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Benzimidazole derivatives. Part 5: Design and synthesis of new benzimidazole–arylpiperazine derivatives acting as mixed 5-HT_{1A}/5-HT_3 ligands

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Dedicated to Professor José L. Soto for a whole life devoted to Organic Chemistry

Abstract—A series of new mixed benzimidazole–arylpiperazine derivatives were designed by incorporating in general structure **III** the pharmacophoric elements of 5-HT_{1A} and 5-HT_3 receptors. Compounds **1**–**11** were synthesized and evaluated for binding affinity at both serotoninergic receptors, all of them exhibiting high 5-HT_3R affinity (K_i = 10–62 nM), and derivatives with an *o*-alkoxy group in the arylpiperazine ring showing nanomolar affinity for the 5-HT_{1A}R (K_i = 18–150 nM). Additionally, all the synthesized compounds were selective over α_1-adrenergic and dopamine D_2 receptors (K_i>1000–10,000 nM). Compound **3** was selected for further pharmacological characterization due to its interesting binding profile as mixed 5-HT_{1A}/5-HT_3 ligand with high affinity for both receptors (5-HT_{1A}: K_i = 18.0 nM, 5-HT_3: K_i = 27.2 nM). In vitro and in vivo findings suggest that this compound acts as a partial agonist at 5-HT_{1A}Rs and as a 5-HT_3R antagonist. This novel mixed 5-HT_{1A}/5-HT_3 ligand was also effective in preventing the cognitive deficits induced by muscarinic receptor blockade in a passive avoidance learning test, suggesting a potential interest in the treatment of cognitive dysfunction.

1. Introduction

The discovery of ligands with affinity for the family of serotonin receptors (5-HTRs) is an area of intense research in medicinal chemistry because of the potential to find new therapeutic drugs due to their involvement in numerous physiological and pathophysiological processes.[1–4] At present seven classes of serotonin receptors (5-$HT_{1–7}$) including fourteen subtypes have been found,[5,6] and most of them belong to the superfamily of G protein-coupled receptors (GPCRs); only the 5-HT_3R is a ligand-gated cation channel receptor.[7] Among 5-HTRs, the 5-HT_{1A} subtype is the best studied and it is generally accepted that it is involved in psychiatric disorders[8–11] such as anxiety, depression and memory loss. On the other hand, the 5-HT_3 subtype is present within the central and peripheral nervous systems, and antagonists of this receptor are of special interest not only because of their wide clinical use as antiemetic agents in cancer patients,[12,13] but also due to their promising therapeutic potential in the treatment of CNS disorders[14–16] such as anxiety, drug abuse and withdrawal, and cognitive dysfunction. In light of the potential utility in anxiety and cognitive disorders of 5-HT_{1A} and 5-HT_3 receptor ligands, it would be of interest, in principle, the development of compounds with affinity at both 5-HTR subtypes.

In the course of a wide program aimed at the discovery of new serotoninergic agents, we have synthesized a series of arylpiperazines[17–21] **I** with high affinity for 5-HT_{1A}Rs in which QSAR studies[22,23] and computational simulations[23–25] have allowed us to determine the structural elements that are optimal for 5-HT_{1A}R–ligand

Keywords: Serotonin ligands; 5-HT_{1A} receptor; 5-HT_3 receptor; Benzimidazole derivatives; Arylpiperazines; Cognitive dysfunction.
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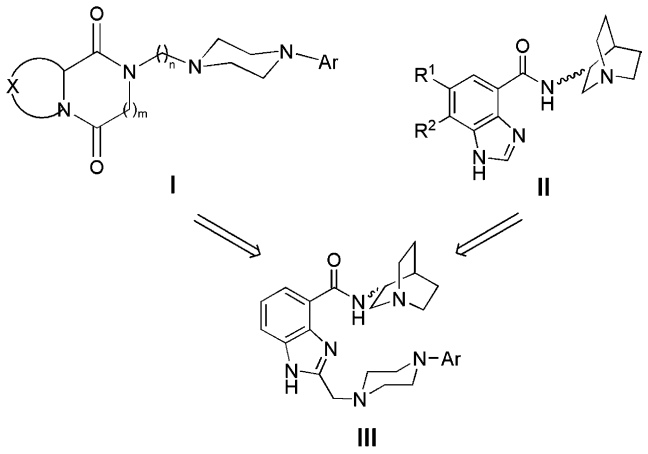
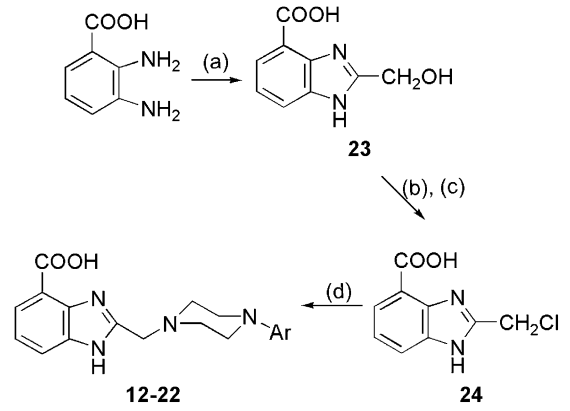


Figure 1. Structure of compounds **I**–**III**.

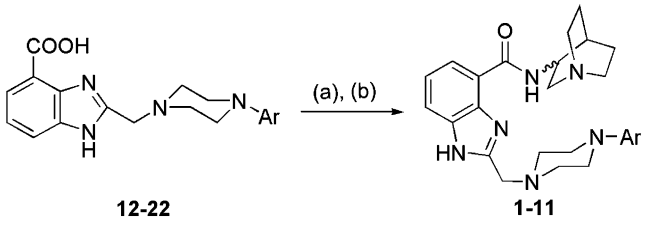


Scheme 2. Reagents and conditions: (a) $HOCH_2COOH$, HCl; (b) $SOCl_2$, 80 °C; (c) HCl/H_2O, Δ; (d) arylpiperazine, acetonitrile, NEt_3, 60 °C.

interaction, as well as a class of azabicyclic benzimidazole derivatives **II** characterized as potent and selective 5-HT_3R antagonists[26,27] (Fig. 1). In the present work we have designed a series of new mixed benzimidazole–arylpiperazines of general structure **III**[28] in which we have incorporated the structural elements of 5-HT_{1A} and 5-HT_3 pharmacophores[29,30] (Fig. 1). Herein we report the synthesis of designed compounds **1**–**11** and their affinities for serotoninergic 5-HT_{1A} and 5-HT_3, adrenergic α_1 and dopaminergic D_2 receptors, obtained by radioligand binding assays. Among them, analogue **3** was selected for further pharmacological characterization: functional activity at 5-HT_{1A} and 5-HT_3 receptors, and behavioural effects in anxiety and cognitive dysfunction models.

2. Chemistry

The general procedure for the preparation of target compounds **III** is shown in Scheme 1. Starting benzimidazolecarboxylic acids **12**–**22** were suitably activated with 1,1′-carbonyldiimidazole (CDI), and subsequently coupled with (±)-3-aminoquinuclidine in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in *N*,*N*-dimethylformamide (DMF) solutions, to afford the desired amides **1**–**11**. 2-[(4-Arylpiperazin-1-yl)methyl]-benzimidazole-4-carboxylic acids **12**–**22** were obtained by reaction of 2-(chloromethyl)benzimidazole-4-carboxylic acid (**24**) with the corresponding arylpiperazines in the presence of NEt_3 (Scheme 2). The intermediate **24** was prepared by condensation of 2,3-diaminobenzoic acid with glycolic acid followed by treatment of 2-(hydroxymethyl)benzimidazole-4-carboxylic acid (**23**) with $SOCl_2$ and hydrolysis in acidic conditions (Scheme 2). As for intermediate arylpiperazines, those with Ar = phenyl, *o*-methoxyphenyl, *m*-(trifluoromethyl)phenyl, and *m*-chlorophenyl were commercial, whereas those with Ar = *o*-ethoxyphenyl, *o*-propoxyphenyl, *o*-*iso*propoxyphenyl, *o*-butoxyphenyl, naphth-1-yl, 2,3-dihydro-1,4-benzodioxan-5-yl, and 1-tritylbenzimidazol-4-yl were synthesized according to previously described methods (see Experimental Section).



Scheme 1. Reagents and conditions: (a) CDI, DMF, 40 °C; (b) (±)-3-aminoquinuclidine, DBU, DMF, 50 °C.

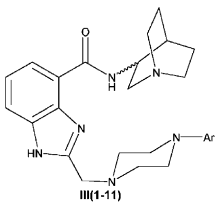
All new compounds were characterized by IR and ^1H and ^{13}C NMR spectroscopy and gave satisfactory combustion analyses (C, H, N).

3. Biological results and discussion

3.1. Affinity data

Target compounds **1**–**11** were assessed for in vitro affinity at serotoninergic 5-HT_{1A} and 5-HT_3 receptors by radioligand binding assays, using [^{3}H]-8-OH-DPAT and [^{3}H]LY 278584, respectively, in rat cerebral cortex membranes. The ligands were also evaluated for in vitro affinity at α_1-adrenergic receptors ([^{3}H]prazosin) and dopamine D_2 receptors ([^{3}H]raclopride), in rat cerebral cortex and striatum membranes, respectively. In the experimental binding assays the compounds were first tested at fixed dose of 10^{-6} M, and for those that in the screening process presented high affinity (displacement of the radioligand ⩾55%), the dose–response curves were determined. The affinity constants obtained for the tested compounds are listed in Table 1. All the synthesized compounds **1**–**11** exhibited high 5-HT_3R affinity (K_i = 10−62 nM), and derivatives with an *o*-alkoxy group in the arylpiperazine ring have shown nanomolar affinity for the 5-HT_{1A}R (K_i = 18−150 nM). Only analogue **11** is an exception in the series (5-HT_{1A}: K_i = 148 nM, 5-HT_3: K_i>1000 nM). The weak 5-HT_{1A}R affinity of derivative **9** was unexpected, according to reported data for arylpiperazines bearing a benzodioxan-5-yl group.[25,31] Additionally, all the

Table 1. Binding data of compounds **III (1–11)**



Compd	Ar	K_i (nM)	
		5-HT_{1A}	5-HT_3
1	Phenyl	>1000	23.1 ± 1.5
2	*o*-Methoxyphenyl	150 ± 33	10.3 ± 1.1
3	*o*-Ethoxyphenyl	18.0 ± 1.7	27.2 ± 0.9
4	*o*-Propoxyphenyl	56.1 ± 2.2	24.6 ± 3.2
5	*o*-*iso*Propoxyphenyl	34.4 ± 3.0	62.1 ± 1.3
6	*o*-Butoxyphenyl	45.9 ± 3.5	15.1 ± 4.8
7	*m*-(Trifluoromethyl)phenyl	>10,000	23.9 ± 2.9
8	*m*-Chlorophenyl	>10,000	18.3 ± 0.4
9	Benzodioxan-5-yl	467 ± 14	24.0 ± 1.0
10	Naphth-1-yl	>1000	32.5 ± 5.3
11	Benzimidazol-4-yl	148 ± 7	>1000

Values are means ± SEM from 2–4 separate experiments performed in triplicate.

compounds were selective over α_1-adrenergic and dopamine D_2 receptors (K_i> 1000–10,000 nM). Compound **3** was selected for further pharmacological characterization due to its interesting binding profile as mixed 5-HT_{1A}/5-HT_3 ligand with high affinity for both receptors (5-HT_{1A}: K_i = 18.0 nM, 5-HT_3: K_i = 27.2 nM).

3.2. Functional characterization of compound 3 at 5-HT_{1A} and 5-HT_3 receptors

The ability of compound **3** to increase binding of [^{35}S]GTPγS to rat hippocampal membranes as well as the antagonism to binding stimulated by the 5-HT_{1A}R agonist 8-OH-DPAT were evaluated. The effect of different concentrations of **3** on [^{35}S]GTPγS binding to hippocampal membranes is shown in Figure 2A. Compound **3** (10^{-10}–10^{-7} M) induced minimal effects on the basal binding of the GTP analogue. Only at a high concentration (10^{-6} M) a moderate increase in basal binding (19%) was observed, and no further enhancement in basal binding was found after a 10-fold higher concentration, 10^{-5} M (not shown). This was in contrast to the marked stimulation observed with the typical 5-HT_{1A}R agonist 8-OH-DPAT (92% at 10^{-6} M). On the other hand, as shown in Figure 2B, compound **3** only inhibited the stimulation of [^{35}S]GTPγ binding induced by 8-OH-DPAT at the low concentrations of 10^{-10} and 10^{-9} M but this effect was not concentration-dependent, and was much weaker than that of the 5-HT_{1A}R antagonist WAY-100635 (IC_{50} = 6 nM).

Since agonists at 5-HT_{1A}Rs consistently induce hypothermia in rodents,[32] the intrinsic effect of compound **3** on rectal temperature and on the hypothermia response to the 5-HT_{1A}R agonist 8-OH-DPAT was studied in mice. Compound **3** (1 mg/kg) also reduced body temperature in mice by approximately 1 °C at 60 and 120 min after administration, but only a slightly higher effect was found when increasing the dose by 10-fold (Table 2). The hypothermic potency of 8-OH-DPAT was much more pronounced, and the effect of this 5-HT_{1A}R agonist was not prevented by compound **3** (Table 3). Altogether, in vitro and in vivo findings suggest that compound **3** is a partial agonist at 5-HT_{1A}Rs.

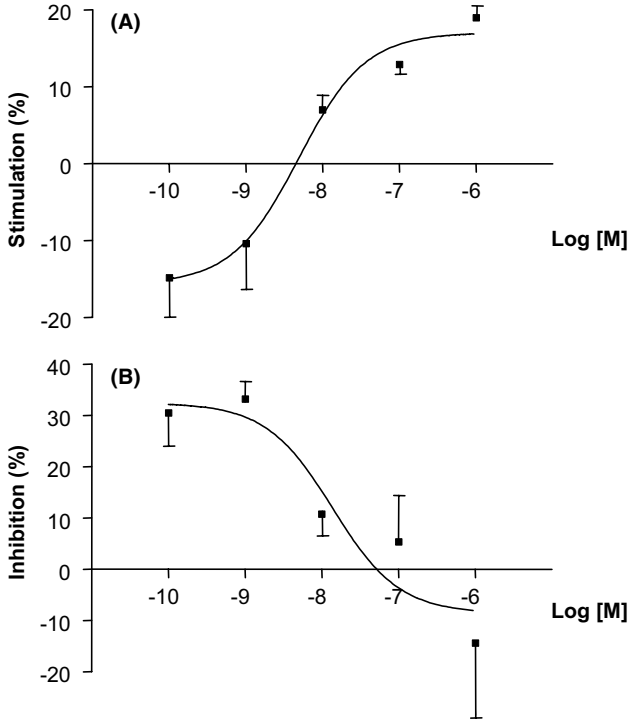


Figure 2. Stimulation of [^{35}S]GTPγS binding (**A**) and inhibition of 8-OH-DPAT-stimulated [^{35}S]GTPγS binding (**B**) in rat hippocampal membranes by compound **3**. Values are the means ± SEM from 5 separate experiments performed in duplicate.

Table 2. Effect of compound **3** on rectal temperature in mice

Dose (mg/kg sc)	Δ Temperature (°C)		
	30 min	60 min	120 min
1	−0.09 ± 0.1	−0.7 ± 0.2	−1.1 ± 0.2
10	−0.9 ± 0.1	−1.3 ± 0.3	−1.2 ± 0.2

Values are means ± SEM from 8 animals.

Table 3. Effect of compound **3** on 8-OH-DPAT-induced hypothermia in mice

Treatment	Δ Temperature (°C)		
	30 min	60 min	120 min
8-OH-DPAT (0.5 mg/kg)	−1.6 ± 0.2	−1.0 ± 0.2	−1.4 ± 0.4
3 (1 mg/kg) + 8-OH-DPAT	−2.2 ± 0.5	−1.5 ± 0.2	−1.6 ± 0.1
3 (10 mg/kg) + 8-OH-DPAT	−1.7 ± 0.2	−1.1 ± 0.1	−1.2 ± 0.1

Values are means ± SEM from 8 animals.

Table 4. Effect of compound **3** and ondansetron on 2-Me-5-HT-induced contraction in guinea pig LMMP

Compd	Concentration (M)	Inhibition (%)	IC_{50} (M)
Ondansetron	10^{-8}	9.6	2.4×10^{-7}
	10^{-7}	40.5	
	10^{-6}	95.4	
	10^{-5}	97.0	
3	10^{-8}	−31.3	1.9×10^{-7}
	10^{-7}	39.2	
	10^{-6}	90.0	
	10^{-5}	93.4	

Values are means ± SEM from 4–5 separate experiments.

The functional profile of compound **3** at 5-HT_3Rs was characterized in the isolated longitudinal muscle-myenteric plexus (LMMP) preparation from guinea-pig ileum. The contraction induced in this preparation by the 5-HT_3R agonist 2-Me-5-HT was inhibited by compound **3** in the concentrations range of 10^{-7}–10^{-5} M, a full blockade of the contractions being observed at the highest concentrations tested (Table 4). Similar effects were found in this preparation with the 5-HT_3R antagonist, ondansetron. The estimated IC_{50}'s for compound **3** and ondansetron were virtually identical, 0.19 and 0.24 μM, respectively.

3.3. Behavioural effects of compound 3

The anxiolytic-like effect of compound **3** was evaluated. As shown in Table 5, on the second day of exposure to the light–dark exploration test, control animals spent less time in the white compartment and the number of line crossings in this area also diminished. This behaviour was effectively prevented by administration of a typical anxiolytic drug such as diazepam (Table 5). However, no changes in behavioural parameters studied were observed after administration of compound **3**, indicating that this compound is not endowed with an anxiolytic-like effect in this test, at least at the doses used.

5-HT_{1A}R agonists may exert anxiolytic- or anxiogenic-like effects by acting on presynaptic regions such as the dorsal raphe nucleus or 5-HT terminal fields such as the hippocampus, respectively.[33] Bell-shaped dose–response curves with enormous differences in the effective dose–range have been reported, on the other hand, for 5-HT_3R antagonists.[34] These dual effects may explain the apparent lack of anxiolytic-like effect in the present study for compound **3**, a 5-HT_{1A}R partial agonist and 5-HT_3R antagonist.

Table 5. Anxiolytic-like effect of compound **3** and diazepam in the light–dark exploration test in mice

Treatment	Dose (mg/kg)	% Time in white area	% Locomotion in white area
Saline	—	35.8 ± 9.0	35.0 ± 7.5
Diazepam	1	72.7 ± 12.8[a]	78.5 ± 11.6[a]
Saline/tween80	—	69.3 ± 5.6	61.1 ± 6.7
Compound **3**	0.1	60.2 ± 7.3	56.3 ± 6.8
	1	65.8 ± 8.3	59.1 ± 5.7

Values are means ± SEM from 6–8 animals.
[a] $p<0.05$ versus the corresponding vehicle-treated controls (ANOVA followed by Dunnett's test).

Table 6. Effect of compound **3** on scopolamine-induced impairment of passive avoidance learning in rats

Treatment	Retention latency (s)
Control	291.8 ± 3.4
Compound **3** (1 mg/kg)	289.5 ± 8.2
Scopolamine (1 mg/kg)	23.9 ± 5.3[a]
Compound **3** + Scopolamine	204.5 ± 21.6[b]

Scopolamine and compound **3** given 30 and 45 min before the acquisition trial, respectively. Retention latency measured 24 h later. Values are means ± SEM from 10–20 animals.
[a] $p<0.05$ versus control.
[b] $p<0.05$ versus scopolamine-treated group (ANOVA followed by Student–Newman–Keuls test).

The effect of compound **3** on learning and retention was also evaluated. In the passive avoidance learning test, the muscarinic antagonist scopolamine, administered 30 min before the acquisition session, reduced significantly the retention latency 24 h later (Table 6). Compound **3**, given at the dose of 1 mg/kg, had no effect by itself in the passive avoidance test. However, when this compound was given 15 min before scopolamine it significantly prevented the retention impairment induced by cholinergic blockade, suggesting the potential interest of ligand **3** in the treatment of cognitive dysfunction.

4. Conclusion

In the present paper, we have designed and synthesized a series of new mixed benzimidazole–arylpiperazine derivatives with affinity for serotoninergic 5-HT_{1A} and 5-HT_3 receptors and selectivity over α_1-adrenergic and dopamine D_2 receptors. Among them, compound **3** was identified as a high-affinity 5-HT_{1A}/5-HT_3 ligand, acting as a partial agonist at 5-HT_{1A}Rs and a 5-HT_3R antagonist, and with ability to counteract the cognitive deficit induced by cholinergic blockade. Consequently, this novel mixed 5-HT_{1A}/5-HT_3 ligand could be a potential substrate to be developed for treatment of cognitive dysfunction, and this therapeutic option is currently under investigation.

5. Experimental

5.1. Chemistry

Melting points were determined on a Gallenkamp electrothermal apparatus. Infrared spectra (IR) were obtained on a FTIR-8300 Shimadzu spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Varian VXR-300S or Bruker 300-AM instrument at 300 and 75 MHz, respectively, or on a Bruker AC-200 spectrometer at 200 and 50 MHz, respectively. Chemical shifts (δ) are expressed in parts per million relative to internal tetramethylsilane, coupling constants (J) are in

hertz (Hz). The following abbreviations are used to describe peak patterns when appropriate: s (singlet), d (doublet), t (triplet), q (quartet), qt (quintet), sx (sextet), st (septet), m (multiplet), br (broad). Elemental analyses (C, H, N) were determined at the UCM's analysis services and were within ±0.4% of the theoretical values. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60 F-254) with detection by UV light, iodine, acidic vanillin solution, or 10% phosphomolybdic acid solution in ethanol. For flash chromato-graphy, Merck silica gel type 60 (size 230–400 mesh) was used. Unless stated otherwise, all starting materials and reagents were high-grade commercial products purchased from Aldrich, Fluka or Merck.

The following intermediates were synthesized according to described procedures: 2-(hydroxymethyl)benzimidazole-4-carboxylic acid (**23**),[35] 1-(*o*-ethoxyphenyl)piperazine,[36] 1-(*o*-propoxyphenyl)piperazine,[37] 1-(*o*-*iso*propoxyphenyl)piperazine,[36] 1-(*o*-butoxyphenyl)piperazine,[36] 1-(2,3-dihydro-1,4-benzodioxan-5-yl)piperazine,[38] 1-(naphth-1-yl)piperazine,[39] and 1-(1-tritylbenzimidazol-4-yl)piperazine.[40]

5.1.1. Synthesis of 2-(chloromethyl)benzimidazole-4-carboxylic acid (24). A solution of 2.0 g (8.7 mmol) of **23** in 45 mL of thionyl chloride was heated at 80 °C for 2 h. After removing the thionyl chloride by co-distillation with toluene under reduced pressure, the crude was taken up in 20 mL of 15% hydrochloric acid and the solution was refluxed for 15 min. The solvent was evaporated under vacuum to afford 2.0 g (95%) of **24** as a hydrochloride salt: mp 232–234 °C (MeOH/Et_2O); IR (KBr) 3420, 2900, 1720, 1575, 1510, 1440, 1245 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 5.24 (s, 2H, CH_2), 7.68 (t, $J = 7.5$, 1H, H_6), 8.11 (d, $J = 7.5$, 1H, H_5), 8.14 (d, $J = 7.8$, 1H, H_7); ^{13}C NMR (Me_2SO-d_6) δ 34.7 (CH_2), 117.0 (C_4), 120.8 (C_7), 124.9 (C_6), 127.3 (C_5), 130.8 (C_{3a}), 135.3 (C_{7a}), 150.8 (C_2), 165.7 (COOH).

5.1.2. General procedure for the synthesis of 2-[(4-arylpiperazin-1-yl)methyl]benzimidazole-4-carboxylic acids (12–22). A mixture of **24** (740 mg, 3 mmol), the appropriate 1-arylpiperazine (5 mmol), triethylamine (1.1 mL, 5 mmol), and acetonitrile (6 mL) was stirred at 60 °C for 20–24 h. After cooling, the solvent was removed under reduced pressure, and the crude was taken up in water and extracted with CH_2Cl_2 (3 × 30 mL). The organic layers were dried over Na_2SO_4 and evaporated to afford the crude product, which was purified by column chromatography and recrystallization from the appropriate solvent. Spectral data of all described compounds **12**–**22** were consistent with the proposed structures.

5.1.3. 2-[(4-Phenylpiperazin-1-yl)methyl]benzimidazole-4-carboxylic acid (12). From **24** and 1-phenylpiperazine following the general procedure was obtained **12**. Yield 504 mg (50%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 272–273 °C (d) (MeOH/Et_2O); IR (KBr) 3200, 1630, 1595, 1500, 1450, 1250 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.66–2.75 (m, 4H, $2CH_{2\text{-PIPERAZINE}}$), 3.12–3.20 (m, 4H, $2CH_{2\text{-PIPERAZINE}}$), 3.91 (s, 2H, CH_2), 6.79 (t, $J = 7.2$, 1H, Ph-H_4), 6.94 (d, $J = 8.4$, 2H, Ph-H_2, Ph-H_6), 7.22 (t, $J = 7.5$, 2H, Ph-H_3, Ph-H_5), 7.30 (t, $J = 7.8$, 1H, H_6), 7.81 (d, $J = 7.8$, 1H, H_5), 7.88 (d, $J = 7.8$, 1H, H_7); ^{13}C NMR (Me_2SO-d_6) δ 48.2 ($2CH_{2\text{- PIPERAZINE}}$), 52.5 ($2CH_{2\text{-PIPERAZINE}}$), 54.5 ($CH_2$), 115.4 (Ph-$C_2$,), 118.8 ($C_4$), 120.9 (Ph-$C_4$, C_6), 123.0 (C_7), 124.1 (C_5), 128.9 (Ph-C_3, Ph-C_5), 134.4 (C_{3a}), 143.5 (C_{7a}), 151.0 (Ph-C_1), 153.0 (C_2), 167.1 (COOH).

5.1.4. 2-[[4-(*o*-Methoxyphenyl)piperazin-1-yl]methyl]-benzimidazole-4-carboxylic acid (13). From **24** and 1-(*o*-methoxyphenyl)piperazine following the general procedure was obtained **13**. Yield 725 mg (66%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 186–187 °C (d) (MeOH/Et_2O); IR (KBr) 3210, 1620, 1590, 1560, 1520, 1500, 1450, 1255; ^1H NMR (Me_2SO-d_6) δ 2.58–2.70 (m, 4H), 2.87–3.00 (m, 4H), 3.74 (s, 3H), 3.86 (s, 2H), 6.80–6.95 (m, 4H), 7.20 (t, $J = 7.8$, 1H), 7.69–7.79 (m, 2H); ^{13}C NMR (Me_2SO-d_6) δ 50.1, 52.8, 54.8, 55.3, 111.9, 118.0, 120.7, 120.8, 121.7, 122.4, 123.8, 134.6, 141.2, 143.4, 152.0, 152.5, 168.4.

5.1.5. 2-[[4-(*o*-Ethoxyphenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxylic acid (14). From **24** and 1-(*o*-ethoxyphenyl)piperazine following the general procedure was obtained **14**. Yield 753 mg (66%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 145–147 °C (d) (MeOH/Et_2O); IR (KBr) 3200, 1620, 1600, 1500, 1480, 1450, 1245; ^1H NMR (Me_2SO-d_6) δ 1.35 (t, $J = 6.9$, 3H), 2.62–2.76 (m, 4H), 2.95–3.08 (m, 4H), 3.89 (s, 2H), 4.02 (q, $J = 6.9$, 2H), 6.84–6.98 (m, 4H), 7.24 (t, $J = 7.8$, 1H), 7.78 (d, $J = 7.7$, 2H); ^{13}C NMR (Me_2SO-d_6) δ 14.8, 49.9, 52.9, 54.8, 63.2, 113.1, 117.9, 118.1, 120.6, 120.8, 121.6, 122.2, 123.7, 134.5, 141.4, 143.3, 151.1, 152.5, 168.0.

5.1.6. 2-[[4-(*o*-Propoxyphenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxylic acid (15). From **24** and 1-(*o*-propoxyphenyl)piperazine following the general procedure was obtained **15**. Yield 793 mg (67%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 228–230 °C (d) (MeOH); IR (KBr) 3421, 1591, 1500, 1458, 1238; ^1H NMR (Me_2SO-d_6) δ 1.05 (t, $J = 6.9$, 3H), 1.76 (sx, $J = 6.8$, 2H), 2.62–2.78 (m, 4H), 2.95–3.08 (m, 4H), 3.88 (s, 2H), 3.95 (t, $J = 6.1$, 2H), 6.87–6.92 (m, 4H), 7.29 (t, $J = 7.5$, 1H), 7.81 (d, $J = 7.5$, 1H), 7.87 (d, $J = 8.0$, 1H); ^{13}C NMR (Me_2SO-d_6) δ 10.5, 22.1, 49.7, 52.7, 54.4, 68.8, 112.5, 115.1, 117.6, 120.5, 120.6, 121.9, 122.6, 123.7, 134.5, 141.0, 143.3, 151,0, 152.0, 166.8.

5.1.7. 2-[[4-(*o*-Isopropoxyphenyl)piperazin-1-yl]methyl]-benzimidazole-4-carboxylic acid (16). From **24** and 1-(*o*-*iso*propoxyphenyl)piperazine following the general procedure was obtained **16**. Yield 828 mg (70%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 188–191 °C (d) (MeOH); IR (KBr) 3436, 1590, 1496, 1450, 1238; ^1H NMR (Me_2SO-d_6) δ 1.23 (d, $J = 6.1$, 6H), 2.62–2.78 (m, 4H), 2.95–3.08 (m, 4H), 3.87 (s, 2H), 4.57 (st, $J = 6.1$, 1H), 6.87–6.92 (m, 4H), 7.20 (t, $J = 7.7$, 1H), 7.73 (d, $J = 7.6$, 1H), 7.74 (d,

$J = 7.8$, 1H); ^{13}C NMR (Me_2SO-d_6) δ 22.0, 49.9, 53.0, 54.7, 69.5, 116.1, 118.2, 120.8, 121.3, 122.1, 122.7, 124.0, 134.4, 142.5, 143.3, 149.7, 153.1, 167.3.

5.1.8. 2-[[4-(*o*-Butoxyphenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxylic acid (17). From **24** and 1-(*o*-butoxyphenyl)piperazine following the general procedure was obtained **17**. Yield 833 mg (68%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 139–141 °C (d) (CH_2Cl_2/Et_2O); ^{1}H NMR (Me_2SO-d_6) δ 0.92 (t, $J = 7.3$, 3H), 1.44 (sx, $J = 7.5$, 2H), 1,70 (qt, $J = 7.9$, 2H), 2.48–2.51 (m, 4H), 2.90–3.03 (m, 4H), 3.84 (s, 2H), 3.93 (t, $J = 6.2$, 2H), 6.33–6.89 (m, 4H), 7.19 (t, $J = 7.8$, 1H), 7.72 (d, $J = 7.9$, 2H); ^{13}C NMR (Me_2SO-d_6) δ 13.8, 19.0, 31.1, 50.1, 53.0, 54.8, 67.3, 113.0, 115.0, 118.0, 120.8, 120.9, 122.3, 122.4, 124.0, 134.1, 141.5, 143.1, 151.4, 153.1, 166.8.

5.1.9. 2-[[4-(*m*-(Trifluoromethyl)phenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxylic acid (18). From **24** and 1-(*m*-(trifluoromethyl)phenyl)piperazine following the general procedure was obtained **18**. Yield 619 mg (51%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 263–264 °C (d) (MeOH); IR (KBr) 3225, 1625, 1595, 1495, 1450, 1250; ^{1}H NMR (Me_2SO-d_6) δ 2.61–2.72 (m, 4H), 3.15–3.27 (m, 4H), 3.87 (s, 2H), 7.03 (d, $J = 7.6$, 1H), 7.13 (s, 1H), 7.16 (d, $J = 7.8$, 1H), 7.23 (t, $J = 7.8$, 1H), 7.38 (t, $J = 7.8$, 1H), 7.76 (d, $J = 7.3$, 1H), 7.80 (d, $J = 7.3$, 1H); ^{13}C NMR (Me_2SO-d_6) δ 47.7, 52.4, 54.5, 110.9 (q, $^3J_{C-F} = 3.5$), 114.6 (q, $^3J_{C-F} = 3.8$), 116.0, 118.8, 120.8, 122.6, 124.0, 124.5 (q, $^1J_{C-F} = 272.5$), 129.9 (q, $^2J_{C-F} = 30.9$), 130.0, 134.5, 143.5, 151.3, 152.8, 167.6.

5.1.10. 2-[[4-(*m*-Chlorophenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxylic acid (19). From **24** and 1-(*m*-chlorophenyl)piperazine following the general procedure was obtained **19**. Yield 623 mg (56%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 247–248 °C (d) (MeOH/Et_2O); IR (KBr) 3300, 1625, 1595, 1570, 1480, 1450, 1255; ^{1}H NMR (Me_2SO-d_6) δ 2.56–2.68 (m, 4H), 3.08–3.20 (m, 4H), 3.85 (s, 2H), 6.74 (d, $J = 7.9$, 1H), 6.83 (d, $J = 8.3$, 1H), 6.88 (s, 1H), 7.16 (t, $J = 8.0$, 1H), 7.21 (t, $J = 7.7$, 1H), 7.77 (d, $J = 7.7$, 2H); ^{13}C NMR (Me_2SO-d_6) δ 47.5, 52.2, 54.4, 113.5, 114.4, 117.5, 117.9, 120.6, 121.9, 123.8, 130.2, 133.7, 134.4, 143.8, 152.1, 152.4, 167.9.

5.1.11. 2-[[4-(Naphth-1-yl)piperazin-1-yl]methyl]benzimidazole-4-carboxylic acid (20). From **24** and 1-(naphth-1-yl)piperazine following the general procedure was obtained **20**. Yield 568 mg (49%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 236–238 °C (d) (MeOH); IR (KBr) 3225, 1620, 1590, 1575, 1520, 1510, 1490, 1455, 1255; ^{1}H NMR (Me_2SO-d_6) δ 2.77–2.90 (m, 4H), 2.99–3.10 (m, 4H), 3.95 (s, 2H), 7.10 (d, $J = 7.3$, 1H), 7.27 (t, $J = 7.8$, 1H), 7.40 (t, $J = 8.0$, 1H), 7.45–7.53 (m, 2H), 7.57 (d, $J = 8.0$, 1H), 7.80 (d, $J = 7.6$, 1H), 7.83–7.91 (m, 2H), 8.04–8.12 (m, 1H); ^{13}C NMR (Me_2SO-d_6) δ 52.5, 52.9, 114.5, 116.2, 120.8, 122.5, 123.0, 123.2, 124.0, 125.3, 125.8, 125.9, 128.0, 128.2, 134.2, 134.5, 143.3, 149.2, 152.9, 167.6.

5.1.12. 2-[[4-(2,3-Dihydro-1,4-benzodioxan-5-yl)piperazin-1-yl]methyl]benzimidazole-4-carboxylic acid (21). From **24** and 1-(2,3-dihydro-1,4-benzodioxan-5-yl)piperazine following the general procedure was obtained **21**. Yield 674 mg (57%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.06; mp 212–214 °C (d) (MeOH/Et_2O); IR (KBr) 3235, 1620, 1600, 1520, 1485, 1470, 1455, 1255; ^{1}H NMR (Me_2SO-d_6) δ 2.57–2.70 (m, 4H), 2.88–3.01 (m, 4H), 3.84 (s, 2H), 4.14–4.24 (m, 4H), 6.42 (d, $J = 7.8$, 1H), 6.46 (d, $J = 8.1$, 1H), 6.67 (t, $J = 8.1$, 1H), 7.16 (t, $J = 7.8$, 1H), 7.68 (d, $J = 7.8$, 2H); ^{13}C NMR (Me_2SO-d_6) δ 50.1, 52.6, 54.6, 63.7, 63.8, 110.2, 111.0, 117.6, 120.2, 120.7, 122.0, 123.8, 134.4, 136.2, 141.6, 143.4, 143.8, 152.5, 167.7.

5.1.13. 2-[[4-(1-Tritylbenzimidazol-4-yl)piperazin-1-yl]methyl]benzimidazole-4-carboxylic acid (22). From **24** and 1-(1-tritylbenzimidazol-4-yl)piperazine following the general procedure was obtained **22**. Yield 1.15 g (62%); chromatography CH_2Cl_2/EtOH/NH_3, from 9:1:0.05 to 8:2:0.01; mp 138–140 °C (d) (MeOH/hexane); IR (KBr) 3651–3384, 1654, 1593, 1556, 1490, 1446, 1269, 1234; ^{1}H NMR (Me_2SO-d_6) δ 2.62–2.65 (m, 4H), 3.20–3.30 (m, 4H), 3.78 (s, 2H), 5.84 (d, $J = 8.1$, 1H), 6.31 (d, $J = 7.7$, 1H), 6.63 (t, $J = 8.1$, 1H), 7.02–7.06 (m, 5H), 7.16 (t, $J = 7.2$, 1H), 7.23–7.26 (m, 10H), 7.69 (dd, $J = 11.5$, 7.1, 2H), 7.76 (s, 1H).

5.1.14. General procedure for the synthesis of (±)-*N*-(1-azabicyclo[2.2.2]oct-3-yl)-2-[(4-arylpiperazin-1-yl)methyl]-benzimidazole-4-carboxamides (1–11). To a solution of acids **12**–**22** (2.5 mmol) in dry DMF (2.5 mL) under an argon atmosphere was added 1,1′-carbonyldiimidazole (CDI, 400 mg, 2.5 mmol). The mixture was stirred at 40 °C for 1 h, then a solution of (±)-3-aminoquinuclidine (2.5 g, 20 mmol) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU, 380 mg, 2.5 mmol) in DMF (5 mL) was added dropwise, and the reaction mixture was stirred at 50 °C for 20–24 h. The solvent was removed under reduced pressure, and the crude was taken up in $CHCl_3$ (25 mL) and washed with water (10 mL) and 20% aqueous K_2CO_3 (10 mL). The organic layer was dried over Na_2SO_4 and evaporated to afford the crude product, which was purified by column chromatography and recrystallization from the appropriate solvents. Spectral data of all described compounds **1**–**11** were consistent with the proposed structures.

5.1.15. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[(4-phenylpiperazin-1-yl)methyl]benzimidazole-4-carboxamide (1). From **12** following the general procedure was obtained **1**. Yield 789 mg (71%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 146–147 °C ($CHCl_3$/Et_2O); IR (KBr) 3430, 3255, 1645, 1610, 1600, 1560, 1495, 1450 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.38–1.52 (m, 1H, $H_{5\text{-QUINUCLIDINE}}$ or $H_{8\text{-QUINUCLIDINE}}$), 1.54–1.68 (m, 2H, $2H_{5\text{-QUINUCLIDINE}}$ or $2H_{8\text{-QUINUCLIDINE}}$), 1.84–1.97 (m, 2H, $H_{5\text{-QUINUCLIDINE}}$ or $H_{8\text{-QUINUCLIDINE}}$, $H_{4\text{-QUINUCLIDINE}}$), 2.54–2.59 (m, 1H, $H_{2\text{-QUINUCLIDINE}}$), 2.64–2.89 (m, 8H, $2CH_{2\text{-PIPERAZINE}}$, $2H_{6\text{-QUINUCLIDINE}}$, $2H_{7\text{-QUINUCLIDINE}}$), 3.12–3.23 (m, 4H, $2CH_{2\text{-PIPERAZINE}}$), 3.25–3.34 (m, 1H, $H_{2\text{-QUINUCLIDINE}}$), 3.91 (s, 2H, CH_2), 3.98–4.12 (m, 1H,

$H_{3\text{-QUINUCLIDINE}}$), 6.77 (t, $J = 7.3$, 1H, Ph-H_4), 6.92 (d, $J = 7.8$, 2H, Ph-H_2, Ph-H_6), 7.20 (t, $J = 7.3$, 2H, Ph-H_3, Ph-H_5), 7.29 (t, $J = 7.8$, 1H, H_6), 7.67 (dd, $J = 7.8$, 1.2, 1H, H_7), 7.81 (dd, $J = 7.6$, 1.2, 1H, H_5), 10.35 (s, 1H, CONH); ^{13}C NMR (Me_2SO-d_6) δ 20.3, 25.6 ($C_{5\text{-QUINUCLIDINE}}$, $C_{8\text{-QUINUCLIDINE}}$), 25.8 ($C_{4\text{-QUINUCLIDINE}}$), 46.1, 47.1 ($C_{6\text{-QUINUCLIDINE}}$, $C_{7\text{-QUINUCLIDINE}}$), 46.3 ($C_{3\text{-QUINUCLIDINE}}$), 48.3 ($2CH_{2\text{-PIPERAZINE}}$), 52.6 ($2CH_{2\text{-PIPERAZINE}}$), 55.4 ($CH_2$), 56.5 ($C_{2\text{-QUINUCLIDINE}}$), 115.0, 115.5 (Ph-$C_2$, Ph-$C_6$, C_7), 118.9 (Ph-C_4), 121.8 (C_5, C_6), 121.9 (C_4), 129.0 (Ph-C_3, Ph-C_5), 135.3 (C_{7a}), 140.2 (C_{3a}), 151.0, 152.8 (Ph-C_1, C_2), 164.4 (CONH). Anal. ($C_{26}H_{32}N_6O$) C, H, N.

5.1.16. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(*o*-methoxyphenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (2). From **13** following the general procedure was obtained **2**. Yield 757 mg (63%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 122–123 °C (acetone); IR (KBr) 3425, 3260, 1650, 1610, 1575, 1560, 1500, 1450; ^{1}H NMR (Me_2SO-d_6) δ 1.41–1.55 (m, 1H), 1.59–1.68 (m, 2H), 1.88–1.96 (m, 2H), 2.54–2.60 (m, 1H), 2.66–2.89 (m, 8H), 2.96–3.04 (m, 4H), 3.21–3.28 (m, 1H), 3.74 (s, 3H), 3.92 (s, 2H), 4.02–4.11 (m, 1H), 6.86–6.95 (m, 4H), 7.31 (t, $J = 7.8$, 1H), 7.69 (d, $J = 7.5$, 1H), 7.82 (dd, $J = 7.5$, 1.2, 1H), 10.45 (d, $J = 7.0$, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.1, 25.4, 25.7, 46.0, 46.9, 49.9, 52.7, 55.2, 56.4, 111.8, 114.8, 117.8, 120.7, 121.8, 121.9, 122.4, 134.3, 140.5, 141.0, 151.9, 152.4, 164.1. Anal. ($C_{27}H_{34}N_6O_2$)C, H, N.

5.1.17. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(*o*-ethoxyphenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (3). From **14** following the general procedure was obtained **3**. Yield 769 mg (68%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 143–144 °C ($CHCl_3$/AcOEt); IR (KBr) 3440, 3260, 1655, 1615, 1560, 1500, 1450; ^{1}H NMR (Me_2SO-d_6) δ 1.34 (t, $J = 7.2$, 3H), 1.52–1.64 (m, 1H), 1.66–1.78 (m, 2H), 1.94–2.09 (m, 2H), 2.60–2.67 (m, 1H), 2.69–2.78 (m, 4H), 2.82 (t, $J = 7.5$, 2H), 2.88–2.98 (m, 2H), 3.01–3.12 (m, 4H), 3.32–3.38 (m, 1H), 3.94 (s, 2H), 4.01 (q, $J = 6.9$, 2H), 4.10–4.20 (m, 1H), 6.80–7.00 (m, 4H), 7.35 (t, $J = 7.8$, 1H), 7.72 (d, $J = 7.8$, 1H), 7.86 (d, $J = 7.5$, 1H), 10.50 (br d, $J = 7.2$, 1H); ^{13}C NMR (Me_2SO-d_6) δ 14.8, 19.9, 25.0, 25.5, 45.9, 46.0, 46.8, 49.9, 52.9, 55.3, 56.2, 63.2, 113.1, 114.9, 117.9, 120.9, 121.8, 121.9, 122.3, 134.6, 140.5, 141.3, 151.1, 152.5, 164.3. Anal. ($C_{28}H_{36}N_6O_2$) C, H, N.

5.1.18. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(*o*-propoxyphenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (4). From **15** following the general procedure was obtained **4**. Yield 817 mg (65%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 101–104 °C ($CHCl_3$/AcOEt); IR (KBr) 3421, 1650, 1550, 1498, 1450; ^{1}H NMR (Me_2SO-d_6) δ 0.99 (t, $J = 7.3$, 3H), 1.42–1.60 (m, 1H), 1.70 (sx, $J = 7.1$, 2H), 1.85–2.05 (m, 3H), 2.18–2.30 (m, 2H), 2.62–2.88 (m, 6H), 2.92–3.12 (m, 6H), 3.18–3.20 (m, 1H), 3.84–3.97 (m, 4H), 4.32–4.48 (m, 1H), 6.80–7.00 (m, 4H), 7.35 (t, $J = 7.6$, 1H), 7.72 (d, $J = 7.6$, 1H), 7.84 (d, $J = 7.6$, 1H), 10.50 (br s, 1H), 13.10 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 10.8, 17.6, 21.6, 24.4, 26.4, 44.0, 45.0, 45.4, 50.0, 53.0, 57.8, 59.8, 69.0, 112.8, 115.5, 117.9, 120.8, 120.9, 122.0, 122.4, 134.5, 140.6, 141.2, 151.3, 152.9, 170.3. Anal. ($C_{29}H_{38}N_6O_2$) C, H, N.

5.1.19. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(*o*-*iso*-propoxyphenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (5). From **16** following the general procedure was obtained **5**. Yield 792 mg (63%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 125–127 °C ($CHCl_3$/AcOEt); IR (KBr) 3429, 1654, 1560, 1496, 1450; ^{1}H NMR (Me_2SO-d_6) δ 1.27 (d, $J = 6.0$, 6H), 1.49–1.60 (m, 1H), 1.61–1.75 (m, 2H), 1.90–2.00 (m, 2H), 2.28–2.36 (m, 1H), 2.65–2.80 (m, 6H), 2.82–2.92 (m, 2H), 3.00–3.12 (m, 4H), 3.18–3.22 (m, 1H), 3.93 (s, 2H), 4.55–4.65 (m, 1H), 4.61 (st, $J = 6.3$, 1H), 6.87–6.96 (m, 4H), 7.35 (t, $J = 7.8$, 1H), 7.71 (d, $J = 7.2$, 1H), 7.86 (d, $J = 7.2$, 1H), 10.47 (d, $J = 6.6$, 1H), 12.97 (br s, 1H). Anal. ($C_{29}H_{38}N_6O_2$) C, H, N.

5.1.20. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(*o*-butoxyphenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (6). From **17** following the general procedure was obtained **6**. Yield 839 mg (65%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; (acetone/hexane); mp 139–141 °C ($CHCl_3$/AcOEt); ^{1}H NMR (Me_2SO-d_6) δ 0.90 (t, $J = 7.3$, 3H), 1.16–1.23 (m, 1H), 1.38–1.42 (m, 3H), 1.45–1.52 (m, 5H), 1.64–1.72 (m, 2H), 2.69–2.79 (m, 8H), 2.92–3.02 (m, 4H), 3.15–3.23 (m, 1H), 3.89–3.96 (m, 4H), 4.32–4.38 (m, 1H), 6.85–7.90 (m, 4H), 7.31 (t, $J = 8.1$, 1H), 7.69 (d, $J = 7.6$, 1H), 7.82 (d, $J = 7.6$, 1H), 10.45 (d, $J = 7.8$, 1H,); ^{13}C NMR (Me_2SO-d_6) δ 13.6, 18.8, 20.2, 25.4, 25.7, 30.0, 46.0, 46.9, 49.9, 52.9, 55.3, 56.5, 67.2, 112.8, 114.8, 117.7, 120.7, 121.8, 122.2, 134.3, 140.5, 141.2, 151.2, 152.5, 164.5. Anal. ($C_{30}H_{40}N_6O_2$) C, H, N.

5.1.21. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(*m*-(trifluoromethyl)phenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (7). From **18** following the general procedure was obtained **7**. Yield 1.04 g (81%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 173–175 °C (d) ($CHCl_3$); IR (KBr) 3435, 3260, 1655, 1610, 1565, 1495, 1450; ^{1}H NMR (Me_2SO-d_6) δ 1.46–1.58 (m, 1H), 1.61–1.71 (m, 2H), 1.90–2.05 (m, 2H), 2.57–2.60 (m, 1H), 2.68–2.70 (m, 6H), 2.81–2.91 (m, 2H), 3.22–3.32 (m, 4H), 3.33–3.38 (m, 1H), 3.96 (s, 2H), 4.05–4.14 (m, 1H), 7.10 (d, $J = 7.8$, 1H), 7.20 (s, 1H), 7.25 (d, $J = 8.1$, 1H), 7.34 (t, $J = 7.8$, 1H), 7.45 (t, $J = 8.1$, 1H), 7.71 (d, $J = 7.5$, 1H), 7.86 (dd, $J = 7.5$, 0.6, 1H), 10.44 (d, $J = 6.6$, 1H), 12.95 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.2, 25.4, 25.6, 46.0, 46.1, 46.9, 47.5, 52.3, 55.1, 56.4, 110.8 (q, $^3J_{C-F} = 3.9$), 114.4 (q, $^3J_{C-F} = 4.2$), 114.9, 118.5, 121.6, 121.8, 124.3 (q, $^1J_{C-F} = 272.2$), 129.8 (q, $^2J_{C-F} = 30.8$), 129.9, 134.3, 140.4, 151.1, 152.4, 164.1. Anal. ($C_{27}H_{31}F_3N_6O$) C, H, N.

5.1.22. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(*m*-chlorophenyl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (8). From **19** following the general procedure was obtained **8**. Yield 1.03 g (86%); chromatography

CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 182–183 °C (acetone); IR (KBr) 3420, 3260, 1660, 1615, 1595, 1560, 1490, 1450; ^{1}H NMR (Me_2SO-d_6) δ 1.46–1.57 (m, 1H), 1.61–1.71 (m, 2H), 1.91–2.05 (m, 2H), 2.56–2.61 (m, 1H), 2.67–2.78 (m, 6H), 2.81–2.91 (m, 2H), 3.21–3.28 (m, 4H), 3.29–3.35 (m, 1H), 3.95 (s, 2H), 4.05–4.14 (m, 1H), 6.81 (d, J = 7.8, 1H), 6.92 (d, J = 8.1, 1H), 6.97 (s, 1H), 7.23 (t, J = 8.1, 1H), 7.34 (t, J = 7.8, 1H), 7.71 (d, J = 7.8, 1H), 7.86 (d, J = 7.8, 1H), 10.44 (br s, 1H), 12.95 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.3, 25.5, 25.7, 46.0, 46.1, 46.9, 47.6, 52.3, 55.2, 56.3, 113.6, 114.5, 114.8, 118.1, 121.8, 121.9, 130.4, 133.8, 134.2, 140.4, 152.2, 152.5, 164.2. Anal. ($C_{26}H_{31}ClN_6O$) C, H, N.

5.1.23. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(naphth-1-yl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (9). From **20** following the general procedure was obtained **9**. Yield 878 mg (71%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 215–217 °C (d) ($CHCl_3$); IR (KBr) 3425, 3265, 1660, 1615, 1590, 1575, 1560, 1510, 1490, 1450; ^{1}H NMR (Me_2SO-d_6) δ 1.41–1.55 (m, 1H), 1.57–1.69 (m, 2H), 1.88–2.05 (m, 2H), 2.58–2.60 (m, 1H), 2.73 (t, J = 7.4, 4H), 2.78–2.93 (m, 4H), 3.02–3.17 (m, 4H), 3.23–3.28 (m, 1H), 4.00 (s, 2H), 4.05–4.09 (m, 1H), 7.12 (d, J = 6.8, 1H), 7.32 (t, J = 7.8, 1H), 7.42 (t, J = 7.8, 1H), 7.46–7.51 (m, 2H), 7.61 (d, J = 8.3, 1H), 7.70 (d, J = 7.5, 1H), 7.83–7.90 (m, 2H), 8.06–8.11 (m, 1H), 10.45 (d, J = 5.8, 1H), 12.96 (br s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.1, 25.4, 25.6, 45.9, 46.0, 46.9, 52.4, 52.8, 55.1, 56.3, 114.3, 114.8, 121.7, 121.8, 122.9, 123.0, 125.2, 125.7, 125.8, 127.9, 128.1, 134.1, 140.3, 149.0, 152.4, 164.1. Anal. ($C_{30}H_{33}N_6O$) C, H, N.

5.1.24. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(2,3-dihydro-1,4-benzodioxan-5-yl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (10). From **21** following the general procedure was obtained **10**. Yield 917 mg (73%); chromatography CH_2Cl_2/EtOH/NH_3, from 9.3:0.7:0.03 to 9:1:0.05; mp 196–197 °C (d) ($CHCl_3$/AcOEt); IR (KBr) 3420, 3255, 1650, 1610, 1595, 1560, 1470, 1450; ^{1}H NMR (Me_2SO-d_6) δ 1.50–1.63 (m, 1H), 1.65–1.77 (m, 2H), 1.91–2.08 (m, 2H), 2.59–2.66 (m, 1H), 2.68–2.76 (m, 4H), 2.81 (t, J = 7.5, 2H), 2.87–2.96 (m, 2H), 2.97–3.11 (m, 4H), 3.30–3.36 (m, 1H), 3.95 (s, 2H), 4.07–4.18 (m, 1H), 4.19–4.28 (m, 4H), 6.49 (d, J = 7.8, 1H), 6.52 (d, J = 8.1, 1H), 6.74 (t, J = 8.1, 1H), 7.35 (t, J = 7.8, 1H), 7.71 (d, J = 7.5, 1H), 7.86 (d, J = 7.8, 1H), 10.47 (d, J = 7.1, 1H), 13.00 (s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 20.0, 25.1, 25.7, 45.9, 46.0, 46.9, 50.1, 52.7, 55.2, 56.1, 63.8, 63.9, 110.2, 111.1, 115.0, 120.4, 121.8, 121.9, 134.5, 136.2, 140.6, 141.6, 143.9, 152.6, 164.3. Anal. ($C_{28}H_{34}N_6O_3$) C, H, N.

5.1.25. (±)-*N*-(1-Azabicyclo[2.2.2]oct-3-yl)-2-[[4-(benzimidazol-4-yl)piperazin-1-yl]methyl]benzimidazole-4-carboxamide (11). The product obtained from **22** following the general procedure was detritylated by refluxing with a solution of THF/H_2O/acetic acid, 1:1:1 for 3 h to afford final compound **11**, which was isolated as hydrochloride salt: yield 606 mg (45%); chromatography $CHCl_3$/MeOH/NH_3, 8:2:0.1; (MeOH/hexane); ^{1}H NMR (Me_2SO-d_6) δ 1.35–1.39 (m, 2H), 1.60–1.67 (m, 2H), 1.77–1.80 (m, 2H), 2.09–2.12 (m, 2H), 2.71–2.77 (m, 1H), 2.89–2.93 (m, 8H), 2.99–3.02 (m, 2H), 3.61–3.64 (m, 1H), 4.08 (s, 2H), 4.21–4.23 (m, 1H), 6.66 (br s, 1H), 7.19 (d, J = 2.9, 2H), 7.45 (t, J = 3.2, 1H), 7.82 (d, J = 3.1, 1H), 7.96 (d, J = 2.9, 1H), 8.19 (s, 1H); ^{13}C NMR (Me_2SO-d_6) δ 25.2, 30.4, 45.9, 46.0, 46.9, 49.2, 52.8, 55.2, 63.4, 114.5, 115.0, 121.9, 122.8, 129.9, 134.5, 140.5, 145.7, 152.7, 164.2. Anal. ($C_{27}H_{32}N_8O$·HCl·H_2O) C, H, N.

5.2. Radioligand binding assays

For all receptor 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 and homogenized on a Polytron PT-10 homogenizer. Membrane suspensions were centrifuged on a Beckman J2-HS instrument.

5.2.1. 5-HT$_{1A}$ receptor. Binding assays were performed by a modification of the procedure previously described by Clark et al.[41] 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.

5.2.2. 5-HT$_3$ receptor. Binding assays were performed according to the procedure previously described by Wong et al.[42] The cerebral cortex was homogenized in 9 volumes of 0.32 M sucrose and centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 17,000*g* for 20 min. The membrane pellet was washed twice by resuspension in 60 volumes of 50 mM Tris–HCl buffer (pH 7.4 at 25 °C) and was centrifuged at 48,000*g* for 10 min. Membranes were resuspended in 2.75 volumes of assay buffer (50 mM Tris–HCl, 10 μM pargyline, 0.6 mM ascorbic acid, and 5 mM $CaCl_2$, pH 7.4 at 25 °C). Fractions of 100 L of the final membrane suspension (about 2 mg/mL of protein) were incubated at 25 °C for 30 min with 0.7 nM [^{3}H]LY 278584 (83 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 10 μM 5-HT.

5.2.3. α$_1$ Adrenoceptor. Binding assays were performed by a modification of the procedure previously described

by Ambrosio et al.[43] 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.

5.2.4. D_2 receptor. Binding assays were performed by a modification of the procedure previously described by Hall et al.[44] The striatum was homogenized in 50 mM Tris–HCl (pH 7.7 at 25 °C) and centrifuged at 48,000*g* for 10 min. The pellet was resuspended and centrifuged as before. The final pellet was resuspended in 50 mM Tris–HCl (pH 7.7 at 25 °C) containing 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, and 0.1% ascorbic acid. Fractions of the final membrane suspension (125–150 μg of protein) were incubated at 25 °C for 60 min with 0.8 nM [^{3}H]raclopride (77 Ci/mmol), in the presence or absence of six concentrations of the competing drug, in a final volume of 1.1 mL of the assay buffer (pH 7.4 at 25 °C). Nonspecific binding was determined with 1 μM (+)-butaclamol.

For all binding assays, 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. 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.[45] The protein concentrations of the rat cerebral cortex and the rat striatum were determined by the method of Lowry et al.,[46] using bovine serum albumin as the standard.

5.3. [^{35}S]GTPγS binding

[^{35}S]GTPγS binding was performed in hippocampal membranes from male Wistar rats (220–240 g) as previously described[47] with minor modifications. Briefly, rats were killed and the hippocampus was dissected and homogenized in cold Tris buffer (50 mM Tris base, pH 7.4). The homogenates were centrifuged at 23,000 rpm for 10 min at 4 °C, and the remaining pellet resuspended in the same Tris buffer and incubated at 37 °C for 10 min in a shaking water bath. After a second centrifugation in the same conditions, the pellet was resuspended in 67 mM Tris base (pH 7.4) containing 4 mM $MgCl_2$, 160 mM NaCl and 0.267 mM EGTA. The binding assay was carried out by incubation of membranes with [^{35}S]GTPγS (0.1 nM) in the presence of 300 μM GDP for 20 min at 37 °C. The reaction was quenched by rapid filtration through Whatman GF/B filters followed by four washes with ice-cold 50 mM Tris base (pH 7.4). Non-specific binding was determined in presence of 10 M unlabeled GTPγS.

The ability of compound **3** (10^{-10}–10^{-5} M) to stimulate basal [^{35}S]GTPγS binding was studied in comparison with the typical 5-HT_{1A}R agonist, 8-OH-DPAT, at the same concentrations. This new compound was also studied at identical concentrations for antagonism to the stimulation of [^{35}S]GTPγS binding induced by 8-OH-DPAT (10^{-6} M), using comparatively the selective 5-HT_{1A}R antagonist WAY-100635.

5.4. Temperature measurements

Rectal temperature was measured in male Swiss mice (24–28 g) with a lubricated digital thermistor probe (pb0331, Panlab, Barcelona) inserted to a depth of 2 cm into the rectum and maintained until the temperature stabilized. Readings were taken immediately before drug injection and 30, 60 and 120 min thereafter. The effect of compound **3** on rectal temperature was studied at doses of 1 and 10 mg/kg sc. To study the possible antagonism to 8-OH-DPAT-induced hypothermia, this compound was administered at the same doses 30 min before 8-OH-DPAT (0.5 mg/kg sc). Results were expressed as changes in rectal temperature (△) over basal values.

5.5. Isolated longitudinal muscle-myenteric plexus preparation (LMMP) from guinea pig ileum

The ileum from male guinea pig (300–350 g) was excised and longitudinal muscle strips with the myenteric plexus attached (LMMP) were prepared as described.[48] LMMP strips were suspended in an organ bath containing Tyrodeás solution aerated with O_2/CO_2, 95%/5% and maintained at 37 °C. Contractile responses were isometrically recorded with a resting tension of 0.5 g. Tissues were equilibrated for 30 min and then stimulated by the 5-HT_3R agonist 2-Me-5-HT, 10^{-5} M. After a period of stabilization, the 5-HT_3R antagonist effect of compound **3** was determined by adding to the bath different concentrations 30 min before 2-Me-5-HT. Ondansetron was used as reference drug. The effect on the contractile response induced by 2-Me-5-HT was expressed as IC_{50} calculated from the corresponding concentration–response curves.

5.6. Light–dark exploration test

A modified light–dark exploration test was used[49] in which the time spent by male Swiss mice (24–28 g) in the white compartment on the second day of exposure was comparatively measured. Experiments were always performed between 09.00 and 15.00 h. The apparatus consisted of an open-topped rectangular box divided into a black small area under red light and a large white area brightly illuminated with an opening door located in the centre of the partition at floor level. The floor of each compartment was marked into 9 cm squares.

Each mouse was placed individually in the centre of the white area and behaviour was recorded over a 5 min period. Two behavioural parameters were measured: the percentage of time spent and the number of line crossings in each area. Mice were exposed to the test for two consecutive days, the first without any treatment and the second day after compound **3** (0.1 and 1 mg/kg sc) given 30 min before the test. Diazepam (1 mg/kg ip) was used as a reference drug. Control mice received vehicle injection.

5.7. Passive avoidance learning

Male Wistar rats weighing 200–220 g were used. Experiments were always performed between 09.00 and 15.00 h. The test was performed according to previously described procedures, using a two-compartment (white/dark) passive avoidance apparatus.[50] The rat was placed in the illuminated area and 3 s later the door was raised. During 90 s the animal explored the apparatus freely (habituation trial). After 10 min, the rat was placed again in the illuminated chamber. When the rat entered the dark compartment, a guillotine door was closed and after 10 s the animal was returned to its home cage. After 60 min, the animal was placed again in the white compartment. When the rat entered the dark chamber, the guillotine door was closed again and, after 10 s, an inescapable 2 mA scrambled electrical foot shock was delivered for 3 s through the grid floor using a shock generator (acquisition trial). A retention trial was given 24 h after the acquisition trial by placing the rat in the illuminated compartment and measuring the response latency to re-enter the dark compartment using a cut-off time of 300 s. The intrinsic effect of compound **3** was determined by administration of a dose of 1 mg/kg sc 30 min before the acquisition trial. In other experiments, learning was impaired by treatment with the muscarinic antagonist scopolamine (1 mg/kg ip) 30 min before acquisition. The effect of compound **3** on retention impairment induced by cholinergic blockade was evaluated by administration of the compound (1 mg/kg sc) 15 min before scopolamine. Control rats received saline or saline/tween 80 injections.

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