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# Novel, flexible, and conformationally defined analogs of gepirone: synthesis and 5-$HT_{1A}$, 5-$HT_{2A}$, and $D_2$ receptor activity

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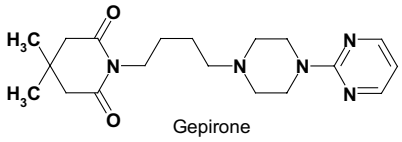
**Abstract**—Novel, flexible arylpiperazine gepirone analogs (**1a**–**3a**) with a mixed 5-$HT_{1A}$/5-$HT_{2A}$ receptor profile, low $D_2$ receptor affinity, and agonistic (**2a**) or partial agonistic (**1a**, **3a**) activity toward 5-$HT_{1A}$ receptor sites were synthesized. Their conformationally restricted counterparts (**1b**–**3b**) were selective 5-$HT_{1A}$ ligands (over 5-$HT_{2A}$ and $D_2$ receptors), which turned out to be agonists (**2b**, **3b**), or partial agonist (**1b**) of 5-$HT_{1A}$ receptors.


## 1. Introduction

Gepirone, a structural analog of the nonbenzodiazepine anxiolytic buspirone belongs to long-chain arylpiperazine (LCAP) 5-$HT_{1A}$ receptor ligands and is a selective agonist of these receptor sites showing anxiolytic/antidepressant properties.[1] It has been demonstrated that compared to buspirone, gepirone possesses a much greater selectivity for 5-$HT_{1A}$ receptors over dopamine $D_2$ sites.[2] Recent clinical trials have shown that the extended-release formulation of gepirone is effective and well-tolerated in the treatment of major depressive disorder; actually, it affects the core symptoms of depression, including lethargy and anxiety[3,4] (Chart 1).



Gepirone

**Chart 1.**

Our research group has successfully developed the concept of restricting the conformational freedom of various LCAP 5-$HT_{1A}$ receptor agents, such as NAN190,[5] MP3022,[5] or MM77,[6] by replacing their flexible aliphatic spacer with a disubstituted cyclohexane linker. Such rigid molecules may serve as conformationally defined templates in future QSAR studies. These facts, together with the finding that the rigid analog of gepirone described by Chilmonczyk et al.[7] exhibited very low affinity for 5-$HT_{1A}$ receptor sites, prompted us to examine the 5-$HT_{1A}$ receptor activity of different arylpiperazine derivatives of gepirone both in vitro and in vivo. We designed and synthesized new gepirone analogs containing tetramethylene (**1a**–**3a**) or a conformationally defined 1e,4e-cyclohexylene (**1b**–**3b**) spacer which connected 2-methoxy-, 3-chloro- and 3-trifluoromethylsubstituted 1-phenylpiperazine pharmacophore, and the 3,3-dimethylglutaric imide fragment (Table 1). The 5-$HT_{1A}$, 5-$HT_{2A}$ and dopamine $D_2$ receptor affinity, and the 5-$HT_{1A}$ receptor functional profile were determined for all the newly synthesized compounds and gepirone.

## 2. Chemistry

The synthesis routes for the compounds under study are shown in Scheme 1. The essentially required flexible

*Keywords*: Flexible and conformationally defined gepirone analogs; Selective 5-$HT_{1A}$ receptor ligands; 5-$HT_{1A}$/5-$HT_{2A}$ Receptor ligands; 5-$HT_{1A}$ Receptor agonists and partial agonists.
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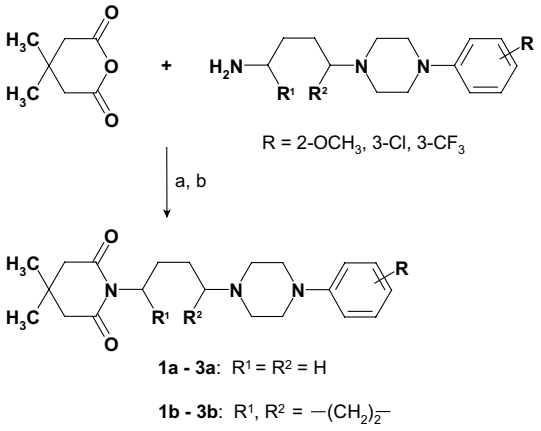
**Table 1.** Structure, 5-$HT_{1A}$, 5-$HT_{2A}$ and $D_2$ receptor affinities of the tested compounds

| Compd | R | $R^1$ | $R^2$ | $K_i \pm$ SEM (nM) | | |
|---|---|---|---|---|---|---|
| | | | | 5-$HT_{1A}$ | 5-$HT_{2A}$ | $D_2$ |
| **1a** | 2-$OCH_3$ | H | H | 8 ± 2 | 83 ± 4 | 310[a] |
| **1b** | 2-$OCH_3$ | –$(CH_2)_2$– | | 22 ± 4 | 4270 ± 34 | 1780[a] |
| **2a** | 3-Cl | H | H | 7 ± 2 | 15 ± 2 | 520[a] |
| **2b** | 3-Cl | –$(CH_2)_2$– | | 15 ± 4 | 247 ± 5 | 5760[a] |
| **3a** | 3-$CF_3$ | H | H | 4 ± 1 | 3.8 ± 0.3 | 2340[a] |
| **3b** | 3-$CF_3$ | –$(CH_2)_2$– | | 9 ± 1 | 426 ± 16 | >10,000[a] |
| Gepirone | | | | 31.8 ± 1.1[b] | 3630[c] | 4320 ± 90 |

[a] The binding experiments to $D_2$ receptors were carried out at two compound concentrations (each run in triplicate) and ligand affinity was expressed as estimated $K_i$ values.
[b] Data from Ref. 8.
[c] Data from Ref. 9.




**Scheme 1.** Reagents and conditions: (a) xylene, reflux, 5 h for **1a**–**3a**, and (a) xylene, reflux, 5 h; (b) $Ac_2O$, AcONa, reflux, 3 h for **1b**–**3b**.

4-(4-arylpiperazin-1-yl)butylamines were prepared according to Glennon et al.[10] by the alkylation of appropriate arylpiperazine with *N*-(4-bromobutyl)phthalimide, followed by the hydrazinolysis of the obtained phthalimides. For the synthesis of the starting constrained 4-(4-arylpiperazin-1-yl)cyclohexylamines, the method described by us previously was applied.[11] The multistage preparation of these amines involved the condensation of benzotriazole, the respective arylpiperazine and 1,4-cyclohexanedione monoethylene ketal, followed by the reduction of the obtained adduct, and the hydrolysis of ketal function. 4-(4-Arylpiperazin-1-yl)cyclohexanones were converted to the corresponding oximes, which were directly reduced to yield the desired amines. Finally, the target analogs of gepirone were synthesized from the appropriate substituted butyl- or cyclohexylamine and 3,3-dimethylglutaric anhydride by heating in xylene. In the case of the compounds of series **b**, intermediate amidoacids were obtained and were then cyclized in acetic anhydride according to a modified procedure for the preparation of 1-phenyl-1H-pyrrole-2,5-dione. [12]

## 3. Pharmacology

New compounds were evaluated for in vitro 5-$HT_{1A}$, 5-$HT_{2A}$, and $D_2$ receptor affinities on the basis of their ability to displace [$^3$H]-8-OH-DPAT [8-hydroxy-2-(di-*n*-propylamino)tetralin], [$^3$H]-ketanserin, and [$^3$H]-spiperone, respectively. The results of the binding studies with flexible **1a**–**3a** and rigid **1b**–**3b** compounds, as well as with gepirone, are presented in Table 1.

The functional 5-$HT_{1A}$ receptor activity of the tested compounds and gepirone was evaluated in in vivo tests. It was previously demonstrated that the hypothermia induced by the 5-$HT_{1A}$ receptor agonist, 8-OH-DPAT, in mice depended primarily on stimulation of presynaptic 5-$HT_{1A}$ receptors[13,14] and was abolished by 5-$HT_{1A}$ receptor antagonists such as, for example, WAY 100635.[15] Thus the hypothermia produced by the tested compounds and reduced by WAY 100635 was regarded as a measure of presynaptic 5-$HT_{1A}$ receptor agonistic activity.

To determine the postsynaptic 5-$HT_{1A}$ receptor agonistic effect of the tested 5-$HT_{1A}$ ligands, their ability to induce lower lip retraction (LLR) in rats was tested. The 8-OH-DPAT-induced LLR was found to be related to the activation of postsynaptic 5-$HT_{1A}$ receptors;[16,17] moreover it was shown that the latter symptom was sensitive to 5-$HT_{1A}$ receptor antagonists.[6,15,18]

## 4. Results and discussion

All the investigated new arylpiperazines exhibited higher 5-$HT_{1A}$ receptor affinity ($K_i$ = 4–22 nM) than did gepirone ($K_i$ = 32 nM; Table 1). On the other hand—in contrast to gepirone—compounds with a tetramethylene spacer (**1a**–**3a**) also bound to 5-$HT_{2A}$ receptor sites; indeed, 3-chloro- and 3-trifluoromethyl substituted 1-phenylpiperazines demonstrated very high 5-$HT_{2A}$ receptor affinity ($K_i$ = 15 and 3.8 nM, respectively), while the $K_i$ value for the 2-methoxy analog (**1a**) was 83 nM. Thus, replacement of the 2-pyrimidyl group in the gepirone structure with a 3-chloro-, 3-trifluoromethyl- or a 2-methoxysubstituted phenyl moiety yielded compounds with a mixed 5-$HT_{1A}$/5-$HT_{2A}$ receptor profile. Restriction of the conformational freedom of those ligands caused a significant decrease in the affinity for 5-$HT_{2A}$ receptors; for compounds **1b**–**3b** the $K_i$ values ranged from 247 to 4270 nM. The new derivatives demonstrated weak or negligible affinity for $D_2$ receptor sites. It is

noteworthy that while *trans*-4,4-dimethyl-1-[4-(4-pyrimidin-2-yl-piperazin-1-yl)cyclohexyl]piperidine-2,6-dione, the rigid analog of gepirone, described by Chilmonczyk et al.[7] showed very low 5-$HT_{1A}$ receptor affinity, we succeeded in obtaining aryl analogs of gepirone with a clearly defined 3-D structure, selective over 5-$HT_{2A}$ and $D_2$ receptor sites.

The results of in vivo studies with the newly synthesized compounds (**1a**–**3a** and **1b**–**3b**) and gepirone are presented in Tables 2–5.

Like gepirone, all the tested compounds induced effects characteristic of presynaptic 5-$HT_{1A}$ receptor agonists in a model of hypothermia in mice; indeed, in doses lower than those of gepirone, they decreased the body temperature of mice (Table 2), that effect being reduced or abolished by WAY 100635 (Table 3). In the LLR test (Table 4), both the 3-chlorophenyl derivatives (**2a**, **2b**) and the rigid 3-trifluoromethylphenyl derivative (**3b**) exhibited feature of postsynaptic 5-$HT_{1A}$ receptor agonists: they induced LLR in rats, but did not affect the LLR evoked by 8-OH-DPAT, whereas the flexible 2-methoxy (**1a**) and 3-trifluoromethyl (**3a**) derivatives behaved like partial agonists in that test, since—when given alone—they induced LLR and simultaneously reduced the LLR evoked by 8-OH-DPAT. In contrast, the constrained 2-methoxysubstituted compound (**1b**) showed features of postsynaptic 5-$HT_{1A}$ receptor antagonist.

Summing up, the modification of the gepirone structure consisting in replacing the 1-(2-pyrimidinyl)piperazine pharmacophore with a 2-methoxy-, a 3-chloro- or a 3-trifluoromethylsubstituted 1-phenylpiperazine moiety led to new, flexible arylpiperazine gepirone analogs (**1a**–**3a**) with a mixed 5-$HT_{1A}$/5-$HT_{2A}$ receptor profile and low $D_2$ receptor affinity, showing agonistic (**2a**) or partial agonistic (**1a**, **3a**) activity toward 5-$HT_{1A}$ receptor sites. On the other hand, their rigid analogs (**1b**–**3b**) were found to be selective 5-$HT_{1A}$ receptor ligands (over 5-$HT_{2A}$ and $D_2$ sites), being agonists (**2b**, **3b**) or partial agonist (**1b**) of 5-$HT_{1A}$ receptors. Hence three new arylpiperazines, that is the flexible **2a** and the constrained **2b** and **3b**, retained the 5-$HT_{1A}$ receptor functional properties of gepirone. Further pharmacological characterization of these compounds are in progress to evaluate their potential anxiolytic/antidepressant properties.

## 5. Experimental

### 5.1. Chemistry

Melting points were determined in a Boetius apparatus and are uncorrected. $^1$H NMR spectra were taken with a Varian EM-360L (60 MHz) or a Varian Mercury-VX (300 MHz) spectrophotometer in $CDCl_3$ solutions with TMS as an internal standard. The spectral data of new compounds refer to their free bases. Chemical shifts were expressed in $\delta$ (ppm) and the coupling constants

**Table 2.** The effect of the tested compounds, gepirone and WAY 100635 on the body temperature in mice

| Treatment | Dose (mg/kg) | $\Delta t \pm$ SEM (°C) | | | |
|---|---|---|---|---|---|
| | | 30 min | 60 min | 90 min | 120 min |
| Vehicle | — | −0.1 ± 0.1 | 0.0 ± 0.2 | 0.1 ± 0.1 | −0.1 ± 0.1 |
| **1a** | 5 | −0.8 ± 0.1[a] | −0.8 ± 0.2[a] | −0.6 ± 0.2[a] | −0.5 ± 0.1 |
| | 10 | −2.0 ± 0.3[b] | −1.3 ± 0.2[b] | −0.9 ± 0.3[b] | −0.8 ± 0.2[a] |
| Vehicle | — | −0.1 ± 0.1 | −0.1 ± 0.1 | −0.1 ± 0.1 | −0.1 ± 0.1 |
| **1b** | 2.5 | −0.9 ± 0.2[b] | −1.0 ± 0.1[b] | −0.9 ± 0.2[b] | −0.6 ± 0.1[b] |
| | 5 | −0.8 ± 0.2[b] | −0.6 ± 0.1[b] | −0.7 ± 0.1[b] | −0.4 ± 0.1[a] |
| Vehicle | — | −0.1 ± 0.1 | −0.1 ± 0.1 | −0.1 ± 0.1 | −0.1 ± 0.1 |
| **2a** | 2.5 | −0.5 ± 0.1 | −0.4 ± 0.1 | −0.3 ± 0.1 | −0.4 ± 0.1 |
| | 5 | −1.3 ± 0.3[b] | −0.7 ± 0.2[a] | −0.4 ± 0.2 | −0.3 ± 0.1 |
| Vehicle | — | −0.2 ± 0.1 | −0.1 ± 0.2 | −0.1 ± 0.1 | −0.2 ± 0.1 |
| **2b** | 2.5 | −1.1 ± 0.2[b] | −0.9 ± 0.2[b] | −0.8 ± 0.1[b] | −0.4 ± 0.1 |
| | 5 | −2.1 ± 0.3[b] | −1.3 ± 0.2[b] | −0.7 ± 0.2[a] | −0.6 ± 0.1[a] |
| Vehicle | — | −0.1 ± 0.1 | −0.1 ± 0.1 | −0.2 ± 0.1 | −0.1 ± 0.1 |
| **3a** | 5 | −0.9 ± 0.1[b] | −0.6 ± 0.1[a] | −0.5 ± 0.1 | −0.3 ± 0.1 |
| | 10 | −1.2 ± 0.1[b] | −1.0 ± 0.2[b] | −0.4 ± 0.1 | −0.3 ± 0.1 |
| Vehicle | — | −0.1 ± 0.1 | −0.2 ± 0.1 | −0.1 ± 0.1 | −0.2 ± 0.1 |
| **3b** | 5 | −0.8 ± 0.2[a] | −0.4 ± 0.2 | −0.6 ± 0.2[a] | −0.6 ± 0.2 |
| | 10 | −1.9 ± 0.2[b] | −1.1 ± 0.2[b] | −1.0 ± 0.2[b] | −0.9 ± 0.2[b] |
| Vehicle | — | 0.1 ± 0.1 | −0.2 ± 0.1 | −0.1 ± 0.1 | −0.1 ± 0.1 |
| Gepirone | 10 | −0.7 ± 0.1[b] | −0.6 ± 0.1[b] | −0.7 ± 0.1[b] | −0.6 ± 0.1[b] |
| | 20 | −1.1 ± 0.1[b] | −1.2 ± 0.1[b] | −1.3 ± 0.1[b] | −1.4 ± 0.1[b] |
| Vehicle | — | 0.0 ± 0.1 | 0.1 ± 0.1 | 0.1 ± 0.1 | 0.0 ± 0.1 |
| WAY 100635 | 0.1 | 0.2 ± 0.1 | 0.2 ± 0.1 | 0.1 ± 0.1 | 0.2 ± 0.1 |

The investigated compounds were administered (ip), gepirone and WAY 100635 (sc) 30 min before the test, $n$ = 7–8 mice per group. The absolute mean initial body temperatures were within a range of 36.5 ± 0.5 °C.

[a] $p < 0.05$ versus vehicle.

[b] $p < 0.001$ versus vehicle.

**Table 3.** The effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by investigated compounds and gepirone in mice

| Treatment and dose (mg/kg) | $\Delta t \pm$ SEM (°C) | |
|---|---|---|
| | 30 min | 60 min |
| Vehicle + vehicle | 0.0 ± 0.1 | 0.0 ± 0.1 |
| Vehicle + **1a** (10) | −2.0 ± 0.3[b] | −1.3 ± 0.2[b] |
| WAY 100635 + **1a** (10) | −1.2 ± 0.2[b,c] | −0.8 ± 0.2[b] |
| Vehicle + vehicle | −0.2 ± 0.1 | −0.2 ± 0.1 |
| Vehicle + **1b** (2.5) | −0.9 ± 0.1[b] | −0.9 ± 0.1[b] |
| WAY 100635 + **1b** (2.5) | −0.3 ± 0.1[d] | −0.4 ± 0.1[c] |
| Vehicle + vehicle | 0.0 ± 0.1 | −0.1 ± 0.1 |
| Vehicle + **2a** (5) | −1.1 ± 0.1[b] | −0.7 ± 0.1[b] |
| WAY 100635 + **2a** (5) | −0.6 ± 0.1[b,d] | 0.0 ± 0.1[d] |
| Vehicle + vehicle | −0.1 ± 0.1 | −0.0 ± 0.1 |
| Vehicle + **2b** (2.5) | −1.6 ± 0.3[b] | −0.7 ± 0.2[a] |
| WAY 100635 + **2b** (2.5) | −0.3 ± 0.2[d] | −0.1 ± 0.1[c] |
| Vehicle + vehicle | −0.2 ± 0.1 | −0.1 ± 0.1 |
| Vehicle + **3a** (10) | −1.2 ± 0.3[b] | −0.9 ± 0.1[b] |
| WAY 100635 + **3a** (10) | −0.5 ± 0.1[c] | −0.5 ± 0.1[c] |
| Vehicle + vehicle | −0.2 ± 0.1 | −0.1 ± 0.1 |
| Vehicle + **3b** (10) | −1.9 ± 0.2[b] | −1.1 ± 0.2[b] |
| WAY 100635 + **3b** (10) | −0.5 ± 0.3[d] | 0.0 ± 0.2[d] |
| Vehicle + vehicle | 0.1 ± 0.1 | −0.2 ± 0.1 |
| Vehicle + gepirone (20) | −1.1 ± 0.1[b] | −1.2 ± 0.1[b] |
| WAY 100635 + gepirone (20) | −0.1 ± 0.1[d] | −0.2 ± 0.1[d] |

WAY 100635 was administered (sc) 15 min before investigated compounds, $n = 7$–8 mice per group. The absolute mean body temperatures were within a range of 36.1 ± 0.5 °C.
[a] $p < 0.05$ versus vehicle + vehicle.
[b] $p < 0.001$ versus vehicle + vehicle.
[c] $p < 0.05$ versus vehicle + investigated compounds.
[d] $p < 0.001$ versus vehicle + investigated compounds.

**Table 4.** Induction of lower lip retraction (LLR) by the tested compounds and gepirone (A) and their effect on the 8-OH-DPAT-induced LLR (B) in rats

| Treatment | Dose (mg/kg) | Mean ± SEM LLR score | |
|---|---|---|---|
| | | A | B |
| Vehicle | — | 0.1 ± 0.1 | 2.7 ± 0.1 |
| **1a** | 5 | 0.4 ± 0.1 | 1.2 ± 0.2[a] |
| | 10 | 1.3 ± 0.4[a] | 0.5 ± 0.2[a] |
| **1b** | 2.5 | 0.1 ± 0.1 | 1.3 ± 0.1[a] |
| | 5 | 0 ± 0 | 0.4 ± 0.2[a] |
| Vehicle | — | 0.1 ± 0.1 | 2.8 ± 0.2 |
| **2a** | 2.5 | 1.3 ± 0.4[a] | 2.4 ± 0.2 |
| | 5 | 2.2 ± 0.3[a] | NT |
| | 10 | 2.3 ± 0.3[a] | NT |
| **2b** | 5 | 1.8 ± 0.2[a] | 2.7 ± 0.2 |
| | 10 | 2.3 ± 0.2[a] | NT |
| Vehicle | — | 0.1 ± 0.1 | 2.8 ± 0.1 |
| **3a** | 5 | 0.8 ± 0.2 | 1.6 ± 0.2[a] |
| | 10 | 1.4 ± 0.2[a] | 0.9 ± 0.3[a] |
| **3b** | 2.5 | 1.5 ± 0.2[a] | 2.5 ± 0.2 |
| | 5 | 2.4 ± 0.2[a] | NT |
| | 10 | 2.4 ± 0.2[a] | NT |
| Vehicle | — | 0.1 ± 0.1 | 2.8 ± 0.1 |
| Gepirone | 5 | 2.2 ± 0.3[a] | NT |
| | 10 | 2.5 ± 0.2[a] | NT |
| | 20 | 2.7 ± 0.2[a] | NT |
| WAY 100635 | 0.1 | 0.1 ± 0.1 | 0.3 ± 0.1[a] |

Compounds **1**–**3** (**a**, **b**) were administered (ip), gepirone and WAY 100635 (sc) 15 min before the test (A) or 45 min before 8-OH-DPAT (sc) (B), $n = 6$ rats per group; NT—not tested.
[a] $p < 0.001$ versus respective vehicle.

**Table 5.** Functional in vivo 5-$HT_{1A}$ activity of the tested compounds and gepirone

| Compd | Presynaptic | Postsynaptic |
|---|---|---|
| **1a** | Agonist | Partial agonist |
| **1b** | Agonist | Antagonist |
| **2a** | Agonist | Agonist |
| **2b** | Agonist | Agonist |
| **3a** | Agonist | Partial agonist |
| **3b** | Agonist | Agonist |
| Gepirone | Agonist | Agonist |

*J* in hertz (Hz). All compounds were routinely checked by TLC using Merck silica gel 60-$F_{254}$ plates (detection at 254 nm). Column chromatography separations were carried out on Merck silica gel 60.

The starting 4-(4-arylpiperazin-1-yl)butylamines were synthesized by published procedures.[10,11] The preparation of 4-[4-(2-methoxyphenyl)piperazin-1-yl]cyclohexylamine,[5] 4-[4-(3-chlorophenyl)piperazin-1-yl]cyclohexylamine, and 4-[4-(3-trifluoromethylphenyl)piperazin-1-yl]cyclohexylamine had been previously published.[11]

**5.1.1. General procedure for the preparation of compounds 1a–3a.** Equimolar amounts (2 mmol) of appropriate 4-(4-arylpiperazin-1-yl)butylamine and 3,3-dimethylglutaric anhydride were refluxed in xylene (20 mL) for 5 h. The solvent was evaporated under reduced pressure and the residue was purified by column chromatography ($SiO_2$, $CHCl_3$/MeOH = 49:1). For pharmacological assays free bases were converted into the hydrochloride salts in acetone (**3a**) or in MeOH (**1a**, **2a**) solutions by the treatment with excess of $Et_2O$ saturated with gaseous HCl.

**5.1.1.1. 1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]butyl}-4,4-dimethylpiperidine-2,6-dione (1a).** The title compound was prepared by the general procedure from 3,3-dimethylglutaric anhydride and 4-[4-(2-methoxyphenyl)piperazin-1-yl]butylamine[10] in 74% yield as yellow oil, $R_f = 0.45$ ($SiO_2$, $CHCl_3$/MeOH = 19:1); $^1$H NMR (60 MHz) $\delta$: 6.9 (s, 4H, Ar–H), 4.0–3.5 [m, 2H, –$(CH_2)_3CH_2$–piperidine-2,6-dione], 3.8 (s, 3H, $OCH_3$), 3.2–2.9 (m, 4H, piperazine $2CH_2$), 2.8–2.2 [m, 6H, piperazine $2CH_2$ and –$(CH_2)_3CH_2$-piperazine], 2.4 (s, 4H, piperidine-2,6-dione $2CH_2$), 1.8–1.3 [m, 4H, –$CH_2(CH_2)_2CH_2$–], 1.0 (s, 6H, $2CH_3$). **1a**·2HCl: colorless crystals, mp 216–218 °C. Anal. Calcd for $C_{22}H_{33}N_3O_3$·2HCl: C, 57.39; H, 7.66; N, 9.13. Found: C, 57.41; H, 7.59; N, 9.30.

**5.1.1.2. 1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]butyl}-4,4-dimethylpiperidine-2,6-dione (2a).** The title compound was prepared by the general procedure from 3,3-dimethylglutaric anhydride and 4-[4-(3-chlorophenyl)piperazin-1-yl]butylamine[11] in 69% yield as yellow

oil, $R_f = 0.57$ ($SiO_2$, $CHCl_3$/MeOH = 19:1); $^1H$ NMR (60 MHz) $\delta$: 7.3–6.9 (m, 4H, Ar–H), 4.0–3.5 [m, 2H, $-(CH_2)_3CH_2-$piperidine-2,6-dione], 3.4–3.0 (m, 4H, piperazine $2CH_2$), 2.7–2.2 [m, 6H, piperazine $2CH_2$ and $-(CH_2)_3CH_2$-piperazine], 2.4 (s, 4H, piperidine-2,6-dione $2CH_2$), 1.9–1.3 [m, 4H, $-CH_2(CH_2)_2CH_2-$], 1.0 (s, 6H, $2CH_3$). **2a**·2HCl: colorless crystals, mp 217–219 °C. Anal. Calcd for $C_{21}H_{30}N_3O_2Cl$·2HCl: C, 54.26; H, 6.94; N, 9.04. Found: C, 54.41; H, 6.70; N, 9.26.

**5.1.1.3. 1-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]butyl}-4,4-dimethylpiperidine-2,6-dione (3a).** The title compound was prepared by the general procedure from 3,3-dimethylglutaric anhydride and 4-[4-(3-trifluoromethylphenyl)piperazin-1-yl]butylamine[11] in 40% yield as yellow oil, $R_f = 0.41$ ($SiO_2$, $CHCl_3$/MeOH = 49:1); $^1H$ NMR (60 MHz) $\delta$: 7.6–6.5 (m, 4H, Ar–H); 4.2–3.6 [m, 2H, $-(CH_2)_3CH_2-$piperidine-2,6-dione], 3.4–3.1 (m, 4H, piperazine $2CH_2$), 2.8–2.2 [m, 6H, piperazine $2CH_2$ and $-(CH_2)_3CH_2$-piperazine], 2.5 (s, 4H, piperidine-2,6-dione $2CH_2$), 2.1–1.4 [m, 4H, $-CH_2(CH_2)_2CH_2-$], 1.0 (s, 6H, $2CH_3$). **3a**·2HCl: colorless crystals, mp 212–214 °C. Anal. Calcd for $C_{20}H_{30}N_3O_2F_3$·2HCl: C, 53.00; H, 6.50; N, 8.40. Found: C, 52.89; H, 6.60; N, 8.33.

**5.1.2. General procedure for the preparation of compounds 1b–3b.** Equimolar amounts (2 mmol) of appropriate 4-(4-arylpiperazin-1-yl)cyclohexylamine and 3,3-dimethylglutaric anhydride were refluxed in xylene (20 mL) for 5 h. The resulting precipitate of noncyclic amidoacid was filtered off and then was heated in acetic anhydride (20 mL) in the presence of anhydrous sodium acetate (30% excess) for 5 h. After cooling the reaction mixture was poured into ice water, neutralized with 10% NaOH, and extracted with $CHCl_3$ ($3 \times 30$ mL). The combined extracts were dried ($K_2CO_3$) and evaporated, to give the oily residue, which was purified by silica gel column chromatography. Free bases were then converted into the hydrochloride salts in $CHCl_3$/MeOH solution by the treatment with excess of $Et_2O$ saturated with gaseous HCl.

**5.1.2.1. *trans*-1-{4-[4-(2-Methoxyphenyl)piperazin-1-yl]cyclohexyl}-4,4-dimethylpiperidine-2,6-dione (1b).** The title compound was prepared by the general procedure from 3,3-dimethylglutaric anhydride and 4-[4-(2-methoxyphenyl)piperazin-1-yl]cyclohexylamine[5] in 50% yield as colorless crystals: mp 210–212 °C, $R_f = 0.32$ ($SiO_2$, $CHCl_3$/MeOH = 19:1); **1b**: $^1H$ NMR (300 MHz) $\delta$: 7.06–6.91 (m, 3H, Ar–H), 6.89 (dd, $J = 8.0$, 1.2 Hz, 1H, Ar–H), 4.59 (tt, $J = 12.2$, 3.8 Hz, 1H, cyclohexane axial H-4), 3.89 (s, 3H, $OCH_3$), 3.20–3,05 (br s, 4H, piperazine $2CH_2$), 2.90–2.75 (br s, 4H, piperazine $2CH_2$), 2.51 (cluster, 5H, piperidine-2,6-dione $2CH_2$ and cyclohexane axial H-1), 2.40 (dq, $J = 12.6$, 3.3 Hz, 2H, cyclohexane axial H-3 and H-3′), 2.10–1.95 (br d, 2H, cyclohexane equatorial H-2 and H-2′), 1.75–1.60 (br d, 2H, cyclohexane equatorial H-3 and H-3′s), 1.45 (dq, $J = 12.4$, 2.8 Hz, 2H, cyclohexane axial H-2 and H-2′), 1.10 (s, 6H, $2CH_3$). **1b**·2HCl: colorless crystals, mp 262–264 °C. Anal. Calcd for $C_{24}H_{35}N_3O_3$·2HCl: C, 59.25; H, 7.67; N, 8.64. Found: C, 59.11; H, 7.57; N, 8.43.

**5.1.2.2. *trans*-1-{4-[4-(3-Chlorophenyl)piperazin-1-yl]cyclohexyl}-4,4-dimethylpiperidine-2,6-dione (2b).** The title compound was prepared by the general procedure from 3,3-dimethylglutaric anhydride and 4-[4-(3-chlorophenyl)piperazin-1-yl]cyclohexylamine[11] in 52% yield as colorless crystals: mp 208–210 °C, $R_f = 0.35$ ($SiO_2$, $CHCl_3$/MeOH = 49:1); $^1H$ NMR (300 MHz) $\delta$: 7.19 (t, $J = 8.1$ Hz, 1H, Ar–H), 6.90 (t, $J = 2.2$ Hz, 1H, Ar–H); 6.82 (dt, $J = 8.3$, 2.6 Hz, 2H, Ar–H), 4.59 (tt, $J = 12.2$, 3.8 Hz, 1H, cyclohexane axial H-4), 3.26–3.18 (br t, 4H, piperazine $2CH_2$), 2.80–2.71 (br t, 4H, piperazine $2CH_2$), 2.56–2.43 (cluster, 5H, piperidine-2,6-dione $2CH_2$ and cyclohexane axial H-1), 2.41 (dq, $J = 12.6$, 3.2 Hz, 2H, cyclohexane axial H-3 and H-3′), 2.06–1.96 (br d, 2H, cyclohexane equatorial H-2 and H-2′), 1.70–1.58 (br d, 2H, cyclohexane equatorial H-3 and H-3′), 1.43 (dq, $J = 12.5$, 3.0 Hz, 2H, cyclohexane axial H-2 and H-2′), 1.09 (s, 6H, $2CH_3$). **2b**·2HCl: colorless crystals, mp 255–258 °C. Anal. Calcd for $C_{23}H_{32}N_3O_2$-Cl·2HCl: C, 56.27; H, 6.98; N, 8.56. Found: C, 56.16; H, 7.06; N, 8.53.

**5.1.2.3. *trans*-1-{4-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]cyclohexyl}-4,4-dimethylpiperidine-2,6-dione (3b).** The title compound was prepared by the general procedure 3,3-dimethylglutaric anhydride and 4-[4-(3-trifluoromethylphenyl)piperazin-1-yl]cyclohexylamine[11] in 40% yield as colorless crystals: mp 198–200 °C, $R_f = 0.35$ ($SiO_2$, $CHCl_3$/MeOH = 49:1); $^1H$ NMR (300 MHz) $\delta$: 7.37 (t, $J = 8.0$ Hz, 1H, Ar–H); 7.14 (s, 1H, Ar–H), 7.09 (d, $J = 8.0$ Hz, 2H, Ar–H), 4.59 (tt, $J = 12.2$, 3.8 Hz, 1H, cyclohexane axial H-4), 3.30–3.22 (br t, 4H, piperazine $2CH_2$), 2.80–2.72 (br t, 4H, piperazine $2CH_2$); 2.57–2.46 (cluster, 5H, piperidine-2,6-dione $2CH_2$ and cyclohexane axial H-1), 2.41 (dq, $J = 12.6$, 3.3 Hz, 2H, cyclohexane axial H-3 and H-3′), 2.08–1.96 (br d, 2H, cyclohexane equatorial H-2 and H-2′), 1.70–1.60 (br d, 2H, cyclohexane equatorial H-3 and H-3′), 1.44 (dq, $J = 12.5$, 3.0 Hz, 2H, cyclohexane axial H-2 and H-2′), 1.09 (s, 6H, $2CH_3$). **3b**·2HCl: colorless crystals, mp 259–261 °C. Anal. Calcd for $C_{24}H_{32}N_3O_2F_3$·2HCl: C, 54.96; H, 6.53; N, 8.01. Found: C, 55.08; H, 6.62; N, 8.11.

### 5.2. In vitro studies—binding experiments

**5.2.1. 5-$HT_{1A}$ and 5-$HT_{2A}$ binding assays.** Radioligand binding experiments were conducted in rat hippocampus for 5-$HT_{1A}$ receptors, and in the cortex for 5-$HT_{2A}$ receptors as previously described.[19] The radioligands used were [$^3$H]-OH-DPAT (170 Ci/mmol, NEN Chemicals) and [$^3$H]-ketanserin (88 Ci/mmol, NEN Chemicals) for 5-$HT_{1A}$ and 5-$H_{2A}$ receptors, respectively. $K_i$ values were determined on the basis of at least three competition binding experiments in which the tested compounds were used in concentrations of $10^{-10}$–$10^{-3}$ M, run in triplicate.

**5.2.2. Dopamine $D_2$ screening assay.** Dopaminergic $D_2$ activity was measured in rat striatal membranes with

[$^3$H]-spiperone (15.70 Ci/mmol, NEN Chemicals) as radioligand according to the previously published procedure.[20] The estimated $K_i$ values were obtained using two compound concentrations (0.1 and 1 μM, each run in triplicate) and were measured in three independent experiments (SEM ± 20%).

Data from binding assays were analyzed using GraphPad Prism.

### 5.3. In vivo studies

The experiments were performed on male Wistar rats (250–300 g) or male Albino Swiss mice (24–28 g). The animals were kept at a room temperature (20 ± 1 °C) on a natural day–night cycle (January–May) and housed under standard laboratory conditions. They had free access to food and tap water before the experiment. Each experimental group consisted of 6–8 animals/ dose, and all the animals were used only once. Gepirone hydrochloride (Bristol–Myers Company), 8-hydroxy-2-(di-*n*-propylamino)tetralin hydrobromide (8-OH-DPAT, Research Biochemical Inc.), and *N*-{2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl}-*N*-(2-pyridinyl)cyclohexanecarboxamide trihydrochloride (WAY 100635, synthesized by Dr. J. Boksa, Institute of Pharmacology, Polish Academy of Sciences, Kraków, Poland) were injected subcutaneously (sc) as aqueous solutions. Suspensions of the compounds **1-3** (**a**, **b**) in a 1% aqueous solution of Tween 80 were injected intraperitoneally (ip). All the compounds were given in a volume of 2 ml/kg (rats) and 10 ml/kg (mice). The obtained data were analyzed by Dunnett's test (when only one drug was given) or by the Newman–Keuls test (when two drugs were administered).

**5.3.1. Body temperature in mice.** The effects of the tested compounds **1-3** (**a**, **b**), gepirone, and WAY 100635 given alone on the rectal body temperature in mice (measured with an Ellab thermometer) were recorded 30, 60, 90, and 120 min after their administration. In an independent experiment, the effect of WAY 100635 (0.1 mg/kg) on the hypothermia induced by compounds **1-3** (**a**, **b**) or gepirone was tested. WAY 100635 was administered 15 min before the tested compounds and the rectal body temperature was recorded 30 and 60 min after their injection. The results were expressed as a change in body temperature ($\Delta t$) with respect to the basal body temperature, as measured at the beginning of the experiment.

**5.3.2. Lower lip retraction (LLR) in rats.** The LLR was assessed according to the method described by Berendsen et al.[16] Compounds **1–3** (**a**, **b**), gepirone, and WAY 100635 were given 15 min before the test. The rats were individually placed in cages (30 × 25 × 25 cm), and they were scored three times (at 15, 30, and 45 min after the administration of all the tested compounds) as follows: 0 = lower incisors not visible, 0.5 = partly visible, 1 = completely visible. The total maximum score amounted to 3/rat. In a separate experiment, the effect of compounds **1–3** (**a**, **b**), gepirone, and WAY 100635 on LLR induced by 8-OH-DPAT (1 mg/kg) was tested. All compounds were administered 45 min before 8-OH-DPAT, and the animals were scored 15, 30, and 45 min after 8-OH-DPAT administration.

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