal areas of the serotoninergic system by preventing the negative feedback of serotonin (5-HT) acting at somatodendritic 5-HT_{1A} receptors [8–11]. In addition, pindolol, a 5-HT_{1A} receptor antagonist has been reported to accelerate the antidepressant action of SSRIs in several open and placebo-controlled clinical trials [12–17]. These observations prompted us to develop the synthesis of new antide

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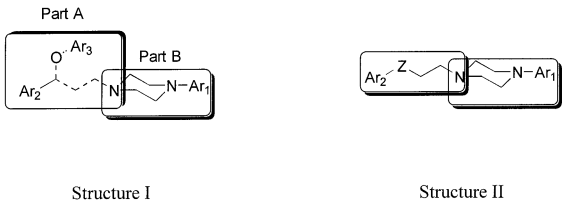


Ar_1= 2-methoxyphenyl (a); 2-hydroxyphenyl (b); 1-naphtyl (c).

Ar_2= 4-methylphenyl (I); 2-methylphenyl (II); 2,4-dimethylphenyl (III)

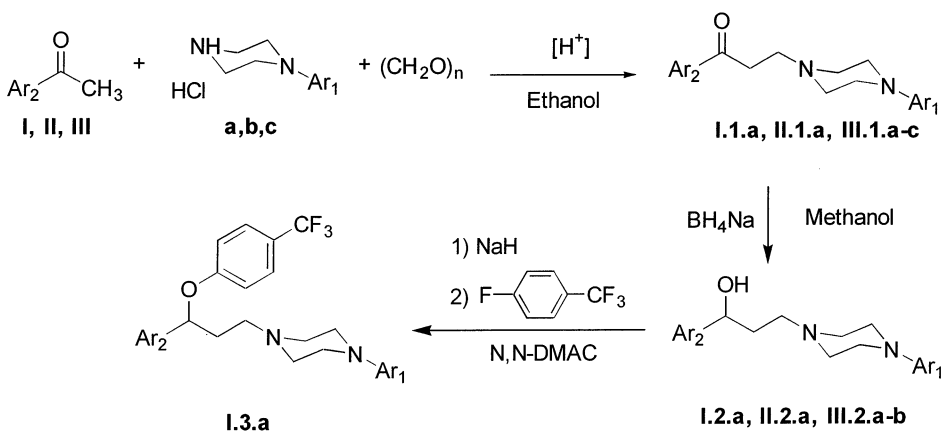
Z= CO (1); CHOH (2); $CHOC_6H_5CF_3$ (3).

Fig. 1.

pressants with a dual mode of action: serotonin reuptake inhibition and 5-HT_{1A} receptor antagonism. Previous works of our group have dealt with this strategy of combining two chemically different structures, associated to the sought activities, in a single chemical entity (Fig. 1): In this figure it can be observed that the nitrogen of the γ-phenoxypropylamine moiety (structure related to the SSRI [18], structure I, part A) has been combined with an arylpiperazine ring (long chain arylpiperazine represent one of the most important classes of 5-HT_{1A} receptors ligands [19], structure I, part B). The results obtained in these previous works [20] showed that compounds without the aromatic ring Ar_3 were endowed with the dual activity, so we synthesized new compounds according to the general structure II (Fig. 1) in which Z consists in different functional groups such as ketones or alcohols. The structural modifications consisted in the introduction of methyl groups on the phenyl ring Ar_2 maintaining the 2-methoxyphenylpiperazine moiety. This modification was proposed with the aim of increasing the electronic density without altering the electronic distribution substantially. This was suggested on the basis of pharmacophores associated to the 5-HT reuptake inhibitors reported previously [21]. Other piperazines, such as 2-hydroxyphenylpiperazine and 1-naphtylpiperazine, were also considered in an attempt to enhance the affinity at the 5-HT_{1A} receptor and 5-HT transporter. All synthesized compounds were screened in rat brain homogenates for their affinity at these two sites. The forced swimming test was carried out subsequently in order to study the antidepressant properties of selected compounds. Compound **III.1.a**, which revealed high effectiveness in this test, was further characterized by means of radioligand binding studies at different central receptors, to define its selectivity profile, and hypothermic studies in rat and mice to investigate agonist/antagonist properties at postsyanptic and presynaptic 5-HT_{1A} receptors [22]. The antidepressant-like property of this new compound was further studied in comparison with other antidepressants, in the learned helplessness test, a widely accepted animal model of depression because of the behavioral analogies with some signs of depression in humans [23].

2. Chemistry

The 3-[(4-aryl)piperazin-1-yl]-1-arylpropane derivatives presented in this work (**I.1**–**3.a**; **II.1**–**2.a**; **III.1**–**2.a**–**c**) were prepared according to methods reported already as shown in Scheme 1. The synthesis of the ketone derivatives was carried out by the condensation of the corresponding acetophenones with the different phenylpiperazine hydrochlorides and paraformaldehyde in ethanol and acid medium (Mannich reaction). The reduction of ketones with sodium borohydride in methanol afforded the corresponding alcohols. Treatment of the alcohols with sodium hydride and 4-fluorotrifluoromethylbenzene led to the phenolic ether (**I.3.a**). All the phenylpiperazines (**a** and **b**) were available commercially, except 1-naphtylpiperazine (**c**), which was obtained by the condensation of 1-naphtylamine

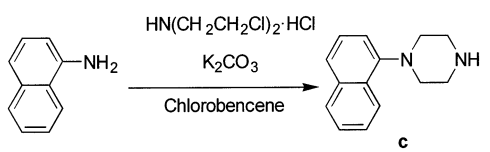


Scheme 1.

with bis-chloroethylamine in basic medium (Scheme 2). Yields and the physical and spectroscopic properties of the compounds are reported in Tables 1 and 2.

2.1. Experimental

Melting points (°C) were determined using a Mettler FP82 + FP80 apparatus and are uncorrected. Infrared spectra were recorded on a Perkin–Elmer 1600 series FTIR apparatus, using potassium bromide pellets for solid products and sodium chloride plates for oil products; the frequencies are expressed in cm^{-1}. The ^1H NMR spectra were obtained with a Brucker AC-200E (200 MHz) instrument, using tetramethylsilane as the internal reference, at a concentration of ca. 0.1 g/ml and with chloroform–$CDCl_3$ as solvents; the chemical shifts are reported in parts per million (ppm) of tetramethylsilane in δ units, and the J values are given in hertz (Hz). Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3–4 mmHg, 24 h, at ca. 80–100°C) with a Leco CHN-900 instrument. The mass spectra were recorded on a Hewlett–Packard 5988-A instrument at 70 eV.



Scheme 2.

Table 1
Formula and physical constants of synthesized compounds

Compound	Formula [a]	M.p. (°C)	Yield [b] (%)	Purification method [c]
I.1.a	$C_{21}H_{26}N_2O_2 \cdot HCl$	160	44	A
I.2.a	$C_{21}H_{28}N_2O_2$	106	45	B
I.3.a	$C_{28}H_{31}F_3N_2O_2$	88	23	C
II.1.a	$C_{21}H_{26}N_2O_2 \cdot HCl$	199	36	A
II.2.a	$C_{21}H_{28}N_2O_2$	102	21	B
III.1.a	$C_{22}H_{28}N_2O_2 \cdot HCl$	157	60	A
III.2.a	$C_{22}H_{30}N_2O_2$	88	40	C
III.1.b	$C_{21}H_{26}N_2O_2$	157	46	C
III.2.b	$C_{21}H_{28}N_2O_2$	149	32	C
III.1.c	$C_{25}H_{28}N_2O \cdot HCl$	240	25	A

[a] All new compounds were analyzed for C, H, N, which were within 0.4% of the theoretical values.

[b] Value of the final transformation is expressed.

[c] Purification method of the final form: A, recrystallized from isopropanol; B, recrystallized from hexane–isopropanol; C, column chromatography (SP, silica gel; MP, *n*-hexane–ethyl acetate 50:50 (v/v)).

2.1.1. Synthesis of 1-aryl-3-(4-arylpiperazin-1-yl)-propan-1-one (*I.1.a*, *II.1.a*, *III.1.a–c*)

A mixture of the appropriated substituted acetophenones (30 mmol), arylpiperazine hydrochloride (30 mmol) and concentrated hydrochloric acid in absolute ethanol (40 ml) was refluxed. Paraformaldehyde (90 mmol) was added in four equal portions over a period of 40 min. The reaction mixture was refluxed for another 8 h, cooled and poured onto crushed ice. The separated solid was filtered and recrystallized from isopropanol (**I.1.a**, **II.1.a**, **III.1.a**, **III.1.c**). For the isolation of compound **III.1.b**, a solution of sodium hydroxide (10%) was added to the reaction mixture until basic pH was reached. It was then extracted with ethyl acetate (3 × 20 ml), washed with brine (3 × 10 ml) and dried (anhydrous sodium sulfate). The solvent was removed under reduced pressure. The impure oil was purified by flash chromatography (SP, silica gel), eluting with *n*-hexane–ethyl acetate 60:40 (v/v).

2.1.2. Synthesis of 1-aryl-3-(4-arylpiperazin-1-yl)-propan-1-ol (*I.2.a*, *II.2.a*, *III.2.a–b*)

An excess of sodium borohydride was added to a well-stirred solution or suspension of the corresponding 1-substituted-3-(1-piperazinyl)-1-propanone (3 mmol) in methanol, over a period of 15 min at 0°C. The stirring was continued for another 4–8 h. The reaction mixture was poured onto water. It was then extracted with ethyl acetate (3 × 20 ml), washed with water (3 × 10 ml), and dried (anhydrous sodium sulfate). The solvent was removed under reduced pressure. The product obtained was further purified by recrystallization or by flash chromatography (SP, silica gel) eluting with *n*-hexane–ethyl acetate 50:50 (v/v) as detailed in Table 1.

2.1.3. Synthesis of 3-[4-(2-methoxyphenyl)piperazin-1-yl]-1-(4-methylphenyl)-1-(4-trifluoromethylphenoxy)-propane (*I.3.a*)

3-[4-(2-Methoxyphenyl)piperazin-1-yl]-1-(4-methylphenyl)propan-1-ol (2.5 mmol) was dissolved in *N*,*N*-dimethylacetamide (15 ml) and heated to 75°C. Sodium hydride (2.5 mmol) was then added and the reaction mixture was maintained at 75°C for 2 h to allow the formation of the salt. After this period of time, 1-fluoro-4-trifluoromethylbenzene was added (2.5 mmol) and the resulting mixture was heated at 110°C for 4 h. The reaction mixture was poured onto crushed ice. It was then extracted with diethyl ether (4 × 10 ml), washed with brine (3 × 10 ml) and dried (anhydrous sodium sulfate). The solvent was removed under re-

Table 2
^{1}H NMR ($CDCl_3$) spectra and IR absorption of the 3-[(4-aryl)piperazin-1-yl]-1-phenyl derivatives

Compound	^{1}H NMR ($CDCl_3$) δ values	IR absorption in KBr (cm^{-1})
I.1.a	(free base): 2.33 (s, 3H, CH_3); 2.75–2.77 (m, 4H, $N^1(CH_2)_2$); 2.92 (t, 2H, N^1CH_2); 3.03–3.19 (m, 4H, $N^4(CH_2)_2$); 3.23 (t, 2H, CH_2–CO); 6.78 (d, 1H, *o*-OCH_3–*Ph*, H_6, $J = 7.2$ Hz); 6.83–6.98 (m, 3H, *o*-OCH_3–*Ph*, $H_3+H_4+H_5$); 7.19 (d, 2H, *p*-CH_3–*Ph*, H_3+H_5, $J = 8.0$ Hz); 7.81 (d, 2H, *p*-CH_3–*Ph*, H_2+H_6, $J = 7.8$ Hz).	2931, 1675, 756
I.2.a	1.83–1.90 (m, 2H, C**H**$_2$CH); 2.34 (s, 3H, CH_3); 2.65–2.83 (m, 6H, $CH_2N^1(CH_2)_2$); 3.07–3.19 (m, 4H, $N^4(CH_2)_2$); 3.86 (s, 3H, OCH_3); 4.93 (t, 1H, CH, $J = 5.6$ Hz); 6.84–7.01 (m, 4H, *o*-OCH_3–*Ph*); 7.15 (d, 2H, *p*-CH_3–*Ph*, H_3+H_5, $J = 8.0$ Hz); 7.28 (d, 2H, *p*-CH_3–*Ph*, H_2+H_6, $J = 8.0$ Hz).	3410, 820
I.3.a	2.11–2.35 (m, 1H, C**H**$_a$CH); 1.25–2.35 (m, 4H, C**H**$_b$CH, CH_3); 2.60–2.74 (m, 6H, $CH_2N^1(CH_2)_2$); 3.08–3.18 (m, 4H, $N^4(CH_2)_2$); 3.85 (s, 3H, OCH_3); 5.28 (dd, 1H, CH, $J = 7.8$ Hz, $J' = 5.0$ Hz); 6.57 (d, 2H, *p*-CF_3–*Ph*, H_2+H_6); 6.80–7.00 (m, 4H, *o*-OCH_3–*Ph*); 7.13 (d, 2H, *p*-CH_3–*Ph*; H_3+H_5, $J = 8.0$ Hz); 7.22–7.44 (m, 4H, *p*-CH_3–*Ph*, H_2+H_6, *p*-CF_3–*Ph*, H_3+H_5).	1327, 1243, 838
II.1.a	(Free base): 2.43 (s, 3H, CH_3); 2.62–2.64 (m, 4H, $N^1(CH_2)_2$); 2.76–2.83 (m, 2H, N^1CH_2); 3.01–3.10 (m, 6H, CH_2–CO, $N^4(CH_2)_2$); 3.78 (s, 3H, OCH_3); 6.76–6.92 (m, 4H, *o*-OCH_3–*Ph*); 7.15–7.33 (m, 3H, *o*-CH_3–*Ph*, $H_3+H_4+H_5$); 7.57 (d, 1H, *o*-CH_3–*Ph*, H_6, $J = 7.3$ Hz).	2937, 1685, 735
II.2.a	1.82–1.85 (m, 2H, C**H**$_2$CH); 2.32 (s, 3H, CH_3); 2.70–2.75 (m, 6H, $CH_2N^1(CH_2)_2$); 3.11–3.23 (m, 4H, $N^4(CH_2)_2$); 3.87 (s, 3H, OCH_3); 5.16 (t, 1H, CH, $J =$ 5.0 Hz); 6.88–6.95 (m, 4H, *o*-OCH_3–*Ph*) 7.12–7.16 (m, 3H, *o*-CH_3–*Ph*, $H_3+H_4+H_5$); 7.55–7.63 (m, 1H, *o*-CH_3–*Ph*, H_6).	3310, 1237, 745
III.1.a	(Free base): 2.27 (s, 3H, CH_3); 2.41 (s, 3H, CH_3); 2.62(t, 4H, $N^1(CH_2)_2$); 2.79 (t, 2H, N^1CH_2, $J = 7.1$ Hz); 3.03–3.10 (m, 6H, $N^4(CH_2)_2$, CH_2–CO); 3.78 (s, 3H, OCH_3); 6.78 (d, 1H, *o*-OCH_3–*Ph*, H_6, $J = 7.4$ Hz); 6.83–7.00 (m, 5H, *o*-OCH_3–*Ph*, $H_3+H_4+H_5$, *o*,*p*-diCH_3–*Ph*, H_3+H_5); 7.53 (d, 1H, *o*,*p*-diCH_3–*Ph*, H_6, $J = 8.4$ Hz).	2926, 1689, 1242
III.2.a	1.80–1.82 (m, 2H, C**H**$_2$CH); 2.27 (s, 3H, CH_3); 2.29 (s, 3H, CH_3); 2.66–2.83 (m, 6H, $CH_2N^1(CH_2)_2$); 3.05–3.21 (m, 4H, $N^4(CH_2)_2$); 3.85 (s, 3H, OCH_3); 5.11 (t, 1H, CH, $J = 5.6$ Hz); 6.62 (b s, 1H, OH); 6.83–6.99 (m, 4H, *o*-OCH_3–*Ph*) 7.00–7.06 (m, 2H, *o*,*p*-diCH_3–*Ph*, H_3+H_5); 7.45 (d, 1H, *o*,*p*-diCH_3–*Ph*, H_6, $J = 7.9$ Hz).	3252, 1240, 747
III.1.b	2.35 (s, 3H, CH_3); 2.50 (s, 3H, CH_3); 2.61–2.69 (m, 4H, $N^4(CH_2)_2$); 2.82–2.90 (m, 6H, $N^1(CH_2)_3$); 3.09–3.16 (m, 2H, CH_2–CO); 6.81–6.95 (m, 4H, *o*-OH–*Ph*); 7.04–7.17 (m, 2H, *o*,*p*-diCH_3–*Ph*, H_3+H_5); 7.58–7.62 (m, 1H, *o*,*p*-diCH_3–*Ph*, H_6).	3373, 1679, 751
III.2.b	1.78–1.86 (m, 2H, C**H**$_2$CH); 2.27 (s, 3H, CH_3); 2.29 (s, 3H, CH_3); 2.60–2.85 (m, 6H, $CH_2N^1(CH_2)_2$); 2.88–2.99 (m, 4H, $N^4(CH_2)_2$); 5.12 (t, 1H, CH, $J = 5.5$ Hz); 6.82–7.18 (m, 6H, *o*-OH–*Ph*, *o*,*p*-diCH_3–*Ph*, H_3+H_5);) 7.44 (d, 1H, *o*,*p*-diCH_3–*Ph*, H_6).	3230, 753
III.1.c	(Free base): 2.27 (s, 3H, CH_3); 2.44 (s, 3H, CH_3); 2.59–2.78 (m, 4H, $N^1(CH_2)_2$); 2.84 (t, 2H, N^1CH_2–, $J = 7.2$ Hz); 3.06–3.13 (m, 6H, $N^4(CH_2)_2$, CH_2–CO); 6.98–7.01 (m, 3H, *o*,*p*-diCH_3–*Ph*, H_3+H_5, *naph*, H_2); 7.27–7.56 (m, 4H, *naph*, $H_3+H_4+H_6+H_7$); 7.71—7.76 (m, 1H, *naph*, H_5); 8.08–8.13 (m, 1H, *naph*, H_8).	2968, 1681

duced pressure. The impure oil was purified by flash chromatography (SP, silica gel), eluting with light petroleum–ethyl acetate 1:1 (v/v).

3. Pharmacology

3.1. Animals

Male Swiss mice (23–28 g) housed in groups of ten and male Wistar rats (180–220 g) housed in groups of five, were used. Animals were kept in conditions of constant temperature (22 ± 1°C) controlled lighting on a 12-h light/dark cycle and free access to food and water.

3.2. Radioligand binding experiments

Binding studies to different receptors were performed as summarized in Table 3, in which the radioligand used, the source of tissue and the corresponding reference are indicated. Displacement curves were analyzed with the receptor fit competition LUNDON program and IC_{50} values, concentration required to inhibit specific binding by 50%, were calculated. Inhibition constants (K_i) were obtained according to the equation of Cheng and Prussoff $K_i = IC_{50}/(1 + [L]/[K_d])$ ([L], ligand concentration; [K_d], dissociation constant). K_i values were determined from at least three competition binding experiments.

3.3. OH-DPAT induced hypothermia in rat and mice

These studies were performed as described previously [22]. Body temperature was measured with a lubricated digital thermometer probe (pb0331, Panlab, Barcelona) inserted to a depth of 2 or 4 cm into the rectum of the mice and rats, respectively. Temperature was recorded at 15, 30 and 60 min for mice and 30, 60 and 90 min for

the rats, after injection of 8-OH-DPAT or other compound. To study the antagonism to 8-OH-DPAT-induced hypothermia, test compounds or vehicle (control) were administered i.p. 30 min before the injection of 8-hydroxy-2-(di-*n*-propylamino)tetraline (8-OH-DPAT) (0.5 mg/kg s.c.). The results were expressed as change in body temperature (Δt) with respect to basal body temperature. The obtained data were analyzed by Anova and Dunnett's test.

3.4. Antidepressant activity

3.4.1. Forced swimming test [34]

Mice were placed individually for 6 min into glass cylinders (height 24 cm, diameter 13 cm) containing 14 cm of water, maintained at 22–23°C. This procedure was repeated for 2 consecutive days. On the second day the animals were treated 30 min before water-immersion and the duration of immobility was recorded the second day during the last 4 min of the 6 min testing period. A mouse was considered to be immobile when it floated in an upright position, and made only small movements to keep its head above water. The tricyclic antidepressant amitriptyline was used as a reference compound.

3.4.2. Learned helplessness test

The test was performed as described previously [35] with minor modifications. Experimental conditions were as follows.

3.4.2.1. Helpless induction. On day 1, rats were placed individually in an operant conditioning chamber with a grid floor connected to a scrambled shock generator (Coulbourn Instruments, USA). Each rat was then exposed to inescapable electric foot shocks (1.1 mA, 10 s) every 30 s during 30 min.

3.4.2.2. Conditioned avoidance training. Animals were placed individually into a shuttle-box (Letica Instruments, Spain) consisting of two compartments of the same size separated by a door. Shocks delivered through the grid floor were terminated as soon as the animal entered into the other compartment. The assay consisted in 30 stimulus-shock trials of 8 s with a 30 s resting period between each trial. During the first 5 s of each trial, a light and a sound were on (conditioned stimulus). If the animal did not cross, within this period to the other compartment, a shock (1 mA, 3 s maximal duration) was delivered. Avoidance sessions were performed during 3 consecutive days and the number of escape failures and of inter-trial crossings were recorded.

Animals received either saline or treatment throughout the 4-day period. On day 1, the drug was given 6 h after the exposure to the inescapable shocks and days 2–4, the drug was administered twice a day, 30 min before shuttle-box exposure and 6 h after. On each day of avoidance testing, differences between control and treated rats were evaluated by the Mann–Whitney *U*-test.

Table 3
Radioligand binding studies and references [a]

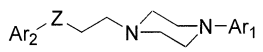
Receptor	^{3}H-Ligand	Tissue	References
5-HT transporter	^{3}H-paroxetina	Rat frontal cortex	[24]
5-HT_{1A} receptor	^{3}H-8-OH-DPAT	Rat frontal cortex	[25]
5-HT_{1D} receptor	^{3}H-5-HT	Bovine caudate	[26]
5-HT_2 receptor	^{3}H-ketanserine	Rat frontal cortex	[27]
5-HT_3 receptor	^{3}H-BRL	Rat frontal cortex	[28]
D_2 receptor	^{3}H-spiroperidol	Rat striatum	[29]
α_1 receptor	^{3}H-prazosin	Rat cortex	[30]
α_2 receptor	^{3}H-clonidina	Rat cortex	[31]
β receptor	^{3}H-DHA	Rat cortex	[32]
M receptor	^{3}H-QNB	Rat cortex	[33]

[a] Abbreviations: ^{3}H-8-OH-DPAT, 8-hydroxy-2-(di-*n*-propylamino)tetraline; ^{3}H-BRL, ^{3}H-BRL 43694; ^{3}H-DHA, ^{3}H-dihydroalprenolol; ^{3}H-QNB, ^{3}H-quinuclidinylbenzylate.

4. Results and discussion

We have synthesized a number of structural analogues of 3-[(4-aryl)piperazin-1-yl]-1-arylpropane derivatives. Their affinities at both, the 5-HT_{1A} receptors and the 5-HT transporter are shown in Table 4. With a single exception, all of the analogues displayed better affinity for the 5-HT_{1A} receptor than for the 5-HT transporter. The best results of affinity at 5-HT_{1A} receptors were obtained by the insertion of the methyl group in position 2 (compounds **II.1.a**. and **II.2.a**). However, the best dual activity was obtained with the 2,4-dimethyl derivatives. Receptor binding studies indicated that compound **III.1.a** exhibited the higher affinity for both the 5-HT transporter ($K_i = 415$ nM) and 5-HT_{1A} receptors (42 nM), however it was approximately 20 times weaker than that showed by fluoxetine ($K_i = 21$ nM) and by 8-OH-DPAT ($K_i = 2$ nM), respectively. The incorporation of the third ring provoked a loss of both activities, probably caused by the excess of volume associated to these ethers (compound **I.3.a**). These results are in accordance with our previous studies [20]. The change of 2-methoxyphenylpiperazine by other piperazines such as 2-hydroxyphenyl and 1-naphtylpiperazine, reduced 5-HT_{1A} receptor binding affinity, although the enhancement of the binding

Table 4
Structure and binding affinity (K_i, nM) at 5-HT_{1A} receptors and 5-HT transporter



Compound	Ar_2	Z	Ar_1	5-HT_{1A} receptor (K_i) [a]	5-HT transporter (λ_i) [a]
I.1.a	4-CH_3-phenyl	C=O	2-OCH_3-phenyl	100 ± 11	>5000
I.2.a	4-CH_3-phenyl	CHOH	2-OCH_3-phenyl	40 ± 5.4	>5000
I.3.a	4-CH_3-phenyl	$CHOC_6H_5CF_3$	2-OCH_3-phenyl	>5000	5000 ± 125
II.1.a	2-CH_3-phenyl	C=O	2-OCH_3-phenyl	25 ± 2.3	5000 ± 120
II.2.a	2-CH_3-phenyl	CHOH	2-OCH_3-phenyl	21.5 ± 1.5	800 ± 110
III.1.a	2,4-di-CH_3-phenyl	C=O	2-OCH_3-phenyl	42 ± 3.5	415 ± 50
III.2.a	2,4-di-CH_3-phenyl	CHOH	2-OCH_3-phenyl	90 ± 8.2	2000 ± 105
III.1.b	2,4-di-CH_3-phenyl	C=O	2-OH-phenyl	115 ± 10	1800 ± 95
III.2.b	2,4-di-CH_3-phenyl	CHOH	2-OH-phenyl	110 ± 9	1700 ± 110
III.1.c	2,4-di-CH_3-phenyl	C=O	1-Naphtyl	500 ± 23	195 ± 13

[a] Results are the mean ± SEM of three experiments except for the K_i values >5.000 nM which were the mean of two separate experiments.

affinity to the 5-HT transporter caused by the introduction of the 1-naphtylpiperazine should be highlighted (**III.1.c**).

Further pharmacological studies were performed with **III.1.a**, the most interesting compound in this series. In the forced swimming test (Table 5), **III.1.a** presented the best result, exhibiting antidepressant-like activity over a wide dose range (0.1–10 mg/kg). In contrast, the SSRI fluoxetine showed only anti-immobility effects at the high dose of 30 mg/kg, in keeping with previous data [36]. There is a good evidence that 5-HT_{1A} receptor agonists are active in the forced swimming test at lower doses than SSRIs indicating that the stimulation of 5-HT_{1A} receptors appears to be important in reducing immobility time [36]. Further studies showed that the 5-HT_{1A} receptors mediating increased mobility are located postsynaptically [37]. We suggest that the mechanism mediating antidepressant activity by **III.1.a** in the forced swimming test is related to an agonist activity to postsynaptic 5-HT_{1A} receptors, however we do not discharge a possible influence also of the inhibition of 5-HT uptake.

In terms of selectivity (Table 6), **III.1.a** also exhibited an interesting biochemical profile, since it was devoid of affinity at other 5-HT receptor subtypes. Compound **III.1.a** exhibited no affinity for muscarinic receptors ($K_i > 5.000$ nM) but showed certain affinity for α_1 adrenoceptors ($K_i = 80$ nM) although 160 times weaker than the reference compound prazosin. Microdialysis studies with compounds that exhibit a dual activity to α_1 adrenoceptors and 5-HT_{1A} receptors have shown that the excitation mediated by the α_1-adrenoceptor is outweighed by simultaneous activation of the latter-5-HT_{1A} receptors [38–40]. These studies have shown that the receptor 5-HT_{1A} is the most important with regard to the interplay between α_1-adrenoceptor and 5-HT_{1A} (auto)receptor mechanisms in the control of 5-HT release in the rat forebrain. Thus it is predictable low implication of the adrenergic component in the pharmacology of compound **III.1.a** after its systemic administration. On the other hand, there is good evidence that the better safety profile of SSRIs over tricyclic antidepressants (TCAs) is due partially to a lack of anticholinergic and cardiovascular side effects, which are derived from the inhibition of muscarinic and α_1 adrenergic receptors, respectively [41,42]. We can suggest that **III.1.a** may show, like SSRIs, a better safety profile than TCAs.

Body temperature measurements revealed that **III.1.a** (5 mg/kg i.p.) antagonized 8-OH-DPAT-induced hypothermia in rats and more weakly in mice suggesting some antagonistic properties of both post and presynaptic 5-HT_{1A} receptors, respectively (Tables 7 and 8).

Table 5
Effects of **II.2.a** and **III.1.a** and antidepressants on immobility time in the forced swimming test [a]

Treatment	Immobility time (s)			
	Vehicle	0.1	1	10
Amitriptyline	128.2 ± 16.8	123.1 ± 14.7	130.5 ± 15.6	19.3 ± 0.1 [c]
Fluoxetine	166.9 ± 9.2	175.0 ± 11.6	160.1 ± 12.3	131.5 ± 12.6
II.2.a	164.1 ± 13.3	177.0 ± 13.6	109.6 ± 14.2 [b]	157.0 ± 19.6
III.1.a	107.9 ± 19.5	38.0 ± 10.9 [c]	22.6 ± 8.1 [c]	28.4 ± 10.2 [c]

[a] Fluoxetine 30 mg/kg i.p. reduced significantly ($P<0.01$) immobility time to 32.8 ± 13.5. Values are means ± SEM of ten mice. Compounds were administered i.p. 30 min before the test.
[b] $P<0.05$.
[c] $P<0.01$ versus vehicle (Student's t-test).

Table 6
Affinity (K_i, nM) of **III.1.a** at 5-HT receptor subtypes, dopamine D_2 receptors, adrenoceptors (α_1, α_2 and β) and muscarinic receptors [a]

Receptor	Standard displacer	Ligand reference	**III.1.a**
5-HT_{1D}	Sumatriptan	12 ± 1.9	660 ± 56
5-HT_2	Ketanserine	0.7 ± 0.09	4870 ± 212
5-HT_3	BRL 43694	0.3 ± 0.01	>5.000
Dopamine D_2	Haloperidol	1.5 ± 1.2	120 ± 10
Adrenoceptor α_1	Prazosin	0.5 ± 0.01	80 ± 8.3
Adrenoceptor α_2	Clonidine	8 ± 0.1	1300 ± 157
Adrenoceptor β	DHA	3.5 ± 0.3	>5.000
Muscarinic	Atropine	2.5 ± 0.1	>5.000

[a] The values represent the mean $\pm$SEM from three independent experiments.

It can not be discarded, however, that at the high dose of 5 mg/kg, partial 5-HT_{1A} agonist properties or another non-specific actions of the compound could be involved.

The results obtained in the learned helplessness test (Table 9) appeared to confirm the antidepressant properties of **III.1.a** shown previously in the forced swimming test. The effect of two typical SSRIs fluoxetine and paroxetine, were also investigated in test and in close analogy to the results obtained in the FST, they were active at higher doses than this new compound.

We can conclude that compound **III.1.a** exhibits the higher affinity for both the 5-HT_{1A} receptors and the 5-HT transporter with a more marked activity at 5-HT_{1A} receptors. Furthermore **III.1.a** has antidepressant-like properties in two widely accepted animal models of depression and it exhibits activity at lower doses than typical SSRIs. Mechanism mediating antidepressant activity by **III.1.a** in the forced swimming and in the learned helplessness test should be related to an agonist activity to postsynaptic 5-HT_{1A} receptors with a possible influence of the inhibition of 5-HT uptake. The work presented have served well in focusing our attention on this class of molecules (3-[(4-aryl)piperazin-1-

Table 7
Time-dependent effects of 8-OH-DPAT (0.5 mg/kg, s.c.), (−)pindolol (10 mg/kg, s.c.) and compound **III.1.a** on rectal temperature and on 8-OH-DPAT-induced hypothermia in the rat [a]

Treatment	Dose (mg/kg/day)	Change in body temperature (°C)		
		30 min	60 min	90 min
Saline		-0.40 ± 0.11	-0.35 ± 0.02	-0.60 ± 0.08
8-OH-DPAT	0.5	-2.07 ± 0.31 [b]	-1.70 ± 0.15 [b]	-1.32 ± 0.21 [b]
(−)Pindolol	10	-0.4 ± 0.21	-0.3 ± 0.15	-0.5 ± 0.11
III.1.a	0.5	-0.45 ± 0.09	-0.45 ± 0.10	-0.76 ± 0.15
	5	-0.48 ± 0.10	-0.40 ± 0.11	-0.72 ± 0.19
Saline/8-OH-DPAT	–/0.5	-2.70 ± 0.25	-1.90 ± 0.18	-1.52 ± 0.17
(−)Pindolol/8-OH-DPAT	10/0.5	-0.62 ± 0.32**	-0.71 ± 0.12**	-0.78 ± 0.15**
III.1.a/8-OH-DPAT	0.5/0.5	-2.83 ± 0.36	-2.01 ± 0.27	-1.47 ± 0.35
	5/0.5	-0.95 ± 0.09**	-0.67 ± 0.14**	-0.57 ± 0.06*

[a] Body temperature was measured immediately before each drug injection and 30, 60 and 90 min later. Values are means $\pm$SEM.
[b] $P<0.01$ versus saline group. *$P<0.05$; **$P<0.01$ versus 8-OH-DPAT group (one-way ANOVA followed by Dunnett's test).

Table 8
Time dependent effects of 8-OH-DPAT (0.5 mg/kg, s.c.), (−)pindolol (10 mg/kg, i.p.) and compound **III.1.a** on rectal temperature and on 8-OH-DPAT induced hypothermia in the mouse [a]

Treatment	Dose (mg/kg/day)	Change in body temperature (°C)		
		15 min	30 min	60 min
Saline	–	-0.55 ± 0.09	-0.67 ± 0.12	-0.78 ± 0.11
8-OH-DPAT	0.5	-3.33 ± 0.28 [b]	-3.50 ± 0.50 [b]	-2.31 ± 0.36 [b]
(−)Pindolol	10	-0.6 ± 0.21	-0.8 ± 0.15	-0.4 ± 0.11
III.1.a	0.5	-0.40 ± 0.08	-0.70 ± 0.10	-0.96 ± 0.15
	5	-0.78 ± 0.09	-0.77 ± 0.16	-0.91 ± 0.16
Saline/8-OH-DPAT	–/0.5	-2.61 ± 0.35	-2.36 ± 0.40	-1.47 ± 0.20
(−)Pindolol/8-OH-DPAT	10/0.5	-0.71 ± 0.21**	-0.95 ± 0.15**	-0.35 ± 0.09**
III.1.a/8-OH-DPAT	0.5/0.5	-2.93 ± 0.38	-2.30 ± 0.47	-1.57 ± 0.43
	5/0.5	-2.53 ± 0.32	-1.47 ± 0.21*	-1.27 ± 0.14

[a] Body temperature was measured immediately before each drug injection and 15, 30 and 60 min later. Values are means $\pm$SEM.
[b] $P<0.01$ versus saline group. *$P<0.05$; **$P<0.01$ versus 8-OH-DPAT group (one-way ANOVA followed by Dunnett's test).

Table 9
Effect of compound **III.1.a** and selected antidepressants on helpless behavior [a]

Treatment	Dose (mg/kg)	Number of escape failures		
		SB_1	SB_2	SB_3
Vehicle	–	15.7 ± 2.1	11.2 ± 3.0	10.7 ± 2.3
Amitriptyline	10	9.0 ± 3.0	3.3 ± 1.7 [c]	1.2 ± 1.0 [c]
Fluoxetine	30	13.0 ± 1.0	4.0 ± 0.8 [b]	3.2 ± 1.0 [b]
Paroxetine	10	3.3 ± 1.4 [b]	3.0 ± 0.9 [b]	2.2 ± 0.6 [b]
III.1.a	1	8.3 ± 2.2 [b]	3.4 ± 1.4 [b]	2.8 ± 1.6 [b]

[a] Data are the means $\pm$ SEM of escape failures in each of the three consecutive shuttle-box sessions (SB 1–3). **III.1.a** was injected (i.p.) on 4 consecutive days, i.e. 6 h after inescapable shocks on day 1 and then twice a day (30 min before shuttle-box sessions and 6 h after).
[b] $P < 0.05$.
[c] $P < 0.01$ versus control (Mann–Whitney U-test).

yl]-1-aryl derivatives) which may serve as a tool for further investigations on new antidepressants with such a dual mechanism of action.

5. Elemental analyses

Compound **I.1.a**	Calc.: C, 67.28; H, 7.20; N, 7.47 Found: C, 67.02; H, 7.46; N, 7.43%
Compound **I.2.a**	Calc.: C, 74.11; H, 8.23; N, 8.23 Found: C, 74.04; H, 8.55; N, 8.27%
Compound **I.3.a**	Calc.: C, 69.42; H, 6.40; N, 5.78 Found: C, 69.15; H, 6.54; N, 5.73%
Compound **II.1.a**	Calc.: C, 67.28; H, 7.21; N, 7.47 Found: C, 66.88; H, 7.30; N, 7.40%
Compound **II.2.a**	Calc.: C, 74.11; H, 8.23; N, 8.23 Found: C, 74.33; H, 8.59; N, 8.08%
Compound **III.1.a**	Calc.: C, 67.95; H, 7.40; N, 7.20 Found: C, 68.10; H, 7.85; N, 7.29%
Compound **III.2.a**	Calc.: C, 74.57; H, 8.47; N, 7.90 Found: C, 74.76; H, 8.92; N, 7.89%
Compound **III.1.b**	Calc.: C, 74.56; H, 7.69; N, 8.28 Found: C, 74.27; H, 7.88; N, 8.01%
Compound **III.2.b**	Calc.: C, 74.11; H, 8.23; N, 8.23 Found: C, 74.03; H, 8.33; N, 8.03%
Compound **III.1.c**	Calc.: C, 73.44; H, 7.01; N, 6.85 Found: C, 72.98; H, 7.31; N, 6.56%

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References

[1] S.M. Stahl, Mixed depression and anxiety: serotonin 1A receptor as a common pharmacological link, J. Clin. Psychiatry 58 (1997) 20–26.
[2] J.F. Heisser, C.S. Wilcox, Serotonin 5-HT1A receptor agonists as antidepressants, CNS Drugs 10 (1998) 343–353.
[3] M. Asberg, B. Eriksson, B. Matenson, L. Traskman-Bendz, A. Wagner, Therapeutic effects of serotonin uptake inhibitors in depression, J. Clin. Psychiatry 47 (1986) 23–35.
[4] C. De Montigny, I. Chaput, P. Blier, Classical and novel targets for antidepressant drugs: new pharmacological approaches to the therapy of depressive disorders, Int. Acad. Biomed. Drug Res. 5 (1993) 8–17.
[5] L.A. Labbate, J.B. Grimes, G.W. Arana, Serotonin reuptake antidepressant effects on sexual function in patients with anxiety disorders, Biol. Psychiatry 43 (1998) 904–907.
[6] J.J. Rutter, C. Gundlah, S.B. Auerbach, Systemic uptake inhibition decreases serotonin release via somatodendritic autoreceptor activation, Synapse 20 (1995) 225–233.
[7] Y. Chaput, C. De Montygny, P. Blier, Effects of a selective 5-HT reuptake blocker, citalopram, on the sensitivity of 5-HT autoreceptors: electrophysiological studies in the rat brain, Naunyn Schmiedebergs Arch. Pharmacol. 333 (1986) 342–348.
[8] S. Hjorth, Serotonin 5-HT_{1A} autoreceptor blockade potenciates the ability of the 5-HT uptake inhibitor citalopram to increase nerve terminal output of 5-HT in vivo: a microdialysis study, J. Neurochem. 60 (1993) 776–779.
[9] S.E. Gartside, V. Umbers, M. Hajos, T. Sharp, Interaction between a selective 5-HT_{1A} receptor antagonist and an SSRIs in vivo: effects on 5-HT cell firing and extracellular 5-HT, Br. J. Pharmacol. 115 (1995) 1064–1070.
[10] L.J. Dreshfield, D.T. Wong, K.W. Perry, E.A. Engleman, Enhancement of fluoxetine-dependent increase of extracellular serotonin (5-HT) levels by (−)-pindolol, an antagonist at 5-HT_{1A} receptors, Neurochem. Res. 21 (1996) 557–562.
[11] T. Sharp, V. Umbers, S.E. Gartside, Effect of a selective 5-HT reuptake inhibitor in combination with 5-HT_{1A} and 5-HT1B receptor antagonists on extracellular 5-HT in rat frontal cortex in vivo, Br. J. Pharmacol. 121 (1997) 941–946.
[12] F. Artigas, V. Perez, E. Alvarez, Pindolol induces a rapid improvement of depressed patients treated with serotonin reuptake inhibitors, Arch. Gen. Psychiatry 51 (1994) 248–251.
[13] M.B. Tome, C.R. Cloninger, J.P. Watson, M.T. Isaac, Serotonergic autoreceptor blockade in the reduction of antidepressant latency: personality variables and response to paroxetine and pindolol, J. Affect. Disord. 44 (1997) 101–109.
[14] V. Perez, I. Gilaberte, D. Faries, E. Alvarez, F. Artigas, Randomised, double blind, placebo-controlled trial of pindolol in combination with fluoxetine antidepressant treatment, Lancet 349 (1997) 1594–1597.
[15] R. Zanardi, F. Artigas, L. Franchini, L. Sforzini, M. Gasperini, E. Smeraldi, J. Perez, How long pindolol should be associated to paroxetine to improve the antidepressant response?, J. Clin. Psychopharmacol. 17 (1997) 446–450.

[16] T. Puzantian, K. Kawase, Does the addition of pindolol accelerate or enhance the response to selective serotonin reuptake inhibitor antidepressants?, Pharmacotherapy 19 (1999) 205–212.
[17] V. Perez, J. Soler, D. Puigdemont, E. Alvarez, F. Artigas, A double-blind, randomized, placebo-controlled trial of pindolol augmentation in depressive patients resistant to serotonin reuptake inhibitors, Arch. Gen. Psychiatry 56 (1999) 375–379.
[18] R.M. Pinder, J.H. Wieringa, Third generation antidepressants, Med. Res. Rev. 13 (1993) 259–325.
[19] R.A. Glennon, Concepts for the design of 5-HT_{1A} serotonin agonists and antagonist, Drug Dev. Res. 26 (1992) 251–274.
[20] A. Monge Vega, J. del Rio Zambrana, B. Lasheras, J.A. Palop, A. Bosch, J.C. del Castillo, J. Roca. (Vita Elan Farma) WO 9902516A1.
[21] A. Rupp, K.A.G. Beuerle, C. Ruf, G. Folkers, A new pharmacophoric model for 5-Ht reuptake inhibitors: differentiation of amphetamine analogues, Pharm. Acta Helv. 68 (1994) 235–244.
[22] D.J. Bill. Knight, E.A. Forster, A. Fletcher, Direct evidence for an important species difference in the mechanism of 8-OH-DPAT-induced hypothermia, Br. J. Pharmacol. 103 (1991) 1857–1864.
[23] P. Willner, Animal models of depression: validity and applications, in: G. Gessa, L Fratta, L. Pani, G. y Serra (Eds.), Depression and mania: From Neurobiology to Treatment, Raven, New York, 1995, pp. 19–41.
[24] E. Habert, D. Graham, L. Tehraqui, Y. Claustre, S.Z. Langer, Characterisation of ^{3}H-paroxetine binding to rat cortical membranes, Eur. J. Pharmacol. 118 (1989) 107–114.
[25] D. Hoyer, G. Engel, H.O. Kalkman, Molecular pharmacology of 5-HT_1 and 5-HT_2 recognition sites in rat and pig brain membranes: radioligand binding sites with ^{3}H-5-HT, ^{3}H-8-OH-DPAT, ^{125}I-iodocyanopindolol, ^{3}H-mesulergine, ^{3}H-ketanserine, Eur. J. Pharmacol. 118 (1985) 13–23.
[26] E. Weisberg, M. Teitler, Novel high-affinity [^{3}H]-serotonin binding sites in rat and bovine brain tissue, Drug Dev. Res. 26 (1992) 225–234.
[27] J.E. Leysen, A. De Chaffoy, D. De Courcelles, F. De Clerck, C.J. Niemegeers, J.M. Van Nueten, 5-HT_2 receptor binding sites and functional correlates, Neuropharmacology 23 (1984) 1493–1501.
[28] D.R. Nelson, D.R. Thomas, [^{3}H]-BRL-43694 (Granisetron), a specific ligand for 5-HT_3 binding sites in rat brain cortical membranes, Biochem. Pharmacol. 38 (1980) 1693–1695.
[29] J.E. Leysen, W. Goumeren, P.M. Laduron, Spiperone: a ligand of choice for neuroleptic receptors: kinetics and characteristics of in vitro binding, Biochem. Pharmacol. 27 (1978) 307–316.
[30] M.C. Michel, E. Brodeo, B. Schnepel, J. Behrendt, R. Tschada, H.J. Montulsky, P.A. Insel, [^{3}H]-idazoxan and some other α_2-adrenergic drugs also bind with high affinity to a non-adrenergic site, Mol. Pharmacol. 35 (1989) 324–330.
[31] A. Pilc, C. Nowan, J. Zak, *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, an irreversible receptor inactivator, as a tool for measurement of α_2-adrenoceptor occupancy in vivo, Eur. J. Pharmacol. 212 (1992) 109–111.
[32] D.H. Bylund, S.H. Snyder, Adrenergic receptor binding in membrane preparations from mammalian brain, Mol. Pharmacol. 12 (1976) 568–580.
[33] A.B. Norman, J.H. Eubanks, I. Creese, Irreversible and quaternary muscarinic antagonists discriminate multiple muscarinic receptor binding sites in rat brain, J. Pharmacol. Exp. Ther. 248 (1989) 1116–1122.
[34] R.D. Porsolt, L.M. Pichon, M. Jalfre, Depression: a new animal model sensitive to antidepressant treatments, Nature 266 (1977) 730–732.
[35] P. Martin, H. Gozlan, A. Puech, 5-HT_3 receptor antagonists reverse helpless behaviour in rats, Eur. J. Pharmacol. 212 (1992) 73–78.
[36] F. Borsini, Role of the serotonergic system in the forced swimming test, Neurosci. Biobehav. Rev. 19 (1995) 377–395.
[37] G.P. Luscombe, K.F. Martin, L.J. Hutchins, J. Gosden, D.J. Heal, Mediation of the antidepressant-like effect of 8-OH-DPAT in mice by postsynaptic 5-HT receptors, Br. J. Pharmacol. 108 (1993) 669–677.
[38] S. Hjorth, H.J. Bengtsson, S. Milano, J.F. Lundberg, T. Sharp, Studies on the role of 5-HT_{1A} autoreceptors and alpha 1-adrenoceptors in the inhibition of 5-HT release — I. BMY7378 and prazosin, Neuropharmacology 34 (1995) 615–620.
[39] H.J. Bengtsson, A. Kullberg, M.J. Millan, S. Hjorth, The role of 5-HT_{1A} autoreceptors and alpha 1-adrenoceptors in the modulation of 5-HT release — III. Clozapine and the novel putative antipsychotic S 16924, Neuropharmacology 37 (1998) 349–356.
[40] L. Johansson, D. Sohn, S.O. Thorberg, D.M. Jackson, D. Kelder, L.G. Larsson, L. Renyi, S.B. Ross, C. Wallsten, H. Eriksson, P.S. Hu, E. Jerning, N. Mohell, A. Westlind-Danielsson, The pharmacological characterization of a novel selective 5-hydroxytryptamine1A receptor antagonist, NAD-299, J. Pharmacol. Exp. Ther. 283 (1997) 216–225.
[41] N.A. Keis, Cardiotoxic side effects associated with tricyclic antidepressant overdose, AACN Clin. Issues Crit. Care-Nurs. 3 (1992) 226–232.
[42] N. Andrews, S. Hogg, L.E. Gonzalez, S.E. File, 5-HT_{1A} receptors in the median raphe nucleus and dorsal hippocampus may mediate anxiolytic and anxiogenic behaviours, respectively, Eur. J. Pharmacol. 264 (1994) 259–264.