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Design and Synthesis of New Benzimidazole-Arylpiperazine Derivatives Acting as Mixed 5-HT_{1A}/5-HT_3 Ligands

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Abstract—A series of new benzimidazole-arylpiperazine derivatives **III** were designed, synthesized and evaluated for binding affinity at serotoninergic 5-HT_{1A} and 5-HT_3 receptors. Compound **IIIc** was identified as a novel mixed 5-HT_{1A}/5-HT_3 ligand with high affinity for both serotonin receptors and excellent selectivity over α_1-adrenergic and dopamine D_2 receptors. This compound was characterized as a partial agonist at 5-HT_{1A}Rs and a 5-HT_3R antagonist, and was effective in preventing the cognitive deficits induced by muscarinic receptor blockade in a passive avoidance learning test.

Introduction

The discovery of new ligands with affinity for the family of serotonin receptors (5-HTRs) is an area of active research in Medicinal Chemistry due to their involvement in numerous physiological and pathophysiological processes.[1–4] At present seven classes of serotonin receptors (5-$HT_{1–7}$) including 14 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} is involved in psychiatric disorders[8–11] such as anxiety, depression and memory loss. On the other hand, 5-HT_3R antagonists are of special interest not only because of their wide clinical use as antiemetic drugs 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 dysfuntion. 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 program aimed at the discovery of new 5-HT_{1A} and 5-HT_3 agents, we have synthesized a series of arylpiperazines[17–22] **I** as potent 5-HT_{1A}R ligands and a class of azabicyclic benzimidazole derivatives **II** which exhibited high affinity for the 5-HT_3R[23] (Fig. 1). In the present work, we have designed a series of new mixed benzimidazole-arylpiperazines of general structure **III** in which we have incorporated the structural elements of 5-HT_{1A} and 5-HT_3 pharmacophores (Fig. 1). Among them, compound **IIIc** has shown high affinity for both 5-HT_{1A} and 5-HT_3 receptors, and has been characterized as a partial agonist at 5-HT_{1A}Rs and a 5-HT_3R antagonist with a potential interest in the treatment of cognitive dysfunction.

Chemistry

The general procedure for the preparation of target compounds **III** is shown in Scheme 1. Starting benzimidazole carboxylic acids **1a–k** were coupled with (±)-3-aminoquinuclidine to afford the desired amides **IIIa–k**. 2-[(4-arylpiperazin-1-yl)methyl]benzimidazole-4-carboxylic acids **1a–k** were obtained by reaction of 2-(chloromethyl)benzimidazole-4-carboxylic acid **3** with the corresponding arylpiperazine. The acid **3** was prepared by condensation of 2,3-diaminobenzoic

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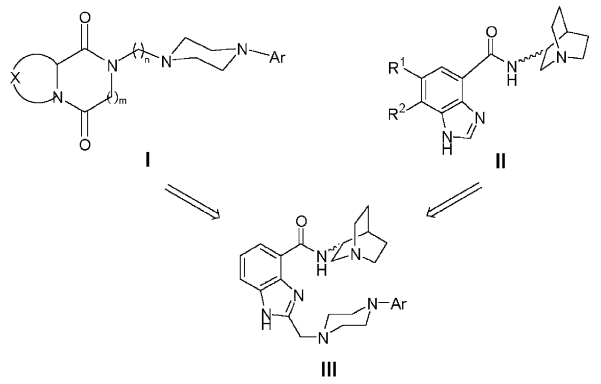
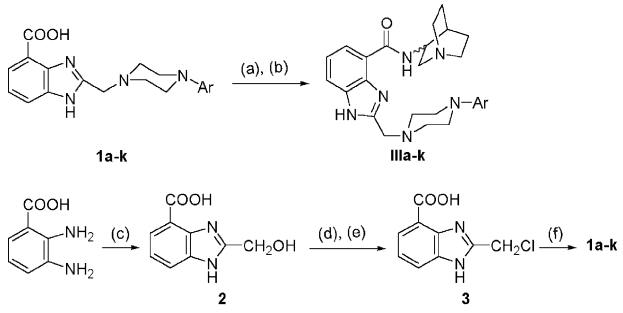


Figure 1. Structure of compounds **I–III**.



Scheme 1. Reagents and conditions: (a) 1,1′-carbonyldiimidazole (CDI), *N*,*N*-dimethylformamide (DMF), 40 °C; (b) (±)-3-aminoquinuclidine, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), DMF, 50 °C; (c) $HOCH_2COOH$, HCl; (d) $SOCl_2$, 80 °C; (e) HCl/H_2O, Δ; (f) arylpiperazine, acetonitrile, NEt_3, 60 °C.

acid[24] with glycolic acid to give 2-(hydroxymethyl)benzimidazole-4-carboxylic acid **2**,[24] which was treated with $SOCl_2$ followed by hydrolysis. The noncommercial arylpiperazines (Ar = *o*-ethoxyphenyl,[25] *o*-propoxyphenyl,[26] *o*-isopropoxyphenyl,[25] *o*-butoxyphenyl,[25] naphth-1-yl,[27] 2,3-dihydro-1,4-benzodioxan-5-yl,[28] 1-tritylbenzimidazol-4-yl)[29] were synthesized according to the literature.

Pharmacology

Affinity data

Target compounds were assessed for in vitro binding affinity at serotoninergic 5-HT_{1A} and 5-HT_3Rs by radioligand binding assays, using [^{3}H]-8-OH-DPAT[30] and [^{3}H]LY 278584,[31] respectively, in rat cerebral cortex membranes. The ligands were also evaluated for in vitro affinity at α_1-adrenergic receptors ([^{3}H]prazosin[32]) and dopamine D_2 receptors ([^{3}H]raclopride[33]), in rat cerebral cortex and striatum membranes, respectively. All the synthesized compounds **IIIa–k** exhibited high 5-HT_3R affinity (K_i = 10–62 nM) (Table 1), 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) (Table 1). Only analogue **IIIk** is an exception in the series (5-HT_{1A}: K_i = 147.8 nM, 5-HT_3: K_i > 1000 nM)

Table 1. Binding data of compounds **III**[a]

Compd	Ar	K_i±SEM (5-HT_{1A})	K_i±SEM (5-HT_3)
IIIa	Phenyl	>1000	23.1±1.5
IIIb	*o*-Methoxyphenyl	149.8±33.4	10.3±1.1
IIIc	*o*-Ethoxyphenyl	18.0±1.7	27.2±0.9
IIId	*o*-Propoxyphenyl	56.1±2.2	24.6±3.2
IIIe	*o*-*iso*Propoxyphenyl	34.4±3.0	62.1±1.3
IIIf	*o*-Butoxyphenyl	45.9±3.5	15.1±4.8
IIIg	*m*-(Trifluoromethyl)phenyl	>10,000	23.9±2.9
IIIh	*m*-Chlorophenyl	>10,000	18.3±0.4
IIIi	Benzodioxan-5-yl	467.1±14.0	24.0±1.0
IIIj	Naphth-1-yl	>1000	32.5±5.3
IIIk	Benzimidazol-4-yl	147.8±6.7	>1000

[a]Values are means of 2–4 experiments performed in triplicate.

(Table 1). The weak 5-HT_{1A}R affinity of derivative **IIIi** was unexpected, according to reported data for arylpiperazines bearing a benzodioxan-5-yl group.[34,35] Additionally, all the compounds were selective over α_1-adrenergic and dopamine D_2 receptors (K_i > 1000–10,000 nM). Compound **IIIc** 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) (Table 1).

Functional characterization of compound IIIc at 5-HT_{1A} and 5-HT_3 receptors

The ability of compound **IIIc** 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.[36] Compound **IIIc** (10^{-10}–10^{-7} M) induced minimal effects on the basal binding of the GTP analogue. Only the highest concentrations tested (10^{-6}–10^{-5} M) produced a moderate increase (20–28%) in [^{35}S]GTPγS binding. A much more marked stimulation was obtained in the presence of the typical 5-HT_{1A}R agonist 8-OH-DPAT (92% at 10^{-6} M). On the other hand, compound **IIIc** blocked only partially the stimulation of [^{35}S]GTPγ binding induced by 8-OH-DPAT at the low concentrations of 10^{-10} and 10^{-9} M but not at higher concentrations. The antagonism to 8-OH-DPAT was considerably less pronounced than that observed with the 'silent' 5-HT_{1A}R antagonist WAY-100635 (IC_{50} = 6 nM). The results obtained in the [^{35}S]GTPγS binding studies tend to suggest that compound **IIIc** behaves as a partial agonist at 5-HT_{1A}Rs.

Since it is known that 5-HT_{1A}R agonists induce hypothermia in rodents, the intrinsic effect of compound **IIIc** on rectal temperature and on the hypothermia response to the 5-HT_{1A}R agonist 8-OH-DPAT was also studied in mice.[37] Compound **IIIc** (1 and 10 mg/kg) reduced body temperature by approximately 1 °C at 60 and 120 min after administration, similar effects being obtained after either dose. This compound did not significantly modify

and rather tended to potentiate the hypothermia induced by 8-OH-DPAT (0.5 mg/kg sc). These in vivo findings appear again to be consistent with a partial agonism at 5-HT_{1A}Rs.

The functional profile of compound **IIIc** at 5-HT_3Rs was characterized in the isolated longitudinal muscle–myenteric plexus preparation from guinea-pig ileum.[38] The contraction induced in this preparation by the selective 5-HT_3R agonist 2-Me-5-HT (10^{-5} M) was inhibited by compound **IIIc** at concentrations 10^{-7}–10^{-5} M, a full blockade of the contraction being observed at the highest concentrations tested. Similar effects were found with the typical 5-HT_3R antagonist, ondansetron. The IC_{50}s for compound **IIIc** and ondansetron in this preparation were almost identical, 0.19 and 0.24 μM, respectively.

Behavioural effects of compound IIIc

The anxiolytic-like effect of compound **IIIc** was evaluated by using a modified light–dark exploration test in mice.[39] In this modified procedure, control animals spend less time in the white compartment on the second day of exposure. This behaviour is effectively prevented by administration of typical anxiolytic drugs. At variance with the results obtained with diazepam (1 mg/kg ip), which approximately doubled the time spent in the white compartment, no significant change was found after administration of compound **IIIc** at doses of 0.1 and 1 mg/kg sc, given 30 min before the test, indicating no anxiolytic-like effect for this compound in this test, at least at the doses used.

The effect of compound **IIIc** on learning and retention was evaluated by using a passive avoidance procedure.[40] As shown in Figure 2, the muscarinic receptor antagonist scopolamine, administered 30 min before the acquisition session, reduced significantly the retention latency 24 h later. Compound **IIIc**, at the dose of 1 mg/kg sc, 30 min before the acquisition trial, had no effect by itself in this test, but significantly prevented the retention impairment induced by cholinergic blockade when given 15 min before scopolamine.

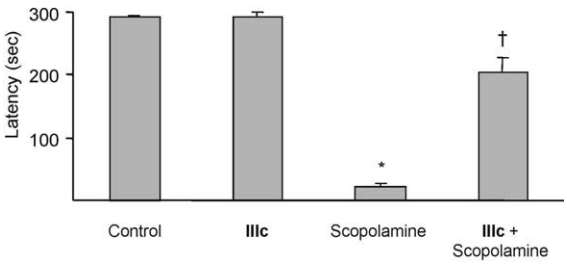


Figure 2. Prevention by compound **IIIc** of scopolamine-induced impairment of passive avoidance learning in rats. Scopolamine and compound **IIIc** (1 mg/kg each) given 30 and 45 min, respectively, before the acquisition trial. Response latency evaluated 24 h later. Values are means ± SEM from 10–20 animals. *$p < 0.05$ versus control; †$p < 0.05$ versus scopolamine-treated group (ANOVA followed by Student–Newman Keuls's test).

Conclusions

In summary, in a series of new benzimidazole-arylpiperazine derivatives compound **IIIc** has been identified as a novel mixed 5-HT_{1A}/5-HT_3 ligand with high affinity for both serotonin receptors, an excellent selectivity profile over α_1-adrenergic and dopamine D_2 receptors, and an ability to counteract the cognitive deficit induced by cholinergic blockade, suggesting a potential interest in the treatment of cognitive dysfunction.

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