The concept of multi-target drugs has arisen studying molecular mechanism of action among several efficient drugs, such as neuroleptics, antidepressants, and antineurodegenerative agents which affect many targets simultaneously. Moreover, the pattern of promiscuous drugs is based on the fact that common central nervous disorders, such as depression, schizophrenia, Alzheimer’s or Parkinson’s diseases, and epilepsy, tend to result from multiple molecular abnormalities, and not from a single defect. This multi-target strategy has expanded tremendously the number of potential targets and has led to the introduction of new classes of drugs with potentially less serious side-effects and lower toxicity (1, 2).

An arylpiperazine moiety is one of the most universal templates used for designing agents active at G-protein coupled receptors (GPCRs). Simple arylpiperazines are classified as non-selective receptor ligands, but long-chain arylpiperazines (LCAPs) have been found to be serotonin receptor ligands, in particular 5-HT₁A and 5-HT₂A. Their general chemical structure contains an alkyl chain (2–4 methylene units) attached to the N₄ atom of the piperazine moiety, and a terminal fragment: an amide or imide. Numerous studies have indicated that even a minor structural modification within the LCAP ring or at the terminal fragment (an amide or imide moiety) strongly affects receptor affinity and selectivity (3–7). For several years, we have been developing LCAP-class agents completed with an amide ring, which were evaluated in functional in vivo models of anxiety and depression (8–11).

Abstract: A series of new arylpiperazinypropyl derivatives of 8(6)-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione and spiro[imidazolidine-4,1’-indene/naphthalene]-2,5-dione was synthesized and their affinity was evaluated toward serotonin 5-HT₁A, 5-HT₁D, 5-HT₂, receptors, dopaminergic D₂, D₃ receptors, adrenergic α₁ receptors, and serotonin transporter (SERT). The highest affinity for serotonin 5-HT₁₆/C receptors was found for compounds containing a tetralin or indane moiety in the imide part. Among these, two compounds (19, 20) were selected for further pharmacological in vivo studies. A binding mode of representative molecule 19, which behaved as a 5-HT₁₆ agonist and weak 5-HT₂ antagonism in the site of 5-HT₁₆/C, was also analyzed in computational studies. Moreover, two highly selective (9 and 11) 5-HT₁₆ receptor antagonists were obtained.

Keywords: imidazolidine-2,4-dione, long-chain arylpiperazines, multi-receptor ligands, spirohydantoin
structure of the imidazolidine-2,4-dione (13). Moreover, imidazolidine-2,4-dione derivatives can also be found as antiarrhythmics (azimilide), antimicrobial drugs (nitrofurantoin), skeletal muscle relaxants (dantrolene) and non-steroidal antiinflammatories (nilutamide), while allantoin (5-ureidohydantoin) is used as a keratolytic, astringent, wound remedy, antacid and antipsoriatic drug (13). Imidazolidine-2,4-dione (hydantoin) can be substituted in several positions and the search for hydantoin-based drugs is ongoing.

In order to obtain compounds acting on multiple biological targets, two pharmacophoric systems (hydantoin and LCAPs) were combined. Following the results of our previous study (9, 11), we extended our studies aimed at verification of the impact of the results of our previous study (9, 11), we extended our studies aimed at verification of the impact of the proposal modifications will improve affinity for serotonin and dopamine receptors as well as allow the linker between spirohydantoin derivatives and the arylpiperazine moiety. The influence on serotonin and dopamine receptor activity of different “spiro” substituents at the 5 position of a hydantoin moiety was studied. For this reason, we proposed to introduce an aromatic ring into the “spiro” substituent as a flexible (9–16) or rigid (17–26) fragment. Furthermore, the arylpiperazine fragment was changed into a 1,2,3,4-tetrahydroisoquinoline moiety to diversify the affinity of the designed compounds for serotonin receptors.

In this paper, we report on the synthesis of new propyl spirohydantoin derivatives and their biological evaluation toward monoaminergic receptors (α1, 5-HT1A, 5-HT2A, 5-HT3, 5-HT7, D1, D2), and a serotonin transporter (SERT). We also discuss whether the proposed modifications will improve affinity for serotonin and dopamine receptors as well as allow suitable multi-receptor profile characteristics for antidepressant or antipsychotic activity to be achieved. Furthermore, the interactions of compound 19 with 5-HT1A receptors are discussed based on molecular modeling study results.

EXPERIMENTAL

Chemistry

The structure of the final compounds 9–26 was established on the basis of the results of elemental (C, H, N) and spectral (1H NMR, 13C NMR) analyses. Additional experiments were recorded on Varian Mercury 300 MHz spectrometer (Varian Inc., Palo Alto, CA, USA). Chemical shifts are expressed in parts per million (ppm), using the solvent (CDCl3 or DMSO-d6) signal as an internal standard. Signal multiplets are represented by the following abbreviations: s (singlet), br s (broad singlet), d (doublet), t (triplet), m (multiplet). Melting points were determined in open capillaries on an Electrothermal 9300 apparatus and were uncorrected. Thin-layer chromatography (TLC) was performed on Merck silica gel 60 F254, aluminium sheets (Merck: Darmstadt, Germany), using the following mixtures of solvents: (S1) benzene/ethyl acetate/acetic acid (10 : 5 : 1, v/v/v) and (S2) acetone/isopropanol/chloroform (20 : 10 : 1, v/v/v). Elemental analyses for C, H, N were carried out on an Elementar Vario EL III apparatus (Hanau, Germany). LC/MS analysis was performed on Waters Acquity TQD system, with a Waters TQD quadrupole mass spectrometer with detection by UV (DAD) using an Acquity UPLC BEH C18 column (1.7 µm, 2.1 mm × 100 mm). Water/acetonitrile gradient with 0.1% TFA was used as a mobile phase at a flow rate of 0.3 mL/min.

The starting spirohydantoins (1–4) and intermediate (7, 8) were prepared according to previously described methods (9, 11).

1-(3-Chloropropyl)-8-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (5)

The free base was obtained in 72% yield as a white powder; m.p. 212–214°C; TLC: Rf = 0.56 (S1); Analysis: calcd. for C17H21N2O2Cl: C 63.64, H 6.60, N 8.73%; found: C 63.66, H 6.65, N 8.50%.

1-(3-Chloropropyl)-6-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (6)

The free base was obtained in 73% yield as a white powder: yield 73%; m.p. 210–214°C; TLC: Rf = 0.64 (S1); Analysis: calcd. for C19H23N2O3Cl: C 63.64, H 6.60, N 8.73%; found: C 63.63, H 6.73, N 8.50%.

General procedure for preparing final compounds 9–26

An intermediate 1-(3-chloropropyl)-spirohydantoin (5 mmol) and the substituted 1-phenylpiperazine or tetrahydroisoquinoline (10 mmol) in ethyl alcohol or 2-methoxyethanol (16) were refluxed for 40 h separately. After cooling, the solvent was evaporated and the residue was extracted with CHCl3 (3 × 15 mL). The combined organic phases were dried, filtered off and evaporated. The obtained oily product was purified either by crystallization from anhydrous ethanol (comp. 14, 15, 17–26) or by column chromatography (comp. 9–13, 16), using a mixture of solvents acetone/isopropanol/chloroform (20 : 10 : 1, v/v/v).

3-[3-(4-Phenylpiperazin-1-yl)propyl]-8-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (9)
The free base was obtained in 59% yield as white powder; m.p. 188–190°C; 'H NMR (300 Hz, CDCl₃, δ, ppm): 8.38 (s, 1H), 7.20–7.37 (m, 7H), 6.83–6.99 (m, 3H), 3.64–3.68 (t, 2H, J = 7.20 Hz), 3.17–3.20 (t, 4H, J = 4.70 Hz), 2.60–2.69 (m, 5H), 2.46–2.51 (t, 2H, J = 7.20 Hz), 1.89–2.08 (m, 5H), 1.70–1.83 (m, 5H). TLC: Rf = 0.07 (S); 0.74 (S); HPLC: Rf = 2.41 (99%). LC/MS (m/z): 447.6 [M + H]+. Analysis: calcd. for C₂₂H₂₈N₂O₂ × H₂O: C 69.80, H 7.81, N 12.06%; found: C 69.68, H 7.43, N 11.98%.

3-[3-(4-(2-Methoxyphenyl)piperazin-1-yl)propyl]-8-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (10)

The free base was obtained in 67% yield as white powder; m.p. 179–181°C; 'H NMR (300 Hz, CDCl₃, δ, ppm): 7.65 (br s, 1H), 7.20–7.33 (m, 5H), 6.83–6.99 (m, 4H), 3.85 (s, 3H), 3.61–3.66 (t, 2H, J = 7.18 Hz), 3.07 (br s, 4H), 2.63 (br s, 5H), 2.44–2.48 (t, 2H, J = 7.18 Hz), 1.93–2.08 (m, 5H), 1.65–1.90 (m, 5H). TLC: Rf = 0.79 (S); HPLC: Rf = 2.43 (99%). Analysis: calcd. for C₂₂H₂₈N₂O₂: C 70.56, H 7.61, N 11.76%; found: C 70.42, H 7.65, N 11.55%.

3-[3-(4-(Chlorophenyl)piperazin-1-yl)propyl]-8-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (11)

The free base was obtained in 55% yield as white powder; m.p. 197–199°C; 'H NMR (300 Hz, CDCl₃, δ, ppm): 8.15 (s, 1H), 7.12–7.33 (m, 6H), 6.73–6.83 (m, 3H), 3.63–3.67 (t, 2H, J = 7.00 Hz), 3.20 (br s, 4H), 2.67–2.69 (m, 5H), 2.15–2.20 (t, 2H, J = 6.90 Hz), 1.97–2.08 (m, 5H), 1.67–1.81 (m, 5H). TLC: Rf = 0.71 (S); HPLC: Rf = 2.64 (96%). Analysis: calcd. for C₂₂H₂₈N₂O₂Cl: C 67.42, H 6.91, N 11.65%; found: C 67.24, H 7.06, N 11.49%.

3-[3-(4-(2-Trifluoromethylphenyl)piperazin-1-yl)propyl]-8-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (12)

The free base was obtained in 69% yield as white powder; m.p. 214–216°C; 'F NMR (300 Hz, CDCl₃, δ, ppm): -62.72 (s, 3F); 'H NMR (300 Hz, CDCl₃, δ, ppm): 8.54 (s, 1H), 7.18–7.36 (m, 6H), 6.99–7.06 (m, 3H), 3.63–3.67 (t, 2H, J = 7.30 Hz), 3.18–3.21 (t, 4H, J = 4.80 Hz), 2.56–2.68 (m, 5H), 2.44–2.49 (t, 2H, J = 7.30 Hz), 1.85–2.06 (m, 6H), 1.72–1.79 (m, 4H). TLC: Rf = 0.73 (S); HPLC: Rf = 2.76 (97%); LC/MS (m/z): 515.5 [M + H]+.

3-[3-(4-Dihydro-1H-isoquinolin-2-yl)propyl]-8-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (13)

The free base was obtained in 54% yield as creamy powder; m.p. 179–181°C; 'H NMR (300 Hz, CDCl₃, δ, ppm): 1.60–1.72 (m, 4H), 1.91–2.02 (m, 6H), 2.56–2.62 (m, 3H), 2.67–2.71 (t, 2H, J = 5.20 Hz), 2.85–2.89 (t, 2H, J = 5.20 Hz), 3.59 (s, 2H), 3.65–3.69 (t, 2H, J = 7.00 Hz), 6.96–7.32 (m, 9H), 7.80 (s, 1H). TLC: Rf = 0.64 (S); HPLC: Rf = 2.38 (99%). Analysis: calcd. for C₂₂H₂₈N₂O₂: C 74.79, H 7.48, N 10.06%; found: C 75.00, H 7.48, N 9.98%.

1-[3-[4-(2-Trifluoromethylphenyl)piperazin-1-yl)propyl]-2’,3’-dihydro-2H,5H-spiro[imidazolidine-4,1’-indene]-2,5-dione (20)
The free base was obtained in 60% yield as white powder; m.p. 140–142°C; $^1$H NMR (300 Hz, CDCl$_3$, δ, ppm): 1.82–1.91 (q, 2H, $J = 7.15$ Hz), 2.18–2.28 (m, 1H), 2.40–2.45 (t, 2H, $J = 7.15$ Hz), 2.56–2.59 (t, 4H, $J = 5$ Hz), 2.65–2.74 (m, 1H), 2.98–3.10 (m, 1H), 3.16–3.20 (t, 4H, $J = 5$ Hz), 3.21–3.24 (m, 1H), 3.58–3.63 (t, 2H, $J = 7.15$ Hz), 6.50 (s, 1H), 7.02–7.11 (m, 3H), 7.17–7.35 (m, 5H).

TLC: $R_f = 0.78$ (S 2); HPLC: $R_t = 2.41$ (99%); LC/MS (m/z) 473.5 [M + H]$^+$.  

Compounds 22–24 were previously described (9).

1-[3-(3,4-Dihydro-1H-isoquinolin-2-yl)propyl]-2',3'-dihydro-2H,5H-spiroimidazolidine-4,1'-indene]-2,5-dione (21)  
The free base was obtained in 57% yield as creamy powder; m.p. 139–140°C; $^1$H NMR (300 Hz, CDCl$_3$, δ, ppm): 1.82–1.91 (q, 2H, $J = 7.15$ Hz), 2.18–2.31 (m, 1H), 2.65–2.77 (t, 2H, $J = 7.00$ Hz), 2.99–3.08 (m, 4H), 3.17–3.34 (m, 3H), 3.66–3.70 (t, 4H, $J = 6.80$ Hz), 5.89 (s, 1H), 7.04–7.34 (m, 8H).

TLC: $R_f = 0.74$ (S 2); HPLC: $R_t = 1.93$ (99%). Analysis: calcd. for C$_{23}$H$_{25}$N$_3$O$_2$: C 73.57, H 6.71, N 11.19%; found: C 73.23, H 7.06, N 11.07%.

Table 1. Binding affinity of investigated compounds for serotonin 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_7$ and dopaminergic D$_2$ receptors.  

<table>
<thead>
<tr>
<th>Compd.</th>
<th>5-HT$_{1A}$ $K_i$ ± SEM [nM]</th>
<th>5-HT$_{2A}$ $K_i$ ± SEM [nM]</th>
<th>5-HT$_7$ $K_i$ ± SEM [nM]</th>
<th>D$_2$ $K_i$ ± SEM [nM]</th>
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<tr>
<td>9</td>
<td>1688 ± 75</td>
<td>20 ± 2</td>
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<tr>
<td>10</td>
<td>132 ± 9</td>
<td>147 ± 12</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>11</td>
<td>5211 ± 268</td>
<td>56 ± 7</td>
<td>NT</td>
<td>&gt;10000</td>
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<tr>
<td>12</td>
<td>2280 ± 88</td>
<td>462 ± 34</td>
<td>NT</td>
<td>NT</td>
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<td>&gt;10000</td>
<td>775 ± 16</td>
<td>NT</td>
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<td>14</td>
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<td>320 ± 34</td>
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<td>15</td>
<td>128 ± 15</td>
<td>570 ± 45</td>
<td>NT</td>
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</tr>
<tr>
<td>16</td>
<td>149 ± 9</td>
<td>284 ± 15</td>
<td>249 ± 12</td>
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<td>17</td>
<td>98 ± 16*</td>
<td>30 ± 2*</td>
<td>NT</td>
<td>&gt;10000</td>
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<td>18</td>
<td>49 ± 2*</td>
<td>653 ± 100*</td>
<td>NT</td>
<td>NT</td>
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<td>25 ± 5*</td>
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<td>284 ± 9*</td>
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<td>145 ± 15</td>
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<td>25</td>
<td>22 ± 2</td>
<td>49 ± 4</td>
<td>146 ± 11</td>
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<td>26</td>
<td>768 ± 54</td>
<td>919 ± 46</td>
<td>172 ± 13</td>
<td>NT</td>
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</table>

* data taken from (9), NT – not tested
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Pharmacology

Radioligand binding studies with native 5-HT\textsubscript{1A}, 5-HT\textsubscript{2A}, 5-HT\textsubscript{7} and D\textsubscript{2} receptors (Table 1) were conducted according to the methods previously described (5). Briefly: 5-HT\textsubscript{1A} assays used rat hippocampal membranes, \(^{[3}H\)-8-OH-DPAT (170 Ci/mmol, NEN Chemicals) and 5-HT for non-specific binding; 5-HT\textsubscript{2A} assays used rat cortical membranes, \(^{[3}H\)-ketanserin (88.0 Ci/mmol, NEN Chemicals) and methysergide for nonspecific binding; 5-HT\textsubscript{7} receptor assay was performed using rat hypothalamic membranes, \(^{[3}H\)-5-CT (34.5 Ci/ml, NEN Chemicals) and 5-HT for non-specific binding and D\textsubscript{2} assays used rat striatal membranes, \(^{[3}H\)-spiperone (15.70 Ci/mmol, NEN Chemicals) and butaclamol for nonspecific binding. Each compound was tested in triplicate at 7–8 concentrations (10\textsuperscript{-11}–10\textsuperscript{-6} M). The radioactivity was measured by liquid scintillation counting (Beckman LS 6500 apparatus) in 4 mL scintillation fluid (Akwascyst, BioCare). Binding isotherms were analyzed by nonlinear regression (Prism, GraphPad Software Inc., San Diego, USA), using the Cheng-Prusoff equation to calculate \(K_i\) values. Results were expressed as means of at least two separate experiments.

The extended in vitro evaluation of selected compounds (Table 2) was based on the standard screening procedure (14–19). Detailed conditions of the assays for respective receptors are shown in Table 3. Briefly, the investigated compounds were tested in screening assay at two final concentrations of 1.0 and 0.1 µM. The analyzed sample consisted of 50 µL of working solution of the tested compound, 50 µL of radioligand and 150 µL of a diluted receptor source and were transferred to a 96-well microplate. The microplate was covered with a sealing tape, mixed and incubated. Reaction mixtures were filtered on UniFilter 96 GF/C plate and rapidly washed with 200 µL of chilled 50 mM Tris-HCl buffer (pH 7.0) using vacuum manifold and 96-well pipettor. The filtered plate was dried and 30 µL liquid scintillator Betaplate Scint was added to each well. The radioactivity was measured by MicroBeta TriLux 1450 scintillation counter (PerkinElmer). Results were expressed as percent inhibition of specific binding.

The functional profiles with respect to 5-HT\textsubscript{1A} and 5-HT\textsubscript{7} receptors were determined at Cerep (Le Bois l’Eveque, 86600 Celle L’Evescault, France) (20). Further methodological details of these studies are available on the company’s web site (www.cerep.fr).

Moreover, the pharmacological studies towards 5-HT\textsubscript{2A} were carried out on male Wistar rats ((KRF.(WI).WU), Animal House, Faculty of Pharmacy, Jagiellonian University Medical College, Kraków) weighing 170–350 g. Treatment of laboratory animals in the present study was in full accor-
dance with the respective Polish regulations. All procedures were conducted according to guidelines of ICLAS (International Council on Laboratory Animal Science) and approved by the Local Ethics Committee on Animal Experimentation.

Molecular modeling

The homology models of human 5-HT\textsubscript{1A} and 5-HT\textsubscript{7} serotonin receptors used herein were generated based on developed and well-validated method and described in previously published papers (10, 21, 22). Glide, induced fit docking, LigPrep and Protein Preparation Wizard were implemented in Schrödinger Suite software, which was licensed for Jagiellonian University Collegium Medicum.

RESULTS AND DISCUSSION

The designed spirohydantoins (9–26) were synthesized in a multi-step procedure summarized in Scheme 1. The core spirohydantoins were obtained in a Bucherer-Berg reaction (1–4), following the alkylation at position-N3 of a heterocyclic ring (5–8) (9, 11). Then, coupling with differently substituted phenylpiperazines (9–12, 15–20, 22–25) or tetrahydroisoquinoline (13, 21, 26) gave the final compounds 9–26 in moderate yields (52–72%). All the final products were obtained as racemic mixtures and for the further pharmacological studies they were transformed into water-soluble hydrochloride salts.

In accordance with the strategy of the multi-receptor ligands, the affinity for serotonin and dopamine receptors and for serotonin transporters was determined (Tables 1, 2). Generally, the comparison of substituent at 5 position of the hydantoin moiety showed a noticeable impact on receptor binding properties. The results show that the fusion of an aromatic area with the cycloalkane ring as a rigid skeleton (17–20, 22–25) significantly increased the binding to serotonin 5-HT\textsubscript{1A} and 5-HT\textsubscript{7} receptor sites (Table 1), whereas the introduction of the phenyl ring to 5-cyclohexane-spirohydantoin as a flexible fragment (9–16) resulted in a decreased affinity for those receptors.

Moreover, it seems that also the secondary amine and the nature of the substituents in phenyl ring had a crucial impact on the affinity to the receptors of the tested compounds. The results presented in Table 1 revealed that
almost all selected spirohydantoin derivatives with a tetralin or indane moiety in the amide part (19–21, 24–26) showed moderate activity toward 5-HT₇ receptors. It seems that this activity toward 5-HT₇ receptors is the result of the occurrence in the structure of both the amide mentioned above and an electron withdrawing group/atom in the phenylpiperazine moiety or tetrahydroisoquinoline fragment. Moreover, some compounds with an unsubstituted phenylpiperazine fragment (9, 17, 22) or their counterparts with 3-chloro (11, 19, 24) or 3-trifluoromethyl substituents (20, 25) possessed a moderate-to-high affinity for 5-HT₁A receptors (11–13). In this screening test, most of the potent serotonin receptor ligands revealed a high-to-moderate affinity for adrenergic α₁ receptors.

For further functional and molecular modeling studies, one (19) of the two counterparts (9, 11) which exhibited a sustainable affinity for serotonin 5-HT₁A receptors and a moderate affinity for dopaminergic D₃ receptors was chosen as an example. Moreover, for functional profile evaluation, the two compounds (9, 11) with the highest selectivity and affinity for 5-HT₁₄ receptors were selected.

The binding mode of the lead compound 19 at the sites of serotonin 5-HT₁₄ and 5-HT₇ receptors was analyzed in detail, as a representative one. To this end, the previously developed homology models of the receptors were used (20). The tested compound was synthesized in a racemic form; nevertheless, predominantly better scores and more favorable interactions in both targets were observed for the S enantiomer, and therefore its binding mode was described. The binding mode of the ligand in the two receptors was shown to be consistent both with the common one for monoaminergic receptor ligands and with previous results (20, 23). The compound 19 molecule in the 5-HT₁₄ receptor adopted linear conformation, extending from the deeper cavity formed by transmembrane helices (TMHs) 3–6 to the second interaction pocket located between TMHs 1, 2 and 7. In the 5-HT₇ receptor, the molecule bent to find interactions in less spatial pocket situated closer to TMH3. The main anchoring interaction in both sites was a charge-reinforced hydrogen bond between the protonated nitrogen atom of the ligand and the carbonyl group of Asp3.32, as well as CH-π interactions of the arylpiperazine and aromatic amino acid cluster of the deeper cavity.
mainly Phe6.52 (Fig. 1). The spirohydantoin fragment of the molecule occupied the additional cavity and found both the hydrophobic and polar, favorable contacts there, which varied depending on the receptor type. For 5-HT$_{1A}$ receptors, the carbonyl oxygen of hydantoin formed an h-bond with the NH$_2$ group of Asn7.39, while the aromatic ring of indane interacted with the phenyl ring of Tyr2.64 ($\pi-\pi$ stacking, Fig. 1A). In the 5-HT$_7$ receptor, the latter fragment formed an analogous interaction with Phe3.28, although the conformation seems to be suboptimal, since the complex lacks additional favorable interactions of h-bond nature (e.g., with Arg7.36), which may contribute to the relatively lower affinity of compound 19 for this site (Fig. 1B). On the other hand, the $m$-Cl substituent at the phenylpiperazine fragment is devoid of polar interactions with, for example, Ser5.42 or Lys191 from the second extracellular loop (ECL), which, if present, might have increased affinity for 5-HT$_{1A}$ receptors.

On the basis of binding affinity results, compound 19 was selected as an example for functional \textit{in vitro} screening toward serotonin 5-HT$_{1A}$ and 5-HT$_7$ receptors. Compound 19 was classified (Fig. 2) as an agonist of 5-HT$_{1A}$ receptors (59.5% in 1.0E-06 M) and a weak antagonist of 5-HT$_7$ receptors (41.1% in 1.0E-06 M).

The antagonist activity of compounds 9 and 11 toward 5-HT$_{3A}$ receptors present in rat aorta was assessed via the inhibition of serotonin-induced contractions (Fig. 3). Both compounds 9 and 11 displayed an ability to block the contractions induced by serotonin, giving a pK$_\text{B}$ value estimate of 7.665 ± 0.034 and 7.110 ± 0.048, respectively. It is noticeable that the affinity from the functional tests for the studied compounds was in the same concentration range as that determined in the radioligand binding assay.

CONCLUSION

In conclusion, we described the synthesis of 8/6-phenyl-1,3-diazaspiro[4.5]decan-2,4-diones and 2í,3í-dihydro-2H,5H/3í,4í-dihydro-2H,2íH,5H-spiro[imidazolidine-4,1í-indene/naphthalene]-2,5-

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**Figure 1.** Binding modes of compound 19 in the binding sites of 5-HT$_{1A}$ (A) and 5-HT$_7$ (B) receptors. Amino acid residues engaged in ligand binding (within 4 Å from the ligand atoms) are shown as thick sticks. Dotted yellow lines represent H-bonds with polar residues. For the sake of clarity a part of ECL2 was hidden. TMH – transmembrane helix; ECL – extracellular loop.
diones connected with an arylpiperazine or tetrahydroisoquinoline fragment by the propylene carbon chain, which have proven to be potent serotonin receptor ligands. The obtained pharmacological results demonstrated that the introduction of an aromatic area into the cycloalkane ring as rigid fragment (indane or tetralin) at position 5 of imidazolidine-2,4-dione noticeably increases the affinity for serotonin receptors. Moreover, the presence of a withdrawing group substituted into a phenylpiperazine moiety had a positive impact on the binding at 5-HT_1A, 5-HT_2A, 5-HT_7 receptor sites. In contrast, the replacement of arylpiperazine fragment with tetrahydroisoquinoline moiety resulted in decreased affinity for 5-HT_1A,5-HT_2A receptors. Therefore, based on preliminary pharmacological research, two com-
pounds (19, 20) which possessed high affinity for serotonin 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_7$ receptors and moderate affinity for dopaminergic D$_3$ receptors were selected for further pharmacological studies. Furthermore, interactions with serotonin 5-HT$_{1A}$/7 were described for compound 19, which behaved as a 5-HT$_{1A}$ agonist and weak 5-HT$_7$ antagonist. Additionally, from among the compounds with multi-receptor profile, we obtained two compounds (9 and 11) with suboptimal affinity which behave as antagonists of 5-HT$_{2A}$ receptors.

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REFERENCES


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