New Serotonin 5-HT<sub>1A</sub> Receptor Agonists with Neuroprotective Effect against Ischemic Cell Damage

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ABSTRACT: We report the synthesis of new compounds 4–35 based on structural modifications of different moieties of previously described lead UCM-2550. The new nonpiperazine derivatives, representing second-generation agonists, were assessed for binding affinity, selectivity, and functional activity at the 5-HT<sub>1A</sub> receptor (5-HT<sub>1AR</sub>). Computational β<sub>2</sub>-based homology models of the ligand–receptor complexes were used to explain the observed structure–affinity relationships. Selected candidates were also evaluated for their potential in vitro and in vivo neuroprotective properties. Interestingly, compound 26 (2-{6-[(3,4-dihydro-2H-chromen-2-ylmethyl)amino]hexyl}-tetrahydro-1H-pyrrolo[1,2-c]imidazole-1,3(2H)-dione) has been characterized as a high-affinity and potent 5-HT<sub>1A</sub>R agonist (K<sub>i</sub> = 5.9 nM, EC<sub>50</sub> = 21.8 nM) and exhibits neuroprotective effect in neurotoxicity assays in primary cell cultures from rat hippocampus and in the MCAO model of focal cerebral ischemia in rats.

INTRODUCTION

G protein-coupled receptors (GPCRs) are the largest family of membrane-bound receptors and transmit chemical signals into a wide array of different cell types. These proteins account for a wide range of opportunities as therapeutic targets in areas including cancer, cardiac dysfunction, diabetes, central nervous system disorders, obesity, inflammation, and pain.† Indeed, drugs targeting members of this integral membrane protein superfamily represent the core of modern medicine because they account for the majority of best-selling drugs and about 40% of all prescription pharmaceuticals on the market. Nevertheless, there is still much to be learned about how GPCRs work and how they can be selectively modulated. Technologies designed specifically to tackle the GPCR challenge, such as cell-based screening assays and structural studies, are blossoming and reinforcing their potential for future drug discovery.‡–§ Consequently, GPCRs are among the most heavily investigated drug targets, and there is broad consensus that they will remain at the hub of drug development activities for the foreseeable future.

Serotonin (5-hydroxytryptamine, 5-HT) receptors constitute an important GPCR family composed of 14 members which have been classified into seven families (5-HT<sub>1–7</sub>) based on amino acid sequences, pharmacology, and intracellular mechanisms.⁵,⁶ The 5-HT<sub>1A</sub> receptor (5-HT<sub>1AR</sub>) subtype has been the most extensively studied, its agonists and partial agonists being clinically used in the treatment of anxiety and depression.⁷–⁹ The 5-HT<sub>1A</sub>R is also responsible for the lack of unwanted side-effects in some atypical antipsychotic drugs.¹⁰,¹¹ Besides these well-established therapeutic areas, other interesting nonpsychiatric perspectives have emerged for 5-HT<sub>1AR</sub> agents in recent years, mostly related to neuroprotection, cognitive impairment, Parkinson’s disease, or pain treatment.¹²–¹⁵ In particular, it is known that the selective 5-HT<sub>1A</sub>R agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT, 1) and other 5-HT<sub>1AR</sub> agonists attenuate excitotoxicity (Chart 1).¹⁶ showing neuroprotective properties in models of global and focal cerebral ischemia in mice, rats, and gerbils.¹⁷,¹⁸ Indeed, the selective 5-HT<sub>1AR</sub> agonists repinotan ([R]−2-{4-[(chroman-2-ylmethyl)amino][butyl]-1,1-dioxobenzo[d]-isothiazolone (BAYx3702, (−)-2]) and piclozotan (SUN N4057) have demonstrated neuroprotective properties in phase IIb clinical trials for treatment of ischemic stroke, although the former has been discontinued.¹⁹–²¹ Stroke is the third leading cause of death in adults and the main neurologic cause of disability in the elderly. It is recognized that cell death...
in the ischemic penumbra may be prevented by different classes of compounds acting at different steps along the ischemic cascade. Yet, pharmacological treatment with available so-called neuroprotective drugs has not been successful to improve clinical outcome in patients. Today, several studies strongly support the potential interest of 5-HT1AR activation in the search for neuroprotective strategies.22−24 Because stroke is such an important cause of mortality worldwide, even small clinical benefits may have a huge positive impact on public health. In this context, our goal is the development of a 5-HT1AR agonist as an anti-ischemic agent.

During the last years, our group has been involved in a wide research program aimed at developing new arylpiperazines with high affinity for the 5-HT1AR.25−31 Some of these ligands have been pharmacologically characterized as potent 5-HT1AR agonists endowed with anxiolytic properties.32,33 In the present work, the previously reported high-affinity ligand 3 (UCM-2550)31 was used as starting point to design and synthesize new compounds 4−35 devoid of piperazine ring (Tables 1−4). In these series, we have systematically modified different structural moieties of lead compound 3: the amide subunit, spacer 1, spacer 2, and the aromatic system (Chart 2). The new synthesized nonpiperazine ligands 4−35, representing second-generation 5-HT1AR agonists, were assessed for binding affinity, selectivity, and functional activity at the receptor. The mode of binding proposed in our computational model of ligand−receptor complex explained the observed structure−affinity relationships. Selected candidates have also been evaluated for their potential in vitro and in vivo neuroprotective properties. In particular, compound 26 [X = (CH2)m, Y = N, n = 0, Z = (CH2)6, m = 1, Ar = chroman-2-yl] was characterized as a potent 5-HT1AR agonist and exhibited neuroprotective effect in neurotoxicity assays in primary cell cultures from rat hippocampus and in a model of focal cerebral ischemia in rats.

RESULTS AND DISCUSSION

Synthesis. Target compounds 4−35 (Tables 1−4) were obtained following the synthetic route described in Scheme 1.

Scheme 1. Synthesis of Target Compounds 4−35

In general, 1,3-thiazolidine-2,4-dione, hydantoin34−36 36−39 [n = 0; Y = N; X = (CH2)3, (CH2)4, CH2SCH2, and S(CH2)2, respectively] or diketopiperazines37 40−41 [n = 1; Y = N; X = (CH2)3, and (CH2)4, respectively] were alkylated with the appropriate commercially available α,ω-dibromo or -dichloro derivative in the presence of sodium hydride and N,N-dimethylformamide (DMF) to yield the corresponding halogenated intermediates 42−60. Subsequent treatment of 42−60 with commercial or previously described amines38−40 61−64 provided final compounds 4−35. Enantiopure amines (+)- and (−)-64 were prepared following a new procedure based on the kinetic resolution of racemic ethyl chromane-2-carboxylate [(±)-65]41 with Pseudomonas fluorescens lipase,42 followed by further conversion of enantiopure esters (+)- and (−)-65 into the corresponding amides and subsequent diborane reduction (Scheme 2).

Binding Affinities. New synthesized compounds 4−35 were assessed for in vitro affinity at serotonin 5-HT1AR receptors by competition binding assays, using [3H]-8-OH-DPAT as
radioligand (see Experimental Section for details). All compounds were assayed as hydrochloride salts. The competitive inhibition assays were first performed at a fixed dose of $10^{-6}$ M, and the complete dose–response curve, at six different concentrations of the ligand, was determined for those compounds that presented a displacement of the radioligand over 55%. The inhibition constant $K_i$ was calculated from the IC$_{50}$ value using the Cheng–Prusoff equation,$^{43}$ and the values in Tables 1–4 are the mean of two to four independent experiments. Compounds exhibiting high affinity for the 5-HT$_{1A}$R ($K_i < 50$ nM) were tested for selectivity over other serotonin receptors as well as $\alpha_1$-adrenergic and dopamine D$_2$ receptors (Tables 2–4), using the following specific radio-ligands: 5-HT$_2A$, [3H]ketanserin; 5-HT$_3$, [3H]LY 278584; 5-HT$_4$, [3H]GR 113808; 5-HT$_7$, [3H]-5-CT; $\alpha_1$, [3H]prazosin; and D$_2$, [3H]spiperone (see Experimental Section for details).

Tables 1–4 show the binding affinities of compounds 4–17, 18–23, and 24–35 obtained from the systematic modification of the different moieties present in the molecule: the amide subunit, spacer 1 (Z), spacer 2 [(CH$_2$)$_m$] and the aromatic (Ar) system (Chart 2). The following structure–affinity relationships can be drawn for each series. Clearly, only bicyclic Ar systems are favorable for 5-HT$_{1A}$R affinity [$K_i(5−9) = 1.23−49$ nM], with the exception of compound 4 [$K_i(4) > 1000$ nM], whereas all derivatives containing a single ring are inactive [$K_i(10−17) > 1000$ nM] (Table 1). Notably, analogue 9–containing a single methylene unit as spacer 2 and chromane as Ar– is the most potent compound. No significant differences were observed between racemic compound (±)-9 ($K_i = 1.23$ nM) and the most active isomer (−)-9 ($K_i = 1.9$ nM). Thus, the (±)-chroman-2-ylmethyl group appeared as the most promising for the Ar and spacer 2 moieties. Using this terminal group, the influence of the amide subunit was studied in compounds 18–23 (Table 3). Modifications in the amide subunit do not have a significant influence because all compounds in the series exhibit high to very high 5-HT$_{1A}$R binding affinity [$K_i(18−23) = 2.4−34$ nM]. Considering both affinity data and synthetic availability, 1,3-dioxoalphapropyrolo[1,2-c]imidazole and 1,3-thiazolidine-2,4-dione were selected as the amide subunits in compounds 24–35 for further optimization of spacer 1. In this series, the best results were obtained for compounds with saturated aliphatic spacers containing 3–6 methylene units ($K_i = 1.23−12.9$ nM) (Table 4). The influence of the cis (29)/trans (28) configuration of the four-methylene spacer was also assessed, the trans isomer being more potent [$K_i(28) = 27$ nM vs $K_i(29) = 114$ nM]. Compound 30, containing a triple bond, is inactive. Substitution of methylene units by a phenyl ring is tolerated [$K_i(31) = 28$ nM; $K_i(32) = 42$ nM]. In general, the new synthesized compounds were inactive or poorly active at other 5-HT receptor subtypes as well as dopamine D$_2$ receptor (Tables 2–4).

**Scheme 2. Synthesis of Enantiopure Amines (+)- and (−)-64$^b$**

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$^a$Values are the mean of two to four experiments performed in triplicate.

Table 1. 5-HT$_{1A}$R Affinity of New Synthesized Compounds 4–17
Computational Model of 5-HT₁AR in Complex with Ligand 26. To date, within the biogenic amine family of GPCRs, the crystal structures of the β₁-adrenergic receptor bound to the antagonist cyanopindolol (Protein Data Bank accession number 2VT4), partial agonists dobutamine (2Y01) or salbutamol (2Y04), or agonists carmoterol (2Y02) or isoprenaline (2Y03), the β₂-adrenergic receptor bound to the partial inverse agonist carazolol (2RH1), the agonist BI-167107 (3P0G), or the irreversible agonist FAUC50 (3PDS), the dopamine D₃ receptor in complex with the antagonist eticlopride (3PBL), and the histamine H₁ receptor in complex with doxepin, have been reported. In adrenergic receptors, all type of compounds (i.e., agonists, partial agonists, antagonists, or inverse agonists) anchor the receptor through a complex hydrogen bond network involving the secondary protonated amine (NH₂⁺) and the β-OH of the ligand and Asp3.32 and Asn7.39 of the receptor. In contrast, the tertiary protonated amine (NH⁺) of eticlopride or doxepin interacts exclusively with Asp3.32 of the dopamine D₃ or histamine H₁ receptor, respectively, due to the presence of a single N–H bond in the ligand and the absence of Asn7.39 in the receptor (Figure 1A). The fact that the Asp3.32 and Asn7.39 combination of side chains is also present in the 5-HT₁AR (Figure 1A) led us to propose a similar hydrogen bond network as in adrenergic receptors. The model depicted in Figure 1B shows the secondary protonated amine (NH₂⁺) and the oxygen atom of the chromane system of 26 anchored between Asp3.32 and Asn7.39, the Ar moiety expanded toward transmembrane helix (TM) 5, and the amide subunit extended toward TM 2. Thus, compound 26 occupies the orthosteric binding pocket that is located between the extracellular segments of TMs 3 and 5−7, and the recently named minor binding pocket located between the extracellular segments of TMs 2, 3, and 7. The binding of 26 to the 5-HT₁AR resembles the binding of FAUC50 covalently bound to the His2.64Cys mutant β₂-adrenergic receptor through a disulfide bond.

This mode of binding of 26 explains the observed structure–affinity relationships. (i) The length of spacer 1 can be extended to eight methylene units without a large decrease in binding affinity. Increase of the number of methylene units...
(cAMP) formation in HeLa cells transfected with the human S-HT1AR (hS-HT1AR). The in vivo test of S-HT1AR stimulation consisted of the hypothermic response in mice (sensitive to the selective S-HT1AR antagonist WAY-100635). (±)-2, a S-HT1AR agonist with marked neuroprotection in animal models of ischemic stroke, was used as reference compound. Most of the assayed compounds behaved as pure agonists in the cell line transfected with the hS-HT1AR, with EC50 values for adenylyl cyclase inhibition in the range of 11.6–34.2 nM (Table 5). In general, a correlation between in vitro and in vivo potency was observed.

Neuroprotective Effect. In Vitro Assays. The in vitro neuroprotective effect was evaluated in primary neuronal cultures from rat hippocampus. Neurotoxicity assays consisted of determining the protection against cell death induced by serum deprivation, glutamate toxicity, or oxygen-glucose deprivation. The 5-HT1AR agonist 1 and the neuroprotective agent 2 were also included in these studies for comparative purposes. The results show that some of the new 5-HT1AR agonists afforded neuroprotection, similar to that of reference compounds, against cell death in primary hippocampal cultures exposed to serum and oxygen-glucose deprivation (Table 6). In particular, ligand (±)-9, endowed with high affinity (Ki = 1.23 nM, Table 1), selectivity (Table 2), and agonist potency (EC50 = 16.3 nM, Table 5) for the S-HT1AR, was approximately equipotent to agonist 1 against apoptotic cell death induced by serum deprivation and against excitotoxic cell death (Table 6). However, all other new S-HT1AR agonists tested were virtually devoid of neuroprotective effect on neuronal cell death induced by glutamate. Notably, compounds 25 and 26 afforded a high neuroprotection against cell death in cultures exposed to oxygen–glucose deprivation, similar to that of reference compound (−)-2 (Table 6). It should be noted that, in general, the protective effect was not strictly concentration-dependent, so only the highest effect found is shown in the

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<th>D2</th>
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Values are the mean of two to four experiments performed in triplicate; nd, not determined.
Such a lack of a concentration- or dose-dependent effect has been reported for other 5-HT1AR agonists in previous studies.\(^53,54\)

### In Vivo Models.

Compound 26, behaving as an effective neuroprotective agent in vitro, was subsequently tested in an in vivo model of neuroprotection. The selected animal model was the focal ischemia in rats induced by the permanent occlusion of the middle cerebral artery (MCAO), which represents an adequate model for ischemic stroke in humans. One day later, brain sections were stained with the mitochondrial dye 2,3,5-triphenyltetrazolium chloride (TTC), and cortical and subcortical infarct volumes were calculated by image analysis. Tested compounds were administered by continuous iv infusion. In these in vivo experiments, the 5-HT1AR agonist (−)-\(^2\), previously reported as neuroprotective agent, was also assayed for comparative purposes. The results are shown in Table 7.

![Figure 1](image-url)

**Figure 1.** (A) Sequence alignment of TMs 2, 3, 5-7 of known crystal structures (\(\beta_1\)- and \(\beta_2\)-adrenergic receptors and dopamine D3 and histamine H1 receptors), serotonin 5-HT1a, 5-HT2a, 5-HT6, and 5-HT7 receptors, and \(\alpha_1\) and D2 receptors. (B) Computational model of the complex between ligand 26 (in white, the aromatic ring of the chromane system is shown in gray) and a \(\beta_2\)-based homology model of the 5-HT1AR. In this model, the protonated amine and the oxygen atom of chromane anchors between Asp3.32 and Asn7.39, the aromatic ring of chromane interacts with Val3.33 and Trp6.48, and 1,3-dioxopyrrolo[1,2-c]imidazole forms hydrogen bond interactions with Tyr2.64, Gln2.65, and Trp7.40. (C) Detailed view of the bicyclic aromatic systems of ligands 26 (white/gray), 4 (orange), 5 (olive), 6 (purple), and 7 (blue) in the orthosteric binding cavity of the 5-HT1AR.

### Table 5. Functional Characterization of New 5-HT1AR Agonists

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\(^a\)Biphasic effect; (PA), partial agonist; nd, not determined.

### Table 6. In Vitro Neuroprotective Effect of New 5-HT1AR Agonists in Primary Neuronal Cultures from Rat Hippocampus

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<tr>
<td>33</td>
<td>10</td>
<td>21 ± 3</td>
<td>0</td>
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<td>34</td>
<td>10</td>
<td>28 ± 2</td>
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<td>9 ± 5</td>
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<tr>
<td>35</td>
<td>1</td>
<td>32 ± 4</td>
<td>nd</td>
<td>32 ± 8</td>
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<td>1</td>
<td>44 ± 1</td>
<td>37 ± 3</td>
<td>54 ± 4</td>
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<tr>
<td>(±)-2</td>
<td>0.1</td>
<td>31 ± 3</td>
<td>nd</td>
<td>78 ± 9</td>
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<tr>
<td>(±)-2</td>
<td>0.1</td>
<td>26 ± 3</td>
<td>16 ± 1</td>
<td>nd</td>
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\(^b\)Compounds were tested at concentrations from 1 nM to 10 \(\mu\)M; nd, not determined.

Neuroprotection was not generally concentration-related so only the lower concentration that induces the highest neuroprotective effect is shown. \(^b\)Compounds were tested at concentrations from 1 nM to 10 \(\mu\)M; nd, not determined.

### In Vivo Models.

Compound 26, behaving as an effective neuroprotective agent in vitro, was subsequently tested in an in vivo model of neuroprotection. The selected animal model was the focal ischemia in rats induced by the permanent occlusion of the middle cerebral artery (MCAO), which represents an adequate model for ischemic stroke in humans. One day later, brain sections were stained with the mitochondrial dye 2,3,5-triphenyltetrazolium chloride (TTC), and cortical and subcortical infarct volumes were calculated by image analysis. Tested compounds were administered by continuous iv infusion. In these in vivo experiments, the 5-HT1AR agonist (−)-2, previously reported as neuroprotective agent, was also assayed for comparative purposes. The results are shown in Table 7.
Administration of a low dose (40 μg/kg/h) of compound 26 for 4 h immediately after MCAO significantly reduced the total infarct volume measured 24 h later (29% reduction), compared to the corresponding saline-treated controls. The protective effect was more marked in cortical areas (35% protection) than in subcortical nuclei, where the protection did not reach statistical significance. Virtually identical results were obtained with the same dose (40 μg/kg/h) of reference agent (−)-2 infused over 4 h (Table 7). The results herein reported suggest the interest of further pharmacological development of compound 26 and related drugs.

**CONCLUSIONS**

Herein we report the synthesis of new compounds 4–35, based on systematic modifications of different structural moieties present in the previously reported lead arylpiperazine 3: the amide subunit, spacer 1, spacer 2, and the aromatic system (Chart 2). The new nonpiperazine derivatives were assessed for binding affinity at the 5-HT1AR and selectivity over other serotonin receptors (Tables 1–4). Computational βi-based homology models revealed that ligands occupy the orthosteric pocket of the receptor between TMs 3, 5–7, and a minor binding pocket between TMs 2 and 7, explaining the observed structure–affinity relationships. Determination of the functional activity at the h5-HT1AR receptor revealed that identified high-affinity ligands (Ki < 25 nM) represent second-generation 5-HT1AR agonists (Table 5). New characterized 5-HT1AR agonists were also evaluated for their potential in vitro and in vivo neuroprotective properties. Compounds (±)-9, 25, and 26 afforded protection against cell death in neurotoxicity assays in primary neuronal cultures from rat hippocampus (Table 6). Interestingly, in the rat model of focal ischemia, iv infusion of compound 26 after MCAO significantly reduced the cerebral infarct volume (~29%), the protection being more marked in cortical areas (~35%). The protective effect of 26 was virtually identical to that of compound (−)-2, a 5-HT1AR agonist that has been previously described as a neuroprotective agent (Table 7). Thus, new compound 26 [X = (CH2)60, Y = N, n = 0, Z = (CH2)60, m = 1, Ar = chroman-2-yl], has been characterized as a high-affinity and potent 5-HT1AR agonist (Ki = 5.9 nM, EC50 = 21.8 nM) that exhibits in vitro and in vivo neuroprotective properties. The results herein reported suggest the interest of further pharmacological development of compound 26 and related drugs.

**EXPERIMENTAL SECTION**

Chemistry. Melting points (mp, uncorrected) were determined on a Stuart Scientific electrothermal apparatus. Infrared (IR) spectra were recorded on a Shimazu-8300 or Bruker Tensor 27 instrument equipped with a Specac ATR accessory of 5200–650 cm⁻¹ transmission range; frequencies (ν) are expressed in cm⁻¹. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance 500 (1H, 500 MHz; 13C, 125 MHz), Bruker Avance 300-AM (1H, 300 MHz; 13C, 75 MHz) or Bruker 200-AC spectrometer (1H, 200 MHz; 13C, 50 MHz) at the UCM’s NMR facilities. 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), m (multiplet), br (broad), 2D NMR experiments (HMOC and HMBC) of representative compounds were carried out to assign protons and carbons of the new elemental structures. Analytical data (C, H, N or C, H, N, S) were obtained on a LECO CHNS-932 apparatus at the UCM’s analysis services and were within 0.5% of the theoretical values, confirming a purity of at least 95% for all tested compounds. Optical rotations were recorded on a Perkin-Elmer 241 polarimeter using a 1 dm path length; concentrations are given as g/100 mL. Analytical thin-layer chromatography (TLC) was run on Merck silica gel plates (Kieselgel 60 F-254) with detection by UV light (254 nm), ninhydrin solution, or 10% phosphomolybdic acid solution in ethanol. Flash chromatography was performed on glass column using silica gel type 60 (Merck, particle size 230–400 mesh, for final compounds) or on a Varian 971-FP flash purification system using silica gel cartridges (Varian, particle size 50 μm, for intermediates). Unless stated otherwise, starting materials, reagents, and solvents were purchased as high-grade commercial products from Sigma-Aldrich, Acros, Lancaster, Scharlau, or Panreac and were used without further purification. Anhydrous tetrahydrofururan (THF) was distilled from sodium benzenophene ketyl and used immediately.

The following compounds were synthesized according to described procedures: tetrahydro-1H-pyrrrolo[1,2-c]imidazole-1,2(3H)-dione (36),55 tetrahydroimidazol[1,5-a]pyridine-1,3(2H,H)-dione (37),35 1H-imidazol[1,5-c][1,3]thiazole-5,7(6H,7,αH)-dione (38),55 hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (40),56 tetrahydro-2H-pyrido[1,2-a]pyrazine-1,4(3H,6H)-dione (41),56 2-(4-bromobutyl)tetrahydro-1H-pyrrrolo[1,2-c]imidazole-1,3(2H)-dione (42),55 2-[(4-bromobutyl)tetrahydroimidazol[1,5-a]pyridine-3,2(3H,H)-dione (43),55 2-(4-bromobutyl)hexahydropyrrolo[1,2-a]pyrazine-1,4-dione (44),56 2-(4-bromobutyl)tetrahydro-2H-pyrido[1,2-c]imidazole-1,2(3H,6H)-dione (45),55 2-(3-bromomethyl)tetrahydro-1H-pyrrrolo[1,2-c]imidazole-1,2(3H)-dione (49),55 2-(1-naphthyl)ethylamine (61),38 2-(naphthyl)ethylamine (62),38 quinolizin-2-ylmethyamine (63),57 3,4-dihydro-2H-chromen-2-ylmethyamine (64),57 and ethyl chro-
mancarboxylate (±)-65.41 Collected data for compounds 4–35 refer to free bases, and then hydrochloride salts were prepared prior to mp determination, elemental analyses, and biological assays. Spectroscopic data of all described compounds were consistent with the proposed structures. For final compounds 4–35, we include the data of 4, 8, 9, 12, 16, 19, 21, 23, 28, 31, and 34. For intermediates 46–48 and 50–60, the data of compounds 46, 53, 55, and 56 are described.

**Synthesis of Dihydroimidazol[5,1-b][1,3]thiazole-5,7-(6H,7αH)-dione (39).** To a suspension of 1,3-thiazolidine-2-carboxylic acid (3.45 g, 26 mmol) in H2O (10 mL), potassium cyanate (3.00 g, 57 mmol) was added and the mixture was refluxed for 1 h, acidified by a solution of 2 equiv of 1,3-thiazolidine-2,4-dione in 60% HCl to pH 2, and refluxed for an additional 2 h. The solvent was evaporated under reduced pressure, and the residue was dried under vacuum overnight. The crude was purified by continuous extraction with EtOAc to afford hydantoin 39 in 60% yield; mp 112–114 °C (EtOAc). IR (KBr) ν 3219, 1772, 1718. 1H NMR (DMso-d4) δ 2.86–3.18 (m, 3H, 3/2CH2), 4.20–4.30 (m, 1H, 1/2CH2), 5.30 (s, 1H, CH), 11.40 (br s, 1H, NH). 13C NMR (DMso-d4) δ 32.4, 47.8 (2CH2), 63.9 (CH), 159.8, 173.1 (2C0).

**General Procedure for the Synthesis of Haloalkyl Der-

**Article**
reduced pressure and the residue was suspended in H2O and extracted with dichloromethane (50 mL). The combined organic layers were dried (Na2SO4), and the solvent was evaporated to dryness. The residue was purified by column chromatography using the appropriate eluant, to afford pure 42–60.

2-(4-Bromomethyl)tetrahydro-1H-pyrrolo[1,2-c]imidazo[1,3](2H)-dione (57). Obtained from 36 and 1,3-bis(bromomethyl)benzene in 42% yield. Chromatography: hexane/EtOAc, 8:2.

2-(5-Bromomethyl)-1,3-thiazolidine-2,4-dione (58). Obtained from 1,3-thiazolidine-2,4-dione and 1,5-dibromopentane in 61% yield. Chromatography: hexane/EtOAc, 9:1.

2-(6-Bromomethyl)-1,3-thiazolidine-2,4-dione (59). Obtained from 1,3-thiazolidine-2,4-dione and 1,6-dibromohexane in 45% yield. Chromatography: hexane/EtOAc, 8:2.

2-(8-Bromocyclooctyl)tetrahydro-1H-pyrrolo[1,2-c]imidazo[1,3](2H)-dione (60). Obtained from 1,3-thiazolidine-2,4-dione and 1,8-dibromooctane in 50% yield. Chromatography: hexane/EtOAc, 9:1.

Synthesis of Ethyl (2R)-(−)- and (2S)-(−)-Chromane-2-carboxylate (|−| and (+)-65). To a solution of racemic ethyl chromane-2-carboxylate (±65) (5.00 g, 24.2 mmol) in THF (20 mL), ion-free H2O (140 mL) and 0.05 M phosphate buffer (133 mL, pH 7.0) were added, and the mixture was stirred at pH 8.0 with an aqueous solution of 0.1 M NaOH at room temperature. Then, lipase P30 (194 mg, 309 U/mg) was added in portions while the reaction was vigorously stirred and the pH was kept at 8.0 by addition of an aqueous solution of 0.01 M NaOH (about 1.5 L). When pH was stable at 8.0, THF was evaporated and the azeotropic mixture was extracted with EtOAc (3 × 750 mL). The organic layers were dried (Na2SO4) and evaporated to afford enantiopure ester (−)-65 as an oil in 42% yield: [α]20D = −8.7 (c 1.24, MeOH) ([lit. [α]20D = −9.3 (c 1.24, MeOH)]. IR, 1H, and 13C NMR spectra were consistent with those described for racemic (±)-65. The enantiomeric excess (ee) was determined by chiral HPLC analysis carried out on an Agilent 1200 series system (Chiralpak IC, hexane/i-PrOH 95:5, 12 mL/min, 38 bar, 280 nm); (S)-enantomer tR = 7.93 min (minor); (R)-enantomer tR = 11.71 min (major); 97% ee. The aqueous layer was acidified with concentrated HCl to pH 1.0 and extracted with EtOAc (3 × 750 mL). The organic layers were dried (Na2SO4) and evaporated to afford the free carboxylic acid, which was esterified by treatment with EtOH and concentrated HCl to pH 1.0 and extracted with EtOAc (3 × 750 mL). The combined organic layers were dried (Na2SO4) and evaporated to afford the free ester (−)-65 as a white solid in 70% and 72% yield, respectively, which were used in the next step without further purification. (2R)-(−)-Chromane-2-carboxylic acid was obtained. Then, this acid was esterified as described previously in this experiment to afford enantiopure ester (+)-65 as an oil in 38% global yield: [α]20D +8.6 (c 1.24, MeOH) ([lit. [α]20D +9.3 (c 1.24, MeOH)] (95% ee).

Synthesis of (2R)-(−)- and (2S)-(−)-3,4-dihydro-2H-chromene-2-ylmethylamine (|−| and (+)-64). To a mixture of enantiopure ester (−)- or (+)-65 (4.7 g, 22.6 mmol) and ammonium chloride (280 mg, 5.5 mmol), aqueous 28% ammonia (63 mL) was added and the mixture was heated at 100 °C for 2 h. After cooling to room temperature, H2O was added (50 mL) and the solution was extracted with dichloromethane (3 × 750 mL). The combined organic layers were dried (Na2SO4) and evaporated to afford (2R)-(−)- or (2S)-(−)-chromene-2-carboxamide as a white solid in 70% and 72% yield, respectively, which were used in the next step without further purification. (2R)-(−)-Chromene-2-carboxamide: [α]20D +39.8 (c 1.2, MeOH). IR (KBr) ν 3400, 1662, 1605, 1585, 1455. 1H NMR (DMSO-d6) δ 6.18–6.98 (m, 8 H, 1 CHcyc), 7.76–8.27 (m, 8 H, 1 CHcyc), 8.75–9.30 (m, 8 H, 1 CHcyc), 13C NMR (DMSO-d6) δ 28.3, 28.2, 62.1, 72.9, 120.7, 132.5, 133.4, 143.9, 150.2, 157.5, 164.1, 164.9, 170.1 (CO).

1H NMR (CDCl3) δ 6.18–6.98 (m, 8 H, 1 CHcyc), 7.76–8.27 (m, 8 H, 1 CHcyc), 8.75–9.30 (m, 8 H, 1 CHcyc), 13C NMR (CDCl3) δ 28.3, 28.2, 62.1, 72.9, 120.7, 132.5, 133.4, 143.9, 150.2, 157.5, 164.1, 164.9, 170.1 (CO).
To an ice-cooled solution of 1 M diborane in THF (45 mL, 45 mmol), a solution of (2R)-(−) or (2S)-(+)−chroman-2-carboxamide (2.11 g, 11.9 mmol) in anhydrous THF (55 mL) was added dropwise under an argon atmosphere, and the mixture was stirred at room temperature overnight. Then, the reaction mixture was refluxed for 1 h and, after cooling to room temperature, 10% HCl (3.4 mL) was added. The reaction was worked up, and the residue was basified to pH 8.5 with aqueous 10% NaOH and extracted with EtO2 (6 × 50 mL). The combined organic layers were dried (Na2SO4) and evaporated. The residue was purified by column chromatography (dichloromethane/THF, 9:1) to afford the corresponding amines (−)−64 or (+)−64 in 82 and 86% yield, respectively. (−)−64: oil. [α]D20 = −119.5 (c 1.0, THF) (lit.[9] [α]D20 = −122.8 (c 1.0, THF)). (+)−64: [α]D20 = +110.5 (c 1.0, THF) (lit.[9] [α]D20 = +128.8 (c 1.0, THF)).

General Procedure for the Synthesis of Final Compounds 4−35. To a solution of 4 equiv of the corresponding aralkylamine (commercially available or 61−64) in dry acetonitrile (1 mL/mmol), a solution of 1 equiv of the appropriate bromo- or chloroalkyl derivative 42−60 in dry acetonitrile (4 mL/mmol) was added dropwise and under an argon atmosphere. The reaction mixture was stirred at 60 °C overnight. Once at room temperature, the solvent was removed under vacuum. The residue was suspended in an aqueous solution of 20% K2CO3 and extracted with dichloromethane (3 × 50 mL). The organic layer was dried (Na2SO4), and the solvent was evaporated to dryness. The residue was purified by column chromatography using the appropriate eluent, to afford pure 4−35. The free amine was characterized (yield, IR, NMR, dissolved in anhydrous Et2O (6 mL/mmol), and a commercial 1 M HCl(g)/EtO2 solution (3 mL/mmol) was added. The hydrochloride salt was isolated by filtration or evaporation, washed with anhydrous Et2O, dried under high vacuum, and characterized (mp, elemental analysis).

2-(1-Naphthyl)ethylamino)butyltetrahydro-1H-pyrrolo[1,2-c]imidazole-1,3(2H)-dione (4). Obtained from 42 and 1-naphthylmethyamine in 42% yield. Chromatography: EtOAc; mp 150−153 °C (chloroform/hexane). IR (CHCl3) ν 3500, 3300, 1770, 1708. 1H NMR (CDCl3) δ 1.64−1.81 (m, 2H, CH2), 1.97−2.06 (m, 2H, CH2), 2.51−2.91 (m, 4H, CH2), 3.73−3.86 (m, 2H, CH2), 4.04 (dd, J = 9.3, 9.1, 1H, CHAr), 4.20 (s, 2H, CH2Ar), 7.13−7.23 (m, 4H, 2CH2, 2CH, Ar), 7.32 (d, J = 8.6, 2H, CH2), 7.40 (d, J = 7.3, 2H, CH), 7.48 (d, J = 7.3, 2H, CH). Anal. (C20H24N4O2·H2O) C, H, N.


2-(2-[1-Naphthyl]ethylamino)butyltetrahydro-1H-pyrrolo[1,2-c]imidazole-1,3(2H)-dione (6). Obtained from 42 and 2-naphthylmethyamine in 44% yield. Chromatography: dichloromethane/EtOAc, 95:5.


2-(4-[Quinolin-2-yl]methylamino)butyltetrahydro-1H-pyrrolo[1,2-c]imidazole-1,3(2H)-dione (8). Obtained from 42 and amine 63 in 40% yield. Chromatography: EtOAc/EtOH, 7:3; mp 126−127 °C (EtOAc). IR (EtOAc) ν 3385, 3204, 1738, 1578, 1387. 1H NMR (CDCl3) δ 1.56−1.57 (m, 2H, CH, Ar), 1.90−2.27 (m, 2H, CH2), 2.50 (t, J = 6.3, 2H, CH, NH), 3.01−3.24 (m, 1H, 1/2CH2), 3.42 (t, J = 6.8, 2H, CH, N), 3.53−3.69 (m, 1H, 1/2CH2), 3.91−4.00 (m, 3H, CH2Ar, CH2), 7.47 (t, J = 7.1, 1H, CHAr), 7.62−7.77 (m, 3H, 3CH2), 8.02 (d, J = 8.3, 1H, CHAr).

2.32 (m, 1H, 1/2CH2), 3.42 (t, J = 6.8, 2H, CH, N), 3.53−3.69 (m, 1H, 1/2CH2), 3.91−4.00 (m, 3H, CH2Ar, CH2), 7.47 (t, J = 7.1, 1H, CHAr), 7.62−7.77 (m, 3H, 3CH2), 8.02 (d, J = 8.3, 1H, CHAr). 13C NMR (CDCl3) δ 24.8, 25.6, 26.8, 26.9, 27.1, 27.5, 38.7, 45.4, 49.3, 54.1 (10CH2), 63.2, 75.0, 116.7, 120.1 (4CH), 121.9 (C), 127.1, 129.4 (2CH), 154.7 (C). (+)−64: oil. [α]D20 = +110.5 (c 1.0, THF) (lit.[9] [α]D20 = +128.8 (c 1.0, THF)).
2-(4-[2-Pyridin-2-yl]amino)butyltetrahydro-1H-pyrrolo[1,2-c]imidazole-1,3(2H)-dione (17). Obtained from 42 and 2-(pyridin-2-yl)ethyamine in 36% yield. Chromatography: EtOAc.


2-(4-[3,4-Dihydro-2H-chromen-2-ylmethyl]amino)butyl-hexahydropyrrolo[1,2-alpyrazine-1,4-dione (19). Obtained from 44 and amine 64 in 35% yield. Chromatography: EtOAc; oil. Rf (EtOAc/EtOH, 9:1) 0.40. IR (CHCl3) ν 3422, 1663. 1H NMR (CDCl3) δ 1.14−2.09 (m, 9H, CH2,N,N), 2.28−3.24 (m, 1H, 1/2CH2cyc), 2.65−2.93 (m, 6H, 2CH2NH, CH2NH2, 3.29−3.56 (m, 4H, CH2,N), 3.71 (d, J = 10.4, 1H, 1/2CH2cyc), 4.04−4.14 (m, 3H, CH2, NH, CH2cyc), 6.67−6.80 (m, 2H, 2CH2), 6.95−

2-(4-[3,4-Dihydro-2H-chromen-2-ylmethyl]amino)butyl-hexahydropyrrolo[1,2-alpyrazine-1,4-dione (19). Obtained from 44 and amine 64 in 35% yield. Chromatography: diethylmethane/EtOAc, 9:1.

tetrahydro-1H-pyrrolo[1,2-c]imidazole-1,3(2H)-dione (17). Obtained from 42 and 2-(pyridin-2-yl)ethyamine in 36% yield. Chromatography: EtOAc.


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tetrahydro-1H-pyrrolo[1,2-c]imidazole-1,3(2H)-dione (17). Obtained from 42 and 2-(pyridin-2-yl)ethyamine in 36% yield. Chromatography: EtOAc.


2-(4-[3,4-Dihydro-2H-chromen-2-ylmethyl]amino)butyl-hexahydropyrrolo[1,2-alpyrazine-1,4-dione (19). Obtained from 44 and amine 64 in 35% yield. Chromatography: diethylmethane/EtOAc, 9:1.
5-HT2A Receptor. Binding assays were performed by a modification of the procedure previously described by Titeler et al. The frontal cortex was homogenized in 60 volumes of ice-cold buffer (50 mM Tris-HCl, 0.5 mM NaN3, 10 mM MgSO4, pH 7.4 at 25 °C) and centrifuged at 3000g for 15 min at 4 °C. The membrane pellet was washed 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 10 volumes of assay buffer (50 mM Tris-HCl, 0.5 mM NaN3, EDTA, 10 mM MgSO4, 0.1% ascorbic acid, 10 μM pargyline, pH 7.4 at 25 °C). Fractions of 100 μL of the final membrane suspension (about 5 mg/mL of protein) were incubated at 37 °C for 15 min with 0.4 nM [3H] 5-HT1AR in the presence or absence of the competing drug, in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 1 μM cinanserin and represented less than 15% of total binding.

5-HT3 Receptor. Binding assays were performed by a modification of the procedure previously described by Wong et al. The cerebral cortex was homogenized in 9 volumes of ice-cold 0.32 M sucrose and centrifuged at 1000g for 10 min at 4 °C. The supernatant was centrifuged at 17000g for 20 min at 4 °C. The membrane pellet was washed twice by resuspension in 60 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) and centrifugation at 48000g for 10 min at 4 °C. After the second wash, the resuspended pellet was incubated at 37 °C for 10 min and centrifuged at 48000g for 10 min at 4 °C. 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 CaCl2, pH 7.4 at 25 °C). Fractions of 100 μL of the final membrane suspension (about 2 mg/mL of protein) were incubated at 25 °C for 30 min with 0.7 nM [3H]LY 278584, in the presence or absence of the competing drug, in a final volume of 2 mL of assay buffer. Nonspecific binding was determined with 10 μM 5-HT and represented less than 20% of total binding.

5-HT7 Receptor. Binding assays were performed by a modification of the procedure previously described by Grossman et al. The striatum was resuspended in 100 volumes of ice-cold 50 mM Tris-HCl, 0.5 mM Na2EDTA, 10 mM MgSO4, pH 7.4 at 25 °C and centrifuged at 30000g for 15 min at 4 °C. The membrane pellet was washed 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 10 volumes of assay buffer (50 mM Tris-HCl, 0.5 mM Na2EDTA, 10 mM MgSO4, pH 7.4 at 25 °C) and centrifuged at 17000g for 20 min at 4 °C. The membrane pellet was washed twice by resuspension and centrifugation. The final pellet was resuspended in 20 volumes of the assay buffer. Fractions of the final membrane suspension (about 250 μg of protein) were incubated at 25 °C for 30 min with 0.2 nM [3H]pazopine, in the presence or absence of six concentrations of the competing drug, in a final volume of 2 mL of the assay buffer. Nonspecific binding was determined with 10 μM phenolamine and represented less than 20% of total binding.

8-OH-DPAT-Induced Hypothermia in Mice. The procedures used for these studies were based on previously described methods. B Briefly, male Swiss mice (23–28 g) were housed in groups of five, and body temperature was measured with a lubricated digital thermometer probe (pH0331, Panlab, Barcelona) inserted to a depth of 2 cm into the rectum of the mice. Temperature was recorded at 15, 30, and 60 min, after injection of 8-OH-DPAT or the compound to be tested. To study the antagonism to 8-OH-DPAT-induced hypothermia, compounds or vehicle (control) were administered intraperitoneally (ip) 30 min before the injection of 8-OH-DPAT (0.5 mg/kg, subcutaneously). The hypothermic response to 8-OH-DPAT was measured as the maximum decrease in body temperature recorded in this time period. The results were expressed as change in body temperature with respect to basal temperature, measured at the beginning of the experiment. The obtained data were analyzed by Anova followed by Student–Newman–Keuls test.

cAMP Formation in HeLa Cells Transfected with the h5-HT1AR. A HeLa cell line permanently expressing the h5-HT1AR gene (kindly donated by Cajal Institute, Madrid) was cultured in Dulbecco's modified Eagle medium (DMEM) supplemented with 2 mM glutamine, 1 mM pyruvate, and 10% heat-inactivated fetal calf serum (FCS). Subcultures were made by using 0.025% trypsin in phosphate buffered saline (PBS). Cultures were maintained at 37 °C in an air/CO2 (95:5) water-saturated atmosphere. cAMP experiments were carried out with cultures grown for 2–3 days in 8-well culture plates with 2 mL medium/well.

Cultures (about 7.5 × 105 cells/well) were washed with PBS and incubated for 10 min with 1 mL of PBS containing 0.5 mM isobutylmethylxanthine and 10 μM forskolin in the presence or absence of test compounds. The medium was then aspirated, and the reaction stopped by addition of 600 μL of ice-cold ethanol. Two hours later, ethanol was taken into an Eppendorf tube to be lyophilized, and the resulting pellet was resuspended in 100 μL of assay buffer (K 113808, in the presence or absence of the competing drug, in a final volume of 1 mL of assay buffer. Nonspecific binding was determined with 30 μM 5-HT and represented less than 20% of total binding.

D2 Receptor. Binding assays were performed by a modification of the procedure previously described by Leysen et al. The striatum was homogenized in 50 mM Tris-HCl (pH 7.4 at 25 °C) and centrifuged at 48000g for 10 min. The pellet was resuspended and centrifuged twice as before. The final pellet was resuspended in 20 volumes of assay buffer (50 mM Tris-HCl (pH 7.1 at 25 °C) containing 120 mM NaCl, 5 mM KCl, 1 mM CaCl2, 1 mM MgCl2, and 5.7 mM ascorbic acid). Fractions of the final membrane suspension (125–150 μg of protein) were incubated at 37 °C for 15 min with 0.11 nM [3H]spiperone, in the presence or absence of six concentrations of the competing drug, in a final volume of 0.55 mL of the assay buffer (pH 7.4 at 25 °C). Nonspecific binding was determined with 1 μM (+)-butaclamol and represented less than 20% of total binding.

α1 Adrenoceptor. Binding assays were performed by a modification of the procedure previously described by Ambrosio et al. The cerebral cortex was homogenized in 20 volumes of ice-cold buffer (50 mM Tris-HCl, 10 mM MgCl2, pH 7.4 at 25 °C) and centrifuged at 30000g for 15 min. The pellet was washed twice by resuspension and centrifugation. The final pellet was resuspended in 20 volumes of the assay buffer. Fractions of the final membrane suspension (about 250 μg of protein) were incubated at 25 °C for 30 min with 0.2 nM [3H]prazosin, in the presence or absence of six concentrations of the competing drug, in a final volume of 2 mL of the assay buffer. Nonspecific binding was determined with 10 μM phenolamine and represented less than 20% of total binding.
deprivation in neuron-enriched cultures was performed as described. Culture medium was replaced by a solution containing (mM): 130 NaCl, 5.4 KCl, 1.8 CaCl2, 26 NaHCO3, 0.8 MgSO4, 1.18 NaH2PO4, and 25 2-deoxy-g-glucose. Cells were transferred to an anaerobic chamber (Forma Scientific, USA) containing 95% N2/5% CO2 at 37 °C for 150 min. After this time, the solution was replaced by DMEM medium supplemented with 33 mM glucose, and plates were incubated for 18 h in 5% CO2 atmosphere. Test compounds were added at the time of initiating oxygen-glucose deprivation. Cell survival was estimated by measuring the activity of mitochondrial dehydrogenase on 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), as described.

Focal Cerebral Ischemia in Rats. Focal cerebral ischemia was produced in male Sprague—Dawley rats by permanent intraluminal occlusion of the middle cerebral artery (MCAO), as previously described. The day before ischemia, anesthesia was induced with 4% halothane. Rats were placed in the prone position on a stereotaxic frame, and anesthesia was maintained at 1.5–2% halothane. The left femoral artery was cannulated to monitor mean arterial blood pressure, and body temperature was maintained at 37.5 °C with a heating blanket connected to a rectal probe. A 2.6 cm length of 3–0 monofilament nylon suture heated-blunted at the tip was introduced into the external carotid artery through a puncture. The nylon suture was gently advanced into the internal carotid artery and circle of Willis until the origin of the MCA was reached, at approximately 22 mm from the carotid bifurcation. Infarct volume was measured 24 h later. Rats were killed while under halothane anesthesia, and the brains were rapidly frozen and kept at −20 °C. Coronal brain sections of 20 μm were obtained with a cryostat and stained with an image analyzing system (AIM Image Research). Test compounds were administered (intravenously bolus injection) one hour before ischemia and one hour later or, alternatively, were given by continuous iv infusion.

Molecular Modeling. Modeler v9.5 was used to build a homology model of the human 5-HT1A receptor using the crystal structure of the β2-adrenergic receptor (PDB code 2RH1) as template. The general Amber force field (GAFF) and HF/6-31G*-derived RESP atomic charges were used for the ligand. Molecular dynamics simulation of the ligand–receptor complex were performed with the Sander module of AMBER 10 using the protocol previously described.

ASSOCIATED CONTENT

Supporting Information Spectral characterization data of compounds 5–7, 10, 11, 13–15, 17, 18, 20, 22, 24–27, 29, 30, 32, 33, 35, 47, 48, 50–52, 54, and 57–60 as well as combustion analysis data of final compounds 4–35. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

DMEM, Dulbecco’s modified Eagle medium; FCS, fetal calf serum; GPCR, G protein-coupled receptor; h5-HT1A-R, human 5-HT1A receptor; ip, intraperitoneally; iv, intravenously; MCAO, occlusion of the middle cerebral artery; PBS, phosphate buffered saline; TM, transmembrane

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