Acta Poloniae Pharmaceutica – Drug Research, Vol. 73 No. 6 pp. 1545–1554, 2016
ISSN 0001-6837
Polish Pharmaceutical Society

DRUG SYNTHESIS

NEW SPIROHYDANTOIN DERIVATIVES – SYNTHESIS, PHARMACOLOGICAL EVALUATION, AND MOLECULAR MODELING STUDY

ANNA CZOPEK[1]*, AGNIESZKA ZAGÓRSKA[1], MARCIN KOŁACZKOWSKI[1], ADAM BUCKI[1], BEATA GRYZŁO[1], JOANNA RYCHTYK[1], MACIEJ PAWŁOWSKI[1], AGATA SIWEK[2], GRZEGORZ SATAŁA[3], ANDRZEJ BOJARSKI[3], MONIKA KUBACKA[4] and BARBARA FILIPEK[4]

[1]Department of Pharmaceutical Chemistry, [2]Department of Pharmacobiology, [4]Department of Pharmacodynamics, Faculty of Pharmacy, Jagiellonian University Medical College, Medyczna 9, 30-688 Kraków, Poland
[3]Department of Medicinal Chemistry, Institute of Pharmacology, Polish Academy of Sciences, Smętna12, 31-343 Kraków, Poland

Abstract: A series of new arylpiperazinylpropyl 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-HT_{1A}, 5-HT_{2A}, 5-HT_7 receptors, dopaminergic D_2, D_3 receptors, adrenergic α_1 receptors, and serotonin transporter (SERT). The highest affinity for serotonin 5-$HT_{1A/2A/7}$ 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-HT_{1A} agonist and weak 5-HT_7 antagonist in the site of 5-$HT_{1A/7}$, was also analyzed in computational studies. Moreover, two highly selective (**9** and **11**) 5-HT_{2A} receptor antagonists were obtained.

Keywords: imidazolidine-2,4-dione, long-chain arylpiperazines, multi-receptor ligands, spirohydantoin

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-HT_{1A} and 5-HT_{2A}. 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).

Imidazolidine-2,4-dione is an important core unit that exhibits a range of central and peripheral biological activities and is incorporated into many drugs with numerous therapeutic applications (12, 13). Therefore, well-known antiepileptic agents such as phenytoin, mephenythoin, norantoin, methetoin, ethotoin, fosphenytoin are based on the

* Corresponding author: e-mail: aczopek@cm-uj.krakow.pl; phone: +48 12 6205450, fax: +48 12 6205450

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 antiandrogens (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 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 (α_1, 5-HT_{1A}, 5-HT_{2A}, 5-HT_6, 5-HT_7, D_2, D_3) 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-$HT_{1A/7}$ 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 (^{1}H NMR, ^{19}F NMR) analyses. NMR spectra 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 ($CDCl_3$ or DMSO-d_6) 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 F_{254} aluminium sheets (Merck; Darmstadt, Germany), using the following mixtures of solvents: (S_1) benzene/ethyl acetate/acetone (10 : 5 : 1, v/v/v) and (S_2) acetone/isopropanol/chloroform (20 : 10 : 1, v/v/v). Elemental analyses for C, H, N were carried 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 white powder ; m.p. 212–214°C; TLC: R_f = 0.56 (S_1); Analysis: calcd. for $C_{17}H_{21}N_2O_2Cl$: C 63.64, H 6.60, N 8.73%; found: C 63.63, H 6.73, 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 white powder: yield 73%; m.p. 210–211°C; TLC: R_f = 0.64 (S_1); Analysis: calcd. for $C_{17}H_{21}N_2O_2Cl$: C 63.64, H 6.60, N 8.73%; found: C 63.66, H 6.65, N 8.55%.

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 (**9**–**12**, **14**, **15**, **17**–**26**) or butyl (**13**) alcohol or 2-methoxyethanol (**16**) were refluxed for 40 h separately. After cooling, the solvent was evaporated and the residue was extracted with $CHCl_3$ (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; ^{1}H NMR (300 Hz, $CDCl_3$, 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: R_f = 0.07 (S_1); 0.74 (S_2); HPLC: R_t = 2.41 (99%); LC/MS (m/z): 447.6 [M + H]$^+$. Analysis: calcd. for $C_{27}H_{34}N_4O_2 \times H_2O$: 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; ^{1}H NMR (300 Hz, $CDCl_3$ 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: R_f = 0.79 (S_2); HPLC: R_t = 2.43 (99%). Analysis: calcd. for $C_{28}H_{36}N_4O_3$: C 70.56, H 7.61, N 11.76%; found: C 70.42, H 7.65, N 11.55%.

***3*-{3-[4-(3-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; ^{1}H NMR (300 Hz, $CDCl_3$ 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: R_f = 0.71 (S_2); HPLC: R_t = 2.64 (96%). Analysis: calcd. for $C_{27}H_{33}N_4O_2Cl$: C 67.42, H 6.91, N 11.65%; found: C 67.24, H 7.06, N 11.49%.

3-{3-[4-(3-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; ^{19}F NMR (300 Hz, $CDCl_3$ δ, ppm): -62.72 (s, 3F); ^{1}H NMR (300 Hz, $CDCl_3$, 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: R_f = 0.73 (S_2); HPLC: R_t = 2.76 (97%); LC/MS (m/z) 515.5 [M + H]$^+$.

3-[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; ^{1}H NMR (300 Hz, $CDCl_3$ 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: R_f = 0.64 (S_2); HPLC: R_t = 2.38 (99%). Analysis: calcd. for $C_{26}H_{31}N_3O_2$: C 74.79, H 7.48, N 10.06%; found: C 75.00, H 7.48, N 9.98%.

3-[3-(4-Phenylpiperazin-1-yl)propyl]-6-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (14)

The free base was obtained in 60% yield as white powder; m.p. 208–209°C; ^{1}H NMR (300 Hz, $CDCl_3$ δ, ppm): 1.14–1.28 (m, 2H) 1.42–1.58 (m, 3H), 1.76–1.91 (m, 5H), 2.04–2.09 (t, 2H, *J* = 7.00 Hz), 2.44–2.47 (t 4H, *J* = 5.00 Hz,), 3.07–3.09 (m, 1H), 3.14–3.18 (t, 4H, *J* = 5.00 Hz), 3.21–3.26 (t, 2H, *J* = 7.00 Hz), 6.81–7.10 (m, 3H), 7.17–7.28 (m, 8H). TLC: R_f = 0.09 (S_1); 0.84 (S_2); HPLC: R_t = 2.27 (97%); LC/MS (m/z) 447.6 [M+H]$^+$. Analysis: calcd. for $C_{27}H_{34}N_4O_2$: C 72.62, H 7.67, N 12.55%; found: C 72.45, H 7.84, N 12.55%.

3-{3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl}-6-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (15)

The free base was obtained in 60% yield as white powder; m.p. 183–184°C; ^{1}H NMR (300 Hz, $CDCl_3$ δ, ppm): 7.16–7.27 (m, 7H), 6.83–7.02 (m, 3H), 3.85 (s, 3H), 3.20–3.25 (t, 2H, *J* = 6.80 Hz), 3.11–3.13 (m, 1H), 3.05 (br s, 4H), 2.51 (br s, 4H), 2.08–2.12 (t, 2H, *J* = 7.20 Hz), 1.76–1.95 (m, 5H), 1.39–1.57 (m, 3H), 1.14–1.26 (m, 2H). TLC: R_f = 0.13 (S_1), 0.77 (S_2); HPLC: R_t = 2.25 (98%). Analysis: calcd. for $C_{28}H_{36}N_4O_3$: C 70.56, H 7.61, N 11.76%; found: C 70.31, H 7.58, N 11.51%.

3-{3-[4-(3-Chlorophenyl)piperazin-1-yl]propyl}-6-phenyl-1,3-diazaspiro[4.5]decan-2,4-dione (16)

The free base was obtained in 54% yield as white powder; m.p. 149–150°C; ^{1}H NMR (300 Hz, $CDCl_3$ δ, ppm): 7.12–7.27 (m, 7H), 6.74–6.86 (m, 3H), 3.19–3.26 (t, 2H, , *J* = 7.10 Hz), 3.13–3.17 (t, 4H, *J* = 5.00 Hz), 3.08–3.09 (m, 1H), 2.42–2.45 (t, 4H, *J* = 5.00 Hz), 2.03–2.08 (t, 2H, *J* = 7.20 Hz), 1.76–1.91 (m, 5H), 1.42–1.58 (m, 3H), 1.13–1.27 (m, 2H). TLC: R_f = 0.66 (S_2); HPLC: R_t = 2.46 (99%). Analysis: calcd. for $C_{27}H_{33}N_4O_2Cl \times H_2O$: C 64.98, H 7.07, N 11.23%; found: C 65.13, H 7.15, N 11.14%.

Compounds **17**-**19** were previously described (9).

1-{3-[4-(3-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; ^{19}F NMR (300 Hz, $CDCl_3$ δ, ppm): -62.76 (s, 3F); ^{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), 3.21–3.29 (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 $[M + H]^+$.

1-[3-(3,4-Dihydro-1H-isoquinolin-2-yl)propyl]-2',3'-dihydro-2H,5H-spiro[imidazolidine-4,1'-indene]-2,5-dione (21)

The free base was obtained in 57% yield as creamy powder; m.p. 139–140°C; 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 $C_{23}H_{25}N_3O_2$: C 73.57, H 6.71, N 11.19%; found: C 73.23, H 7.06, N 11.07%.

Compounds **22**-**24** were previously described (9).

1-{3-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]propyl}-3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione (25)

The free base was obtained in 56% yield as white powder; m.p. 151–153°C; ^{19}F NMR (300 Hz, $CDCl_3$ δ, ppm): -62.74 (s, 3F); ^{1}H NMR (300 Hz, $CDCl_3$, δ, ppm): 7.04–7.36 (m, 8H), 5.78 (s, 1H), 3.40–3.69 (t, 2H, *J* = 7.15 Hz), 3.21–3.24 (t, 4H, *J* = 5 Hz), 2.84–2.90 (m, 2H), 2.59–2.62 (t, 4H, *J* = 5 Hz), 2.45–2.49 (t, 2H, *J* = 7.15 Hz), 2.23–2.31 (m, 2H), 1.74–2.03 (m, 4H). TLC: R_f = 0.81 (S_2); HPLC: R_t = 2.48 (99%); LC/MS (m/z) 487.4 $[M + H]^+$.

1-[3-(3,4-Dihydro-1H-isoquinolin-2-yl)propyl]-3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-naphthalene]-2,5-dione (26)

The free base was obtained in 54% yield as white powder; m.p. 148–151°C; H NMR (300 Hz, $CDCl_3$ δ, ppm): 7.02–7.24 (m, 8H), 5.92 (s, 1H), 3.67–3.73 (t, 4H, *J* = 7.00 Hz), 2.91–3.01 (m, 4H), 2.82–2.86 (t, 4H, *J* = 6.90 Hz), 2.22–2.31 (m, 2H), 1.74–1.99 (m, 4H). TLC: R_f = 0.68 (S_2); HPLC: R_t =

Table 1. Binding affinity of investigated compounds for serotonin 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 and dopaminergic D_2 receptors.

Compd.	Ki ± SEM [nM]			
	5-HT_{1A}	**5-HT_{2A}**	**5-HT_7**	**D_2**
9	1668 ± 75	20 ± 2	NT	>10000
10	132 ± 9	147 ± 12	NT	NT
11	5211 ± 268	56 ± 7	NT	>10000
12	2280 ± 88	462 ± 34	NT	NT
13	>10000	>10000	775 ± 16	NT
14	743 ± 56	320 ± 34	NT	NT
15	128 ± 15	570 ± 45	NT	NT
16	149 ± 9	284 ± 15	249 ± 12	NT
17	98 ± 16*	30 ± 2*	NT	>10000
18	49 ± 2*	653 ± 100*	NT	NT
19	38 ± 1*	53 ± 6*	77 ± 8	>10000
20	24 ± 2	58 ± 7	83 ± 5	NT
21	>10000	>10000	84 ± 7	NT
22	88 ± 21*	25 ± 5*	NT	675 ± 82
23	23 ± 5*	284 ± 9*	NT	965 ± 6
24	350 ± 123*	35 ± 6*	145 ± 15	1800 ± 300
25	22 ± 2	49 ± 4	146 ± 11	>10000
26	768 ± 54	919 ± 46	172 ± 13	NT

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

Table 2. The extended *in vitro* pharmacological results of selected compounds for 5-HT_6, 5-HT_7, D_2, D_3, α_1 receptors and for serotonin transporter (SERT).

Compd.	% of total binding											
	5-HT_6[a]		5-HT_7[b]		D_2[c]		D_3[d]		α_1[e]		SERT[f]	
	10^{-6}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-7}	10^{-6}	10^{-7}
11	79	37	87	25	13	22	72	21	70	5	14	10
12	63	0	70	15	26	7	89	28	15	14	8	0
13	31	0	51	31	17	15	64	35	21	3	0	0
16	3	0	85	63	17	8	39	4	94	39	7	8
19	7	0	98	88	15	21	22	11	87	60	0	0
20	0	0	94	84	12	23	64	34	72	33	0	19
21	13	0	89	79	0	0	32	22	48	36	11	14
24	33	0	91	76	29	19	38	19	90	64	3	2
25	27	0	93	78	32	19	59	27	69	28	0	5
26	26	0	83	63	4	11	15	15	55	34	13	5

% inhibition of specific binding of reference drugs (10^{-6}/10^{-7}): [a] Methiothepin (99/99), Serotonin (75/44); [b] Methiothepin (99/97), Olanzapine (73/54); [c] Haloperidol (97/99), Olanzapine (92/64); [d] Haloperidol (98/99), Olanzapine (90/76); [e] Phentolamine (100/99), Amitriptyline (97/82); [f] Fluoxetine (96/96), Imipramine (96/91)

2.02 (99%). Analysis: calcd. for $C_{24}H_{27}N_3O_2 \times H_2O$: C 70.74, H 7.17, N 10.31%; found: C 70.54, H 6.97, N 10.25%.

Pharmacology *in vitro*

Radioligand binding studies with native 5-HT_{1A}, 5-HT_{2A}, 5-HT_7 and D_2 receptors (Table 1) were conducted according to the methods previously described (5). Briefly: 5-HT_{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_{2A} assays used rat cortical membranes, [^{3}H]-ketanserin (88.0 Ci/mmol, NEN Chemicals) and methysergide for nonspecific binding; 5-HT_7 receptor assay was performed using rat hypothalamic membranes, [^{3}H]-5-CT (34.5 Ci/mmol; NEN) and 5-HT for non-specific binding and D_2 assays used rat striatial 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^{-11}–10^{-4} M). The radioactivity was measured by liquid scintillation counting (Beckman LS 6500 apparatus) in 4 mL scintillation fluid (Akwascynt, 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_{1A} and 5-HT_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_{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-

Table 3. Radioligand screening assay conditions.

Receptor (source)	Radioligand (concentr/Kd)	Nonspecific binding	Assay buffer	Incubation conditions
5-HT$_6$ (14) (human recombinant, HEK-293 cells)	[^{3}H]LSD (2.5/2.2 nM)	50 mM Tris-HCl pH 7.4, 10 µM methiothepin EDTA	10 mM $MgCl_2$; 0.5 mM	60 min, 37°C
5-HT$_7$ (15) (human recombinant, CHO-K1 cells)	[^{3}H]LSD (3.0/2.8 nM)	10 µM methiothepin	50 mM Tris-HCl pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA	60 min, 30°C
D$_2$ (16) (human recombinant, CHO-K1 cells)	[^{3}H]N-methylspiperone (0.4/0.2 nM)	10 µM (+)-butaclamol	50 mM HEPES-HCl, pH 7.4, 50 mM NaCl, 5 mM $MgCl_2$, 0.5 mM EDTA)	60 min, 30°C
D$_3$ (17) (human recombinant cells)	[^{3}H]N-methylspiperone (0.3 nM)	1 µM chlorpromazin	50 mM Tris-HCl pH 7.4, 120 mM NaCl	60 min, 24°C
α_1 (18) (rat cortex)	[^{3}H]prazosin (0.2/0.2 nM)	10 µM phentolamine	50 mM Tris-HCl pH 7.6	30 min, 30°C
SERT (19) (rat cortex)	[^{3}H]citalopram (1.0/1.0 nM)	1 µM imipramine	50 mM Tris-HCl pH 7.7, 150 mM NaCl, 5mM KCl	60 min, 24°C

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$_{1A}$ and 5-HT$_7$ 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 Schro¨dinger 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$_{1A}$ and 5-HT$_7$ 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.

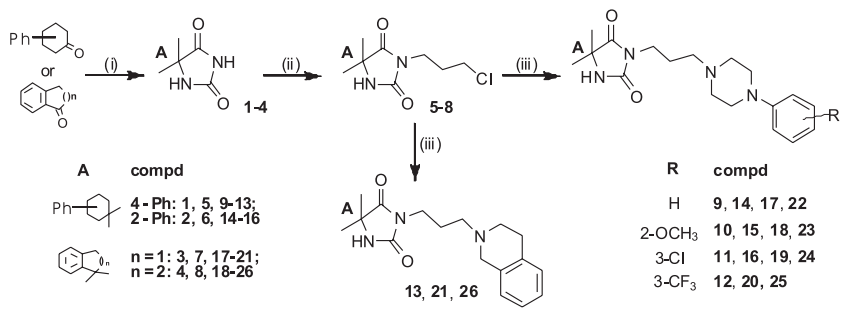
Moreover, it seems that also the secondary amine and the nature of the substituents in phenyl ring had a crucial impact on the affinity to the receptors of the tested compounds. The results presented in Table 1 revealed that

almost all selected spirohydantoin derivatives with a tetralin or indane moiety in the amide part (**19–21**, **24–26**) showed moderate activity toward 5-HT_7 receptors. It seems that this activity toward 5-HT_7 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-trifluromethyl substituents (**20**, **25**) possessed a moderate-to-high affinity for 5-HT_{2A}; two of these show high selectivity for 5-HT_{2A} receptors (**9** and **11**). Furthermore, the presence of an indane and tetralin moiety and a phenylpiperazine fragment in the structure of the investigated compounds had a positive impact on the binding at 5-HT_{1A} receptor sites (**17–20**, **22**, **23**, **25**). The tetrahydroisoquinoline derivatives (**13**, **21**, **26**) showed no affinity for 5-$HT_{1A/2A}$ receptors, but a moderate affinity for 5-HT_7 receptors.

Additionally, the spirohydantoin derivatives with a withdrawing group (Cl, CF_3) substituted into a phenylpiperazine moiety or with a tetrahydroisoquinoline fragment (**11–13**, **16**, **19–21**, **24–25**) were chosen for screening evaluation and their affinity for dopamine (D_2, D_3) and adrenergic (α_1) receptors and serotonin transporters (SERT) was determined (Table 2). In this screening study, all the selected compounds showed a high-to-moderate affinity for 5-HT_7 receptors. However, apart from compound **11** which had weak affinity for 5-HT_6 receptors, the other derivatives were inactive. The tested compounds exhibited a low or no affinity for dopamine D_2 receptors and were practically devoid of any affinity for SERT. Moreover, compounds containing a 3-trifluromethylphenylpiperazine fragment (**12**, **20** and **25**) or a 8-phenyl-cyclohexyl moiety (**11–13**) demonstrated a moderate affinity for dopamine D_3 receptors, unlike the other spirohydantoin derivatives. In this screening test, most of the potent serotonin receptor ligands revealed a high-to-moderate affinity for adrenergic α_1 receptors.

For further functional and molecular modeling studies, one (**19**) of the two counterparts (**19**, **20**) which exhibited a sustainable affinity for serotonin 5-$HT_{1A/2A/7}$ receptors and a moderate affinity for dopaminergic D_3 receptors was chosen as an example. Moreover, for functional profile evaluation, the two compounds (**9**, **11**) with the highest selectivity and affinity for 5-HT_{2A} receptors were selected.

The binding mode of the lead compound **19** at the sites of serotonin 5-HT_{1A} and 5-HT_7 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-HT_{1A} 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-HT_7 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 carboxyl group of Asp3.32, as well as CH-π interactions of the arylpiperazine and aromatic amino acid cluster of the deeper cavity,



Scheme 1. Synthetic pathways of compounds **9–26**. Reagents and conditions: (i) KCN, $(NH_4)_2CO_3$, 50% ethyl alcohol, 28 h, 56°C; (ii) K_2CO_3, KJ, acetone, reflux 20 h (iii), -substituted piperazine derivatives or tetrahydroisoquinoline, 96% ethyl or butyl (compd. **13**) alcohol or 2-methoxyethanol (compd. **16**), reflux 40 h

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 (π-π 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 *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_{2A}-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_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 2',3'-dihydro-2H,5H/3',4'-dihydro-2H,2'H,5H-spiro[imidazolidine-4,1'-indene/naphthalene]-2,5-

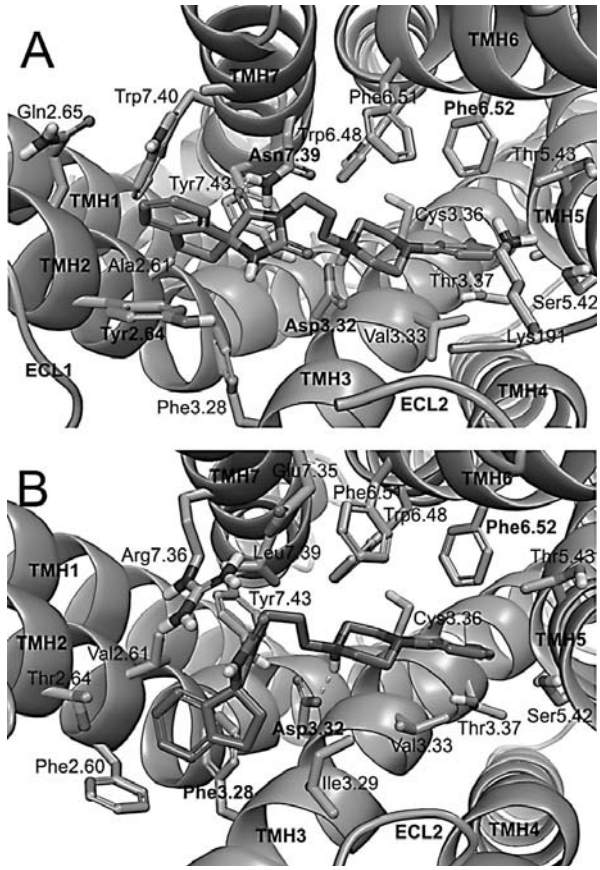


Figure 1. Binding modes of compound 19 in the binding sites of 5-HT_{1A} (A) and 5-HT_7 (B) receptors. Amino acid residues engaged in ligand binding (within 4Å from the ligand atoms) are shown as thick sticks. Dotted yellow lines represent H-bonds with polar residues. For the sake of clarity a part of ECL2 was hidden. TMH – transmembrane helix; ECL – extracellular loop

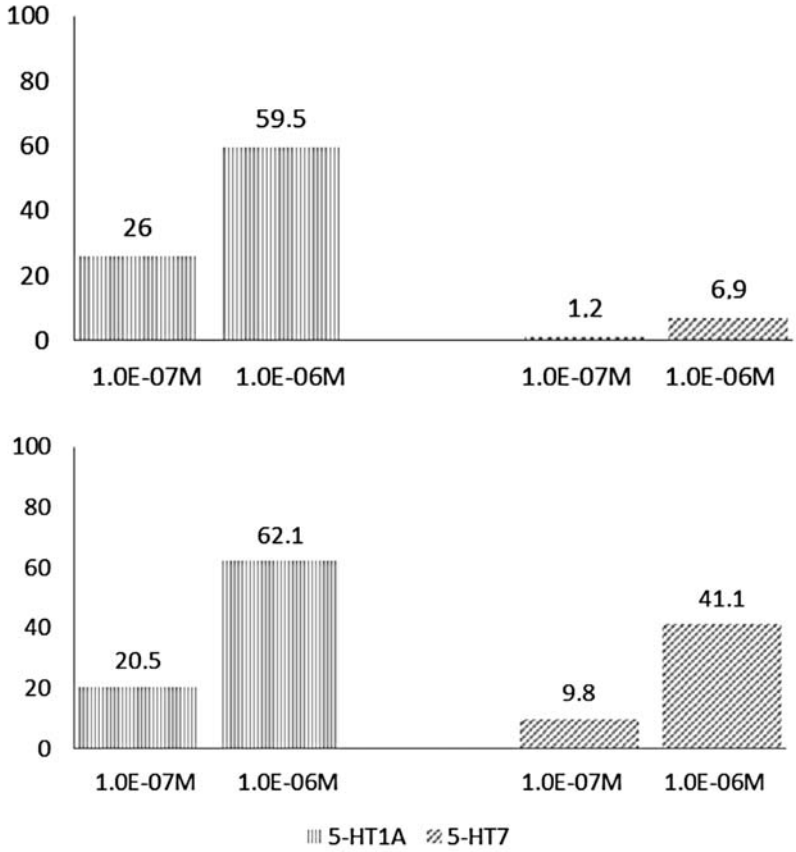


Figure 2. Functional properties for compound **19**, percent of control agonist response (on the top) and percent inhibition of control agonist response (on the bottom)

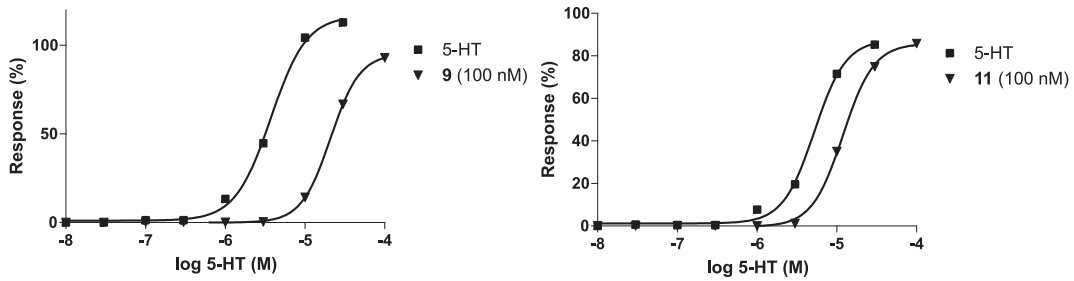


Figure 3. Concentration-response curves to serotonin in the rat aorta in the absence (■) or presence of compound **9** (on the left, ▼100 nM); and 11 (on the right, ▼100 nM); results are expressed as percentage of the maximal response to KCl depolarizing solution. Each point represents the mean ± SEM (n = 4)

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-$HT_{1A/2A}$ 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.

Acknowledgments

This study was financially supported by National Science Center grants (No. DEC–2011/01/B/NZ4/00695, DEC–2012/07/B/NZ7/01173), and by Funds for Statutory Activity of Jagiellonian University Medical College (No. K/ZDS/004652).

REFERENCES

1. Korcsmáros T., Szalay M.S., Böde C., Kovács I.A., Csermely P.: Expert Opin. Drug Discov. 2, 1 (2007).
2. Frantz S.: Nature 437, 942 (2005).
3. Zajdel P., Marciniec K., Maślankiewiecz A., Grychowska K., Satała G. et al.: Eur. J. Med. Chem. 60, 42, (2013).
4. Zagórska A., Pawłowski M., Siwek A., Kazek G., Partyka A. et al.: Arch. Pharm. Chem. Life Sci. 346, 832, (2013).
5. Bojarski A.J., Paluchowska M.H., Duszyńska B., Kłodzińska A., Tatarczyńska E., Chojnacka-Wójcik E.: Bioorg. Med. Chem. 13, 2293 (2005).
6. Kołaczkowski M., Zajdel P., Fhid O., Duszyńska B., Tatarczyńska E., Pawłowski M.: Pharmacol. Rep. 57, 235 (2005).
7. Bojarski A.J., Paluchowska M.H., Duszyńska B., Bugno R., Kłodzińska A. et al.: Bioorg. Med. Chem. 14, 1391 (2006).
8. Byrtus H., Pawłowski M., Czopek A., Bojarski A.J., Duszyńska B. et al.: Eur. J. Med. Chem. 40, 820 (2005).
9. Czopek A., Byrtus H., Kołaczkowski M., Pawłowski M., Dybała M. et al.: Eur. J. Med. Chem. 45, 1295 (2010).
10. Czopek A., Kołaczkowski M., Bucki A., Byrtus H., Pawłowski M. et al.: Arch. Pharm. 346, 98 (2013).
11. Czopek A., Kołaczkowski M., Bucki A., Byrtus H., Pawłowski M. et al.: Bioorg. Med. Chem. 23, 3436 (2015).
12. Avendano C., Menendez J.C.: Hydantoin and Its Derivatives, in Kirk-Othmer Encyclopedia Chemical Technology, 4th edn. pp. 1, John Wiley & Sons, New York 2000.
13. Kleemann A., Engel J., Kutscher B., Reichert D. Pharmaceutical Substances, Synthesis, Patents, Applications, 4th edn., . Georg Thieme, Stuttgart 2001.
14. Monsma F.J.Jr., Shen Y., Ward R.P., Hamblin M.W., Sibley D.R.: Mol. Pharmacol. 43, 320 (1993).
15. Shen Y., Monsma F.J.Jr., Metcalf M.A., Jose P.A., Hamblin M.W., Sibley D.R.: J. Biol. Chem. 268, 18200 (1993).
16. Grandy D.K., Marchionni M.A., Makam H., Stofko R.E., Alfano M. et al.: Proc. Natl. Acad. Sci. USA 86, 9762 (1989).
17. MacKenzie R.G., VanLeeuwen D., Pugsley T.A., Shih Y.H., Demattos S. et al.: Eur. J. Pharmacol. 266, 79 (1994).
18. Greengrass P., Bremner R.: Eur. J. Pharmacol. 55, 323 (1979).
19. Owens M.J., Morgan W.N., Plott S.J., Nemeroff C.B.: J. Pharmacol. Exp. Ther. 283, 1305 (1997).
20. Kołaczkowski M., Marcinkowska M., Bucki A., Pawłowski M., Mitka K. et al.: J. Med. Chem. 57, 4543 (2014).
21. Xu L., Zhou S., Yu K., Gao B., Jiang H. et al.: J. Chem. Inf. Model. 53, 3202 (2013).
22. Kołaczkowski M., Marcinkowska M., Bucki A., Śniecikowska J., Pawłowski M. et al.: Eur. J. Med. Chem. 92, 221 (2015).
23. Partyka A., Chłoń-Rzepa G., Wasik A., Jastrzębska-Więsek M., Bucki A. et al.: Bioorg. Med. Chem. 23, 212 (2015).

Received: 5. 01. 2016