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Synthesis and structure–activity relationships of a new model of arylpiperazines. Part 7: Study of the influence of lipophilic factors at the terminal amide fragment on 5-HT$_{1A}$ affinity/selectivity

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Abstract—The influence of lipophilic factors at the amide fragment of a new series of (±)-7*a*-alkyl-2-[4-(4-arylpiperazin-1-yl)butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazoles **2** and of (±)-7*a*-alkyl-2-[(4-arylpiperazin-1-yl)methyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazoles **3** has been studied. Variations of logP have been carried out by introducing different hydrocarbonated substituents (R^1) at the position 7*a* of the bicyclohydantoin, namely the non-pharmacophoric part. All the new compounds exhibit high potency for the 5-HT$_{1A}$ receptor; however, affinities for the α_1 receptor are high for compounds **2a**–**l** while compounds **3a**–**f** are selective over this adrenergic receptor. On the other hand, differences in logP do not notably affect the K_i values for the above receptors.

1. Introduction

Serotonin (5-HT) is one of the most attractive targets for medicinal chemists and the discovery of ligands with affinity for the family of 5-HT receptors (5-HTRs) is an area of intense research because of the potential to find new therapeutic drugs, due to their involvement in numerous physiological and pathophysiological processes.[1–4] Among the fourteen 5-HTRs identified to date,[5,6] the 5-HT$_{1A}$ subtype is the best studied due to the implication of agonists and partial agonists in anxiety and depression.[7–9] Also, recent studies have suggested that 5-HT$_{1A}$R agonists have neuroprotective properties.[10–12] The 5-HT$_{1A}$R belongs to the G protein-coupled receptors (GPCRs),[13] and the members of this family possess amino acid composition in common. In particular, the transmembrane amino acid sequence of the 5-HT$_{1A}$R presents a high degree of homology with the α_1-adrenergic receptor.[14] Thus, a great number of 5-HT$_{1A}$R ligands exhibit poor selectivity over α_1-adrenergic receptors.

Our group has undertaken a research program aimed at developing new 5-HT$_{1A}$R agents[15–24] with high affinity and selectivity over α_1-adrenergic receptors using as a starting point a series of arylpiperazines **I** (Fig. 1), which showed affinity for 5-HT$_{1A}$ and α_1-adrenergic receptors. Our main objective was to carry out a systematic study based on the non-pharmacophoric and pharmacophoric sites of both receptors, to gain insight into the structural factors that are responsible for 5-HT$_{1A}$/α_1 selectivity.

With respect to the pharmacophore part (arylpiperazine), in a previous study[21,22] we have designed and synthesized a training set of 32 compounds of general structure **I**. The amide moiety is a bicyclohydantoin or a diketopiperazine (X = –$(CH_2)_3$–,–$(CH_2)_4$–; $m = 0$, 1), the spacer length (n) is 3 or 4 methylene units, and the aromatic substituent R occupies the *ortho*- or *meta*- position and it has been selected from a data base of 387 substituents using the EDISFAR program. The 5-HT$_{1A}$ and α_1-adrenergic receptor binding affinities have been used to derive classical QSAR and neural networks models for both receptors which were useful in the design of the new ligand EF-7412 (X = –$(CH_2)_3$–; $m = 0$; $n = 4$; 5-HT$_{1A}$: K_i (nM) = 27; α_1: K_i (nM) > 1000).

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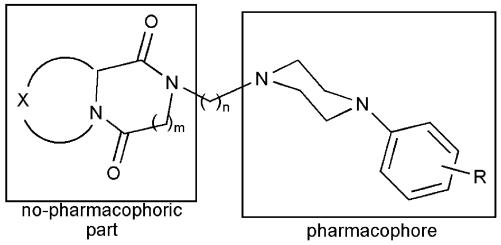
The influence of the spacer between the arylpiperazine and the amide moieties has also been studied[16,19,21,24] in series **I–III** (Figs 1 and 2). We have observed that the length of the spacer is of great importance for 5-HT_{1A}/α_1 affinity and selectivity in these ligands. The maximum affinity at both receptors is reached with $n=3$ or 4, and reduction to $n=2$ leads to inactive or poorly active compounds. A spacer of $n=1$ also decreased affinity especially at α_1 adrenoceptors, improving notably the selectivity for 5-HT_{1A}Rs.

Regarding the non-pharmacophoric sites, we have described[18,19] a series of derivatives **II** and **III** in order to determine the influence of electronic and steric factors on the stabilization of receptor–ligand complex (Fig. 2). SAR studies[18] in compounds **II**, which are devoid of the terminal amide fragment present in related 5-HT_{1A}R ligands but which preserve the steric requeriments of this moiety, suggest that there is influence of electronic factors on the non-pharmacophoric part of the α_1-adrenergic receptor site; however, they have little influence on the stabilization of the 5-HT_{1A}R–ligand complex. In the series of arylpiperazines **III**,[19] in which we have explored some steric requirements by modifying the size and the shape of the amide portion with respect to the bicyclohydantoin **I** ($m=0$), we observed that the non-pharmacophoric pocket in the 5-HT_{1A}R have less restriction than the corresponding pocket in the α_1-adrenergic receptor. These studies allowed us to suggest some differences between the non-pharmacophoric sites of both 5-HT_{1A} and α_1-adrenergic receptors.



I

Figure 1. Arylpiperazines of general structure **I**.

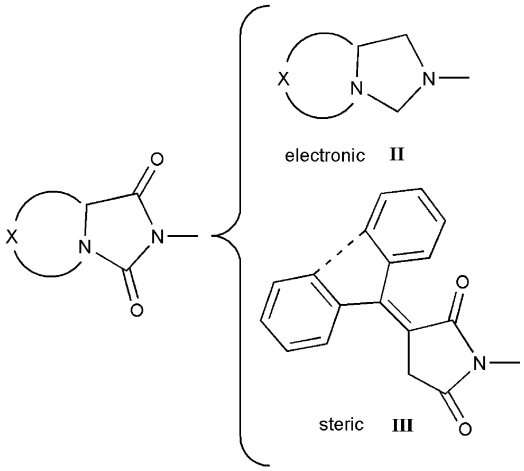
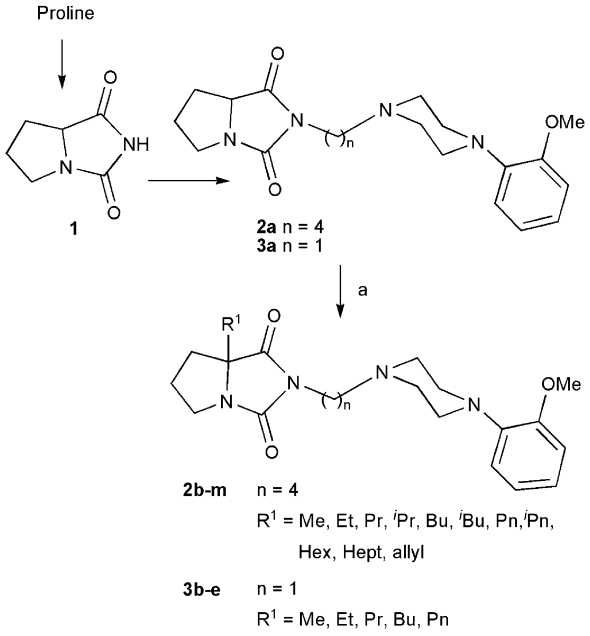


Figure 2. Study of the influence of electronic and steric factors at the non-phamacophoric part.

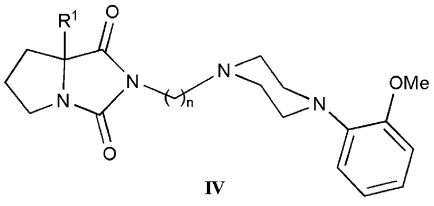


Scheme 1. Synthesis of alkylated bicyclohydantoin derivatives **2** and **3**.

To conclude this systematic study in this work we have considered a new series of arylpiperazines **IV**, in which we will explore the influence of lipophilic factors (Fig. 3). We have taken into account a variation of logP by introducing different substituents (R^1) at the position 7a of the bicyclohydantoin, without increasing the van der Waals volume of the non-pharmacophoric part in more than 200 Å^3, based on the steric requirements of the amide fragment.[19] In the design of compounds **IV** we have selected an *o*-methoxy group as the aromatic substituent R and 1 or 4 methylene units in the spacer.

2. Chemistry

The synthesis of compounds of general structure **IV** has been carried out by *C*-alkylation at the bicyclohydantoin fragment of arylpiperazines **2a** and **3a** by deprotonation with LDA followed by addition of the corresponding alkylating agent (Scheme 1). The synthesis of arylpiperazines **2a** and **3a** was carried out from bicyclohydantoin **1**, which was obtained by cyclization of proline with potassium cyanate.[25] Subsequent alkylation reaction of



IV

n = 1, 4
R^1 = Me, Et, Pr, ^{i}Pr, Bu, ^{i}Bu, Pn, ^{i}Pn, Hex, Hept, allyl

Figure 3. Training set for the study of the lipophilicity at the non-pharmacophoric part.

1 with 4-(4-bromobutyl)-1-(*o*-methoxyphenyl)piperazine provided **2a** ($n=4$) as we have previously reported.[16] The synthesis of **3a** ($n=1$)[16] was performed by Mannich reaction of **1**, formaldehyde and 1-(*o*-methoxyphenyl)-piperazine.

All new compounds (Table 1) were characterized by IR and ^{1}H and ^{13}C NMR spectroscopy, and gave satisfactory combustion analyses (C, H, N).

3. Biochemistry

Target compounds were assessed for in vitro binding affinity at serotoninergic 5-HT$_{1A}$ and α_1-adrenergic receptors by radioligand binding assays, using [^{3}H]-8-OH-DPAT[26] and [^{3}H]prazosin,[27] respectively, in rat cerebral cortex membranes. All the synthesized compounds **2a–l** and **3a–f** exhibited high affinity for the 5-HT$_{1A}$R. However, while compounds **2a-l** display high affinity for the α_1 receptor, compounds **3a–f** are highly selective over this adrenergic receptor. The different behaviour of compounds **2** ($n=4$) and **3** ($n=1$) in terms of selectivity completely agree with the computational models recently proposed[22–24] for the interaction of these arylpiperazines and 5-HT$_{1A}$/α_1 receptors.

4. Influence of lipophilic factors

The introduction of linear and branched alkylic chains (R^1) of increasing number of carbons at the 7*a* carbon of the bicyclohydantoin fragment of the new ligands has allowed to evaluate the influence of lipophilicity at the non-pharmacophoric part of the molecules. Table 1 gathers binding affinities at 5-HT$_{1A}$ and α_1 receptors as well as values of logP, determined by using ACD lab program for each compound.

Table 1. Binding affinity data at 5-HT$_{1A}$ and α_1 receptors and logP of arylpiperazines **2a–l** and **3a–f**

Compd	R^1	*n*	logP[a]	$K_i \pm$SEM (nM)[b]	
				5-HT$_{1A}$ [^{3}H]-8-OH-DPAT	α_1 [^{3}H]Prazosin[c]
2a[c]	H	4	1.16	5.5±0.7	8.3±0.3
2b	Me	4	1.64	2.8±0.1	12±2
2c	Et	4	2.17	2.1±0.4	25±2
2d	Pr	4	2.7	5.4±0.1	8±1
2e	^{i}Pr	4	2.52	3.5±0.2	15±0.1
2f	Bu	4	3.23	2.4±0.4	13±0.2
2g	^{i}Bu	4	3.05	5±0.3	13±1
2h	Pn	4	3.76	21±2	16±1
2i	^{i}Pn	4	3.58	17±2	12±2
2j	Hex	4	4.3	27.9±1.6	15.2±1.8
2k	Hept	4	4.83	40.4±0.6	15±0.2
2l	allyl	4	2.41	4.1±0.2	5.6±0.6
3a[c]	H	1	0.69	34.9±0.7	500±65
3b	Me	1	1.34	42±2	>10,000
3c	Et	1	1.87	13±5	>1000
3d	Pr	1	2.4	25±2	>1000
3e	Bu	1	2.93	16±6	>10,000
3f	Pn	1	3.46	53±8	>1000

[a] logP values were calculated by using ACD lab program.
[b] K_i values are means±SEM of 2–4 assays, performed in triplicate. Inhibition curves were analyzed by a computer-assisted curve-fitting program (Prism GraphPad) and K_i values were determined from the Cheng–Prusoff equation.
[c] Data of **2a** and **3a** were previously reported and are included for comparative purposes.[16]

Within the series of compounds **2a–l** ($n=4$) we have observed equipotent values of K_i for the 5-HT$_{1A}$R when logP increases from 1.16 (R^1 = H) to 3.23 (R^1 = Bu). Although lipophilicity does not exert a significant effect on affinity for compounds **2a–g**, a remarkable decrease in potency was observed for **2h–k**, with values of logP higher than 3.5 (R^1 = Pent, Hex and Hept). Variations of lipophilicity produced by branching (R^1 = ^{i}Pr, ^{i}Bu, ^{i}Pn) or insaturations (R^1 = allyl) did not notably alter 5-HT$_{1A}$R affinity. Similarly, for analogues **3a–f** no significant variations in K_i values at 5-HT$_{1A}$Rs were found, pointing out that lipophilicity of the molecules is not a crucial factor for binding the receptor. Regarding selectivity, from the data gathered in Table 1 there is not a clear influence of lipophilicity on selectivity over α_1-adrenergic receptor for either series of compounds (**2** and **3**), being the different selectivity profile primarily marked by the length of the alkylic spacer ($n=1$ versus $n=4$).[24]

5. Conclusion

In this paper we have designed and synthesized two series of new arylpiperazines ($n=1$ and 4) that exhibit moderate to high potency for binding the 5-HT$_{1A}$R. Lipophilicity of the ligands, increased by introduction of alkylic chains at the non-pharmacophoric part, does not exert a crucial influence neither on the 5-HT$_{1A}$R affinity nor on the selectivity over α_1-adrenergic receptors of the new ligands.

6. Experimental

6.1. Chemistry

All reagents were commercial products purchased from Aldrich, Fluka. All solvents were distilled prior to use. Anhydrous diisopropylamine was obtained by distillation over CaH_2. Analytical TLC was carried out on Merck (Kieselgel 60F–254) silica gel plates with detection by UV light, iodine and acidic vanillin solution. For flash chromatography, Merck silica gel type 60 (size 230–400 mesh) was used. Melting points (uncorrected) were determined on a Gallenkamp electrothermal apparatus. Infrared spectra (IR) were obtained on a FTIR-8300 Shimadzu spectrophotometer. Nuclear magnetic resonance spectra were recorded on a Varian VXR-300S, Bruker AM-300, and Bruker AM-200. Unless otherwise stated, all spectra were recorded in $CDCl_3$. Chemical shifts are reported in ppm using the residual chloroform as internal standard. The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), m (multiplet). Elemental analyses (C, H, N) were carried out on a Perkin Elmer 2400 apparatus at Facultad de Farmacia, UCM. Where analyses are indicated by the symbols of

the elements, results obtained were within 0.4% of the theoretical values.

6.1.1. (±)-2-[4-[4-(*o*-Methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2a). This compound was synthesized according to procedures previously reported.[16]

6.1.2. (±)-2-[4-(*o*-Methoxyphenyl)piperazin-1-ylmethyl]-1,3-dioxoperhydropyrrolo[1,2-*c*] imidazole (3a). This compound was synthesized according to procedures previously reported.[16]

6.1.3. General procedure for the alkylation of *o*-methoxyphenylpiperazine derivatives 2a and 3a. To a cold (0 °C) solution of LDA in 2 mL of THF (generated from 0.22 mL of diisopropylamine and 0.62 mL of *n*-BuLi 2.5 M in hexane) was added 0.7 mmol of **2a** or **3a** in THF (2.3 mL). The mixture was stirred for 30 min at 0 °C and then a solution of 1.6 mmol of the corresponding electrophile in THF (0.78 mL) was added, and the reaction mixture was stirred for 24 h from 0 °C to room temperature. The mixture was then quenched by adding 2 mL of an aqueous saturated solution of NH_4Cl and basified with 20% aqueous solution of K_2CO_3. This mixture was extracted using EtOAc (3×20 mL), the organic extracts were dried (Na_2SO_4) and the solvent removed under vacuum. The crude mixtures were purified by column chromatography on silica gel using the appropriate mixture of solvents (hexane:EtOAc or EtOAc:EtOH) as eluent. The pure isolated compounds were characterized as free bases and then transformed into their hydrochloride salts to perform elemental analyses and binding assays.

6.1.4. (±)-2-[4-[4-(*o*-Methoxyphenyl)piperazin-1-yl]butyl]-7*a*-methyl-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2b). From **2a** and MeI following the general procedure was obtained **2b**. Yield = 79%. mp = 95–97 °C. R_f = 0.1 (EtOAc). IR (KBr) 2945, 1775, 1700, 1600, 1560, 1500, 1460, 1240, 750 cm^{-1}. ^1H NMR δ 1.37 (s, 3H, 7*a*-CH_3), 1.41–1.65 (m, 4H, CH_2-C<u>H</u>$_2$-C<u>H</u>$_2$-CH_2-), 1.69–1.90 (m, 2H, H_6), 1.98–2.20 (m, 2H, H_7), 2.38 (t, *J* = 7.3 Hz, 2H, CH_2-$N_{PIPERAZINE}$), 2.59 (m, 4H, 2 $CH_{2\text{-PIPERAZINE}}$), 3.04 (m, 4H, 2 $CH_{2\text{-PIPERAZINE}}$), 3.21 (ddd, *J* = 11.6, 7.8, 5.6 Hz, 1H, H_5), 3.45 (t, *J* = 6.8 Hz, 2H, CH_2-$N_{HYDANTOIN}$), 3.67 (ddd, *J* = 11.5, 7.8, 5.4 Hz, 1H, H_5), 3.81 (s, 3H,-OCH_3), 6.79–7.01 (m, 4H, Ar-H). ^{13}C NMR δ 21.8 (7*a*-CH_3), 23.9 (CH_2-CH_2-C<u>H</u>$_2$-CH_2-), 26.0 (CH_2-C<u>H</u>$_2$-CH_2-CH_2-, C_6), 33.4 (C_7), 38.8 (CH_2-$N_{HYDANTOIN}$), 44.5 (C_5), 50.5 (2 $OCH_{3\text{-PIPERAZINE}}$), 53.3 (2 $CH_{2\text{-PIPERAZINE}}$), 55.3 (OCH_3), 58.0 (CH_2-$N_{PIPERAZINE}$), 68.8 (C_{7a}), 111.1 (Ar-C_6), 118.1 (Ar-C_3), 120.9 (Ar-C_4), 122.8 (Ar-C_5), 141.3 (Ar-C_1), 152.2 (Ar-C_2), 160.0 (C_3), 176.7 (C_1). Anal. ($C_{22}H_{32}N_4O_3$·2HCl) C, H, N.

6.1.5. (±)-7*a*-Ethyl-2-[4-[4-(*o*-methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2c). From **2a** and EtI following the general procedure was obtained **2c**. Yield = 80%. Oil. R_f = 0.1 (EtOAc). IR ($CHCl_3$) 3020, 2945, 1765, 1700, 1595, 1500, 1445, 1415, 1245, 1215, 760 cm^{-1}. ^1H NMR δ 0.84 (t, *J* = 7.3 Hz, 3H), 1.42–1.69 (m, 3H), 1.72–2.11 (m, 5H), 2.36 (t, *J* = 7.3 Hz, 2H), 2.60 (m, 4H), 3.06 (m, 4H), 3.15 (ddd, *J* = 11.7, 7.6, 6.3 Hz, 1H), 3.47 (td, *J* = 6.6, 2.3 Hz, 2H), 3.77 (ddd, *J* = 11.7, 8.1, 6.7 Hz, 1H), 3.83 (s, 3H), 6.81–7.02 (m, 4H). ^{13}C NMR δ 8.2, 23.9, 25.9, 26.1, 28.1, 32.7, 38.8, 44.7, 50.6, 53.3, 55.3, 58.0, 72.8, 111.1, 118.1, 120.9, 122.7, 141.3, 152.2, 160.7, 176.2. Anal. ($C_{23}H_{34}N_4O_3$·2HCl) C, H, N.

6.1.6. (±)-2-[4-[4-(*o*-Methoxyphenyl)piperazin-1-yl]-butyl]-7*a*-propyl-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2d). From **2a** and *n*-PrI following the general procedure was obtained **2d**. Yield = 77%. Oil. R_f = 0.1 (EtOAc). IR ($CHCl_3$) 3020, 2945, 1765, 1705, 1595, 1500, 1445, 1420, 1240, 1215, 760 cm^{-1}. ^1H NMR δ 0.92 (t, *J* = 7.1 Hz, 3H), 1.05–1.72 (m, 7H), 1.75–2.17 (m, 5H), 2.40 (t, *J* = 7.3 Hz, 2H), 2.61 (m, 4H), 3.06 (m, 4H), 3.16 (ddd, *J* = 11.5, 7.6, 5.9 Hz, 1H), 3.46 (td, *J* = 6.6, 2.3 Hz, 2H), 3.74 (ddd, *J* = 11.5, 8.1, 6.4 Hz, 1H), 3.94 (s, 3H), 6.81–7.02 (m, 4H). ^{13}C NMR δ 14.0, 17.3, 23.9, 26.0, 26.1, 33.0, 37.2, 38.8, 44.8, 50.6, 53.4, 55.3, 58.1, 72.4, 111.1, 118.2, 121.0, 122.8, 141.4, 152.3, 160.6, 176.4. Anal. ($C_{24}H_{36}N_4O_3$·2HCl) C, H, N.

6.1.7. (±)-7*a*-*Iso*propyl-2-[4-[4-(*o*-methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2e). From **2a** and *i*-PrI following the general procedure was obtained **2e**. Yield = 75%. Oil. R_f = 0.2 (EtOAc:EtOH, 9:1). IR ($CHCl_3$) 3020, 2945, 1765, 1700, 1595, 1500, 1440, 1415, 1365, 1335, 1245, 1215, 760 cm^{-1}. ^1H NMR δ 0.94 (d, *J* = 6.8 Hz, 3H), 1.00 (d, *J* = 6.8 Hz, 3H), 1.57-1.60 (m, 4H), 1.80–2.11 (m, 5H), 2.49 (t, *J* = 7.1 Hz, 2H), 2.71 (m, 4H), 3.06 (ddd, *J* = 9.0, 7.1, 3.9 Hz, 1H), 3.10 (m, 4H), 3.45 (td, *J* = 6.6, 2.9 Hz, 2H), 3.82 (ddd, *J* = 9.5, 7.6, 4.2 Hz, 1H), 3.82 (s, 3H), 6.79–7.02 (m, 4H). ^{13}C NMR δ 16.9, 23.3, 25.9, 26.1, 30.2, 33.8, 38.4, 46.1, 50.0, 53.1, 55.3, 57.8, 75.1, 111.1, 118.3, 120.9, 123.0, 140.9, 152.2, 161.2, 176.5. Anal. ($C_{24}H_{36}N_4O_3$·2HCl) C, H, N.

6.1.8. (±)-7*a*-Butyl-2-[4-[4-(*o*-methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2f). From **2a** and *n*-BuI following the general procedure was obtained **2f**. Yield = 60%. Oil. R_f = 0.2 (EtOAc:EtOH, 9:1). IR ($CHCl_3$) 2940, 1765, 1700, 1595, 1500, 1445, 1415, 1240, 1120, 1030, 735 cm^{-1}. ^1H NMR δ 0.85 (t, *J* = 6.6 Hz, 3H), 1.06–1.38 (m, 4H), 1.47–1.68 (m, 5H), 1.75–2.14 (m, 5H), 2.39 (t, *J* = 7.3 Hz, 2H), 2.62 (m, 4H), 3.05 (m, 4H), 3.15 (ddd, *J* = 11.7, 7.8, 6.1 Hz, 1H), 3.45 (td, *J* = 6.8, 2.0 Hz, 2H), 3.74 (ddd, *J* = 11.7, 8.1, 6.6 Hz, 1H), 3.82 (s, 3H), 6.80–7.01 (m, 4H). ^{13}C NMR δ 13.9, 22.6, 24.0, 26.0, 26.1, 33.1, 34.9, 38.8, 44.8, 50.6, 53.4, 55.3, 58.1, 72.4, 111.1, 118.2, 121.0, 122.8, 141.4, 152.3, 160.7, 176.4. Anal. ($C_{25}H_{38}N_4O_3$·2HCl·1/2H_2O) C, H, N.

6.1.9. (±)-7*a*-*Iso*butyl-2-[4-[4-(*o*-methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2g). From **2a** and *i*-BuI following the general procedure was obtained **2g**. Yield = 44%. Oil. R_f = 0.2 (EtOAc:EtOH, 9.5:0.5). IR ($CHCl_3$) 3020, 2825, 1765, 1705, 1595, 1500, 1445, 1415, 1370, 1360, 1240, 1215, 1180, 1030, 760 cm^{-1}. ^1H NMR δ 0.83 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.4 Hz, 3H), 1.41–2.13 (m, 11H), 2.41 (t, *J* = 7.2 Hz, 2H), 2.62 (m, 4H), 3.06 (m, 4H), 3.17 (ddd,

J = 11.5, 7.6, 6.1 Hz, 1H), 3.48 (td, J = 6.6, 2.4 Hz, 2H), 3.75 (ddd, J = 11.7, 7.9, 4.2 Hz, 1H), 3.83 (s, 3H), 6.78–7.01 (m, 4H). ^{13}C NMR δ 23.5, 23.8, 23.9, 24.9, 25.8, 26.0, 34.3, 38.8, 42.7, 44.5, 50.5, 53.3, 55.3, 58.0, 72.1, 111.1, 118.2, 120.9, 122.8, 141.3, 152.2, 160.4, 176.5. Anal. ($C_{25}H_{38}N_4O_3$·HCl) C, H, N.

6.1.10. (±)-2-[4-[4-(*o*-Methoxyphenyl)piperazin-1-yl]-butyl]-7*a*-pentyl-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2h). From **2a** and *n*-$C_5H_{11}I$ following the general procedure was obtained **2h**. Yield = 48%. Oil. R_f = 0.2 (EtOAc:EtOH, 9:1). IR ($CHCl_3$) 3020, 2945, 1765, 1700, 1595, 1500, 1445, 1420, 1245, 1215, 760 cm^{-1}. ^{1}H NMR δ 0.83 (t, J = 6.3 Hz, 3H), 1.11–1.36 (m, 6H), 1.41–1.69 (m, 5H), 1.77–2.14 (m, 5H), 2.39 (t, J = 7.2 Hz, 2H), 2.62 (m, 4H), 3.05 (m, 4H), 3.15 (ddd, J = 11.7, 7.8, 6.1 Hz, 1H), 3.45 (td, J = 6.8, 2.4 Hz, 2H), 3.74 (ddd, J = 11.5, 7.8, 6.8 Hz, 1H), 3.83 (s, 3H), 6.81–7.01 (m, 4H). ^{13}C NMR δ 13.9, 22.4, 23.5, 24.0, 26.0, 26.1, 31.6, 33.0, 35.0, 38.8, 44.8, 50.6, 53.4, 55.3, 58.0, 72.4, 111.1, 118.2, 121.0, 122.8, 141.4, 152.2, 160.6, 176.4. Anal. ($C_{26}H_{40}N_4O_3$·HCl·1/2H_2O) C, H, N.

6.1.11. (±)-7*a*-*Iso*pentyl-2-[4-[4-(*o*-methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2i). From **2a** and *i*-$C_5H_{11}I$ following the general procedure was obtained **2i**. Yield = 65%. Oil. R_f = 0.2 (EtOAc:EtOH, 9:1). IR ($CHCl_3$) 3020, 2950, 1765, 1700, 1595, 1500, 1450, 1415, 1245, 1215, 1120, 760 cm^{-1}. ^{1}H NMR δ 0.84 (d, J = 6.6 Hz, 6H), 0.98 (ddd, J = 12.2, 6.6, 4.9 Hz, 1H), 1.12–1.30 (m, 2H), 1.42–1.69 (m, 6H), 1.78–1.86 (m, 3H), 1.91–2.11 (m, 1H), 2.41 (t, J = 7.2 Hz, 2H), 2.62 (t, J = 4.2 Hz, 4H), 3.02 (t, J = 4.2 Hz, 4H), 3.15 (ddd, J = 11.7, 7.6, 6.1 Hz, 1H), 3.46 (td, J = 6.6, 2.7 Hz, 2H), 3.74 (ddd, J = 11.7, 8.1, 6.6 Hz, 1H), 3.83 (s, 3H), 6.80–7.01 (m, 4H). ^{13}C NMR δ 22.4, 22.5, 23.9, 26.0, 26.1, 28.0, 32.6, 33.0, 33.1, 38.8, 44.8, 50.5, 53.3, 55.3, 58.0, 72.4, 111.2, 118.2, 121.0, 122.9, 141.3, 152.3, 160.7, 176.5. Anal. ($C_{26}H_{40}N_4O_3$·HCl·1/2H_2O) C, H, N.

6.1.12. (±)-7*a*-Hexyl-2-[4-[4-(*o*-methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2j). From **2a** and *n*-$C_6H_{13}I$ following the general procedure was obtained **2j**. Yield = 60%. Oil. R_f = 0.2 (EtOAc:EtOH, 9:1). IR ($CHCl_3$) 3015, 2940, 2825, 1765, 1705, 1595, 1500, 1445, 1415, 1240, 1215, 1030 cm^{-1}. ^{1}H NMR δ 0.84 (t, J = 6.8 Hz, 3H), 1.23 (m, 8H), 1.60 (m, 4H), 1.78–1.92 (m, 4H), 1.97–2.12 (m, 2H), 2.46 (m, 2H), 2.67 (m, 4H), 3.10 (m, 4H), 3.10–3.23 (m, 1H), 3.47 (m, 2H), 3.75 (dt, J = 11.7, 8.0 Hz, 1H), 3.84 (s, 3H), 6.82–7.03 (m, 4H). ^{13}C NMR δ 14.0, 22.5, 23.8, 26.0, 29.1, 31.6, 33.0, 35.1, 38.0, 44.8, 50.3, 53.3, 55.3, 58.0, 72.4, 111.2, 118.3, 121.0, 123.0, 141.0, 152.3, 160.0, 176.5. Anal. ($C_{27}H_{42}N_4O_3$·HCl·2H_2O) C, H, N.

6.1.13. (±)-7*a*-Heptyl-2-[4-[4-(*o*-methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2k). From **2a** and *n*-$C_7H_{15}I$ following the general procedure was obtained **2k**. Yield = 53%. Oil. R_f = 0.3 (EtOAc:EtOH, 9:1). IR ($CHCl_3$) 2930, 2855, 2815, 1770, 1710, 1500, 1440, 1415, 1180, 1030 cm^{-1}. ^{1}H NMR δ 0.84 (t, J = 6.7 Hz, 3H), 1.15–1.33 (m, 10H), 1.44–1.88 (m, 8H), 1.94–2.15 (m, 2H), 2.40 (t, J = 7.3 Hz, 2H), 2.61 (m, 4H), 3.06 (m, 4H), 3.16 (ddd, J = 13.7, 7.6, 6.1 Hz, 1H), 3.47 (td, J = 6.8, 2.7 Hz, 2H), 3.74 (dt, J = 14.6, 7.6 Hz, 1H), 3.84 (s, 3H), 6.81–7.02 (m, 4H). ^{13}C NMR δ 14.1, 22.6, 23.9, 24.0, 26.0 26.1, 29.1, 29.4, 31.7, 33.0, 33.1, 35.1, 38.8, 44.8, 50.6, 53.4, 55.3, 58.1, 72.4, 111.1, 118.2, 121.0, 122.8, 141.4, 152.3, 160.7, 176.5. Anal. ($C_{28}H_{44}N_4O_3$·2HCl·H_2O) C, H, N.

6.1.14. (±)-7*a*-Allyl-2-[4-[4-(*o*-methoxyphenyl)piperazin-1-yl]butyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (2l). From **2a** and allyl bromide following the general procedure was obtained **2l**. Yield = 69%. Oil. R_f = 0.3 (EtOAc:EtOH, 9:1). IR ($CHCl_3$) 2945, 2820, 2250, 1770, 1705, 1500, 1415, 1445, 1240, 910 cm^{-1}. ^{1}H NMR δ 1.47–1.68 (m, 5H), 1.82–2.15 (m, 3H), 2.36–2.44 (m, 2H), 2.51–2.61 (m, 2H), 2.62 (m, 4H), 3.07 (m, 4H), 3.18 (ddd, J = 11.5, 7.1, 5.7 Hz, 1H), 3.41–3.50 (m, 2H), 3.69–3.84 (m, 1H), 3.84 (s, 3H), 5.09–5.19 (m, 2H), 5.58–5.70 (m, 1H), 6.81–7.02 (m, 4H). ^{13}C NMR δ 23.8, 26.1, 32.2, 38.8, 39.5, 45.1, 50.5, 53.3, 55.3, 58.0, 72.1, 111.1, 118.2, 120.2, 121.0, 122.9, 131.2, 141.3, 152.3, 160.4, 175.9. Anal. ($C_{24}H_{34}N_4O_3$·HCl·2H_2O) C, H, N.

6.1.15. (±)-2-[4-(*o*-Methoxyphenyl)piperazin-1-yl]methyl]-7*a*-methyl-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (3b). From **3a** and MeI following the general procedure was obtained **3b**. Yield = 49%. Oil. R_f = 0.2 (EtOAc:Hexane, 1:1). IR ($CHCl_3$) 3020, 1770, 1705, 1595, 1500, 1450, 1310, 1295 cm^{-1}. ^{1}H NMR δ 1.45 (s, 3H, 7*a*-CH_3), 1.76–1.92 (m, 2H, $2H_6$), 2.02–2.20 (m, 2H, $2H_7$), 2.83 (t, J = 4.5 Hz, 4H, 2 $CH_{2\text{-PIPERAZINE}}$), 3.04 (m, 4H, 2 $CH_{2\text{-PIPERAZINE}}$), 3.25 (ddd, J = 11.1, 8.1, 5.1 Hz, 1H, H_5), 3.72 (dt, J = 11.4, 7.5 Hz, 1H, H_5), 3.82 (s, 3H,-OCH_3), 4.50 (s, 2H, N-CH_2-N), 6.81–7.01 (m, 4H, Ar-H). ^{13}C NMR δ 22.1 (7*a*-CH_3), 25.9 (C_6), 33.7 (C_7), 44.6 (C_5), 50.5 (2 $CH_{2\text{-PIPERAZINE}}$), 50.6 (2 $CH_{2\text{-PIPERAZINE}}$), 55.2 (-OCH_3), 60.4 (N-CH_2-N), 69.1 (C_{7a}), 110.9 (Ar-C_6), 118.2 (Ar-C_3), 120.9 (Ar-C_4), 123.0 (Ar-C_5), 141.1 (Ar-C_1), 152.1 (Ar-C_2), 160.5 (C_3), 178.0 (C_1). Anal. ($C_{19}H_{26}N_4O_3$·2HCl·H_2O) C, H, N.

6.1.16. (±)-7*a*-Ethyl-2-[4-(*o*-methoxyphenyl)piperazin-1-yl]methyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (3c). From **3a** and EtI following the general procedure was obtained **3c**. Yield = 48%. Oil. R_f = 0.3 (EtOAc:Hexane, 2:1). IR ($CHCl_3$) 3055, 1770, 1710, 1595, 1500, 1420, 1375, 1295 cm^{-1}. ^{1}H NMR δ 0.85 (t, J = 7.3 Hz, 3H), 1.57–1.74 (m, 1H), 1.81–1.95 (m, 2H), 1.98–2.15 (m, 5H), 2.81 (t, J = 4.8 Hz, 4H), 3.05 (t, J = 4.8 Hz, 4H), 3.17 (ddd, J = 11.5, 6.8, 4.7 Hz, 1H), 3.78 (dt, J = 11.9, 7.3 Hz, 1H), 3.81 (s, 3H), 4.49 (s, 2H), 6.81–7.01 (m, 4H). ^{13}C NMR δ 8.5, 25.9, 27.9, 32.7, 44.7, 50.4, 50.5, 55.1, 60.3, 73.0, 111.2, 118.1, 120.9, 122.8, 141.1, 152.0, 161.2, 177.4. Anal. ($C_{20}H_{28}N_4O_3$·2HCl·2H_2O) C, H, N.

6.1.17. (±)-2-[4-(*o*-Methoxyphenyl)piperazin-1-yl]methyl]-7*a*-propyl-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (3d). From **3a** and EtI following the general procedure was obtained **3d**. Yield = 57%. Oil. R_f = 0.2 (EtOAc:Hexane, 2:1). IR ($CHCl_3$) 3020, 2960, 1770, 1710, 1595, 1500, 1450, 1430, 1350, 1010 cm^{-1}. ^{1}H NMR δ 0.91 (t, J = 6.5 Hz, 3H), 1.10–1.65 (m, 4H), 1.72–1.88 (m, 2H), 1.92–2.12 (m, 2H), 2.80 (t, J = 4.8 Hz, 4H), 3.05 (t, J = 4.8 Hz,

4H), 3.19 (ddd, $J = 11.8$, 7.0, 5.5 Hz, 1H), 3.80 (dt, $J = 11.8$, 6.9 Hz, 1H), 3.81 (s, 3H), 4.50 (s, 2H), 6.80–7.04 (m, 4H). ^{13}C NMR δ 14.0, 17.5, 25.9, 33.2, 37.2, 44.8, 50.6, 50.7, 55.3, 60.5, 72.7, 111.4, 118.2, 121.0, 122.9, 141.2, 152.2, 161.3, 177.6. Anal. ($C_{21}H_{30}N_4O_3 \cdot 2HCl \cdot 2H_2O$) C, H, N.

6.1.18. (±)-7*a*-Butyl-2-[4-(*o*-methoxyphenyl)piperazin-1-yl]methyl]-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (3e). From **3a** and *n*-BuI following the general procedure was obtained **3e**. Yield = 52%. Oil. $R_f = 0.3$ (EtOAc:Hexane, 2:1). IR ($CHCl_3$) 3020, 2960, 1770, 1710, 1595, 1500, 1450, 1430, 1330, 1240, 1220, 1160, 1010, 760 cm^{-1}. ^{1}H NMR δ 0.84 (t, $J = 6.8$ Hz, 3H), 1.12–1.41 (m, 4H), 1.52–1.67 (m, 1H), 1.81–1.95 (m, 2H), 1.99–2.12 (m, 3H), 2.75 (t, $J = 4.8$ Hz, 4H), 2.99 (t, $J = 4.8$ Hz, 4H), 3.14 (ddd, $J = 11.7$, 6.7, 5.1 Hz, 1H), 3.71 (ddd, $J = 11.7$, 8.1, 6.8 Hz, 1H), 3.75 (s, 3H), 4.42 (s, 2H), 6.71–6.98 (m, 4H). ^{13}C NMR δ 13.8, 22.6, 25.9, 26.2, 33.1, 34.7, 44.7, 50.5, 50.6, 55.2, 60.5, 72.6, 111.3, 118.2, 120.9, 122.9, 141.2, 152.2, 161.2, 177.6. Anal. ($C_{22}H_{32}N_4O_3 \cdot 2HCl \cdot 1/2H_2O$) C, H, N.

6.1.19. (±)-2-[4-(*o*-Methoxyphenyl)piperazin-1-yl]methyl]-7*a*-pentyl-1,3-dioxoperhydropyrrolo[1,2-*c*]imidazole (3f). From **3a** and *n*-$C_5H_{11}I$ following the general procedure was obtained **3f**. Yield = 56%. Oil. $R_f = 0.3$ (EtOAc: Hexane, 2:1). IR ($CHCl_3$) 3020, 2930, 1770, 1705, 1597, 1500, 1425, 1120, 1010 cm^{-1}. ^{1}H NMR δ 0.83 (t, $J = 6.2$ Hz, 3H), 1.20–1.30 (m, 6H), 1.50–1.62 (m, 1H), 1.78–1.91 (m, 2H), 2.00–2.08 (m, 3H), 2.79 (t, $J = 4.7$ Hz, 4H), 3.03 (m, 4H), 3.15 (ddd, $J = 11.7$, 7.8, 6.0 Hz, 1H), 3.76 (ddd, $J = 10.8$, 6.9, 5.4 Hz, 1H), 3.79 (s, 3H), 4.46 (s, 2H), 6.81–7.01 (m, 4H). ^{13}C NMR δ 13.9, 22.3, 23.7, 25.9, 31.5, 33.1, 34.9, 44.8, 50.5, 50.6, 55.1, 60.4, 72.6, 111.2, 118.2, 120.9, 122.8, 141.2, 152.1, 161.1, 177.6. Anal. ($C_{23}H_{32}N_4O_3 \cdot 2HCl \cdot 2H_2O$) C, H, N.

6.2. Biochemistry

6.2.1. Radioligand binding assays. Male Sprague–Dawley rats (*Rattus norvegicus albinus*), weighing 180–200 g, were killed by decapitation and the brains rapidly removed and dissected. Tissues were stored at −80 °C for subsequent use. Membrane suspensions were centrifugated on a Beckman XL-90.

6.2.2. 5-HT$_{1A}$ Receptor. Binding assays were performed by a modification of the procedure previously described by Clark et al.[26] The cerebral cortex was homogenized in 10 volumes of ice-cold Tris buffer (50 mM Tris–HCl, pH 7.7 at 25 °C) and centrifuged at 28,000 *g* for 15 min. The membrane pellet was washed twice by resuspension and centrifugation. After the second wash the resuspended pellet was incubated at 37 °C for 10 min. Membranes were then collected by centrifugation and the final pellet was resuspended in 50 mM Tris–HCl, 5 mM $MgSO_4$, and 0.5 mM EDTA buffer (pH 7.4 at 37 °C). Fractions of 100 μL of the final membrane suspension (about 1 mg of protein) were incubated at 37 °C for 15 min with 0.6 nM [^{3}H]-8-OH-DPAT (133 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 1.1 mL of assay buffer (50 mM Tris–HCl, 10 nM clonidine, 30 nM prazosin, pH 7.4 at 37 °C). Nonspecific binding was determined with 10 μM 5-HT.

6.2.3. α_1 Receptor. Binding assays were performed by a modification of the procedure previously described by Ambrosio et al.[27] The cerebral cortex was homogenized in 20 volumes of ice-cold buffer (50 mM Tris–HCl, 10 mM $MgCl_2$, pH 7.4 at 25 °C) and centrifuged at 30,000 *g* for 15 min. Pellets were washed twice by resuspension and centrifugation. Final pellets were resuspended in the same buffer. Fractions of the final membrane suspension (about 250 μg of protein) were incubated at 25 °C for 30 min with 0.2 nM [^{3}H]prazosin (23 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 2 mL of buffer. Nonspecific binding was determined with 10 μM phentolamine.

Competing drug, nonspecific, total and radioligand bindings were defined in triplicate. Incubation was terminated by rapid vacuum filtration through Whatman GF/B filters, presoaked in 0.05% poly(ethylenimine), using a Brandel cell harvester. The filters were then washed with the assay buffer, dried and placed in poly(ethylene) vials to which were added 4 mL of a scintillation cocktail (Aquasol). The radioactivity bound to the filters was measured by liquid scintillation spectrometry on a Packard 2500 TR instrument. The data were analyzed by an iterative curve-fitting procedure (program Prism, Graph Pad), which provided IC_{50}, K_i, and r^2 values for test compounds, K_i values being calculated from the Cheng and Prusoff equation.[28] The protein concentrations of the rat cerebral cortex and the rat striatum were determined by the method of Lowry,[29] using bovine serum albumin as the standard.

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