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RESEARCH ARTICLE

Synthesis and anticonvulsant activity of new *N*-mannich bases derived from benzhydryl- and isopropyl-pyrrolidine-2,5-dione

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Abstract

Synthesis and anticonvulsant properties of 26 new *N*-Mannich bases of 3-benzhydryl-(**5**–**17**) and 3-isopropyl-pyrrolidine-2,5-diones (**18**–**30**) have been described. Initial anticonvulsant screening for these compounds was evaluated in mice after intraperitoneal administration in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizures tests. The acute neurological toxicity was determined by applying the rotorod test. The *in vivo* results in mice showed that the majority of 3-benzhydryl-pyrrolidine-2,5-dione derivatives revealed effectiveness, while 3-isopropyl-pyrrolidine-2,5-dione derivatives were practically devoid of activity. The quantitative evaluation in both tests revealed that the most active were N-[{4-(3-chlorophenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione (**9**) with ED_{50} value =42.71 mg/kg (MES), ED_{50} value >150 mg/kg (*sc*PTZ), and *N*-[{4-(3-trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione (**13**) with ED_{50} value =101.46 mg/kg (MES) and ED_{50} value =72.59 mg/kg (*sc*PTZ). These molecules showed higher potency and lower neurotoxicity than the reference antiepileptic drugs (ethosuximide and valproic acid). To explain the probable mechanism of action of selected active derivatives (**9** and **13**), their influence on $Na_v1.2$ and L-type calcium channel was evaluated *in vitro*.

Keywords

Anticonvulsant activity, *in vitro* studies, *in vivo* studies, pyrrolidine-2,5-diones

History

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Introduction

Epilepsy is a chronic disorder of the brain that affects people in every country of the world. It is characterized by recurrent seizures. Seizures are brief episodes of involuntary tremors which may involve a part of the body (partial) or the entire body (generalized)[1]. Epilepsy actually afflicts approximately 50 million people worldwide[2]. In spite of over 40 antiepileptic drugs (AEDs) currently in clinical use and significant advances that have been made in epilepsy research, especially in molecular genetics, imaging technologies, and experimental models that have expanded our understanding of the mechanisms underlying seizures and epilepsy, convulsions in 30% of epileptics are inadequately controlled by standard drug therapy[3,4]. Besides, currently available anticonvulsant drugs cause serious side effects like diminished attention, executive function, memory, and processing speed[5]. Thus, there is a need for more efficient and less toxic antiepileptic drugs.

Over the past decades, many attempts have been made to identify the structural features essential for anticonvulsant activity. As a result, it is well established that one of the important core fragments is defined by a nitrogen heteroatomic system, usually cyclic imide, with at least one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system[6–8]. The examples of the above-mentioned structures are pyrrolidine-2,5-dione, pyrrolidin-2-one or imidazolidine-2,4-dione. They are present in the molecules of both first generation AEDs such as phenytoin, ethosuximide, and also in newer drugs like levetiracetam, brivaracetam, and seletracetam (Figure 1).

The previous research from our laboratory have demonstrated diversified anticonvulsant activities among the differently substituted pyrrolidine-2,5-dione. The structure–activity relationship analysis among this type of compounds showed that the most promising were N-Mannich bases with aromatic and alkyl groups at the position-3 and the phenylpiperazine moiety with electron withdrawing chlorine atoms or trifluoromethyl group at the position-1 of imide ring (Figure 2)[9–13].

In the current work, we present library consisting of 26 new N-Mannich bases with benzhydryl or isopropyl moiety introduced at the position-3. The proposed modifications enable to establish the effect of introducing methine bridge between two phenyl rings and the imide core, and subsequent replacement of those aromatic rings with two methyl groups, on anticonvulsant activity.

Methods and materials

Chemistry

All the chemicals and solvents were purchased from Sigma Aldrich (St. Louis, MO) and were used without further purification. Melting points (mp) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity and the homogeneity of the compounds were assessed with thin-layer

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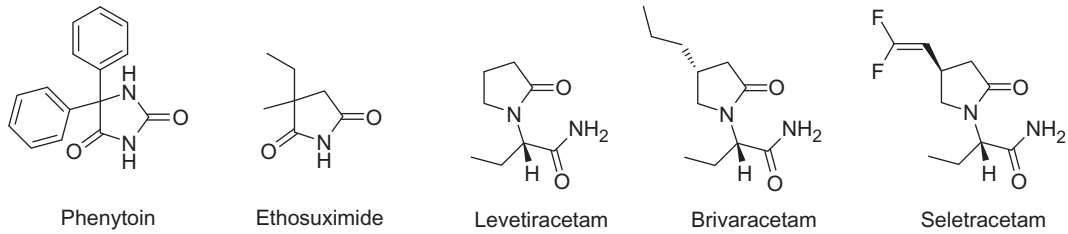
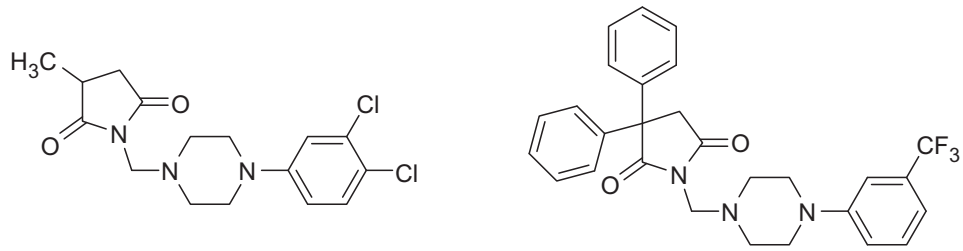


Figure 1. Structures of model AEDs based on the 5-membered heterocyclic rings.



ED_{50} = 133.99 mg/kg (*sc*PTZ, *i.p.*, mice)
TD_{50} > 500 mg/kg (rotorod test, *i.p.*, mice)
PI (TD_{50}/ ED_{50}) = 3.73

ED_{50} = 30.03 mg/kg (MES, *p.o.*, rats)
TD_{50} < 400 mg/kg (rotorod test, *p.o.*, rats)
PI (TD_{50}/ ED_{50}) < 13.2

Figure 2. Structures of active compounds obtained in the previous studies.

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chromatography (TLC). The TLC was performed on Merck silica gel 60 F_{254} aluminum sheets (Merck, Darmstadt, Germany), using developing systems consisted of S_1 chloroform:acetone (9:1, *v/v*). Spots were detected by their absorption under UV light ($\lambda = 254$ nm). Elemental analysis for C, H, and N was carried out by a micro method using the elemental Vario EI III Elemental analyzer (Elementar Analysensysteme Comp., Hanau, Germany). The results of elemental analyses were within ±0.4% of the theoretical values. ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA), in $CDCl_3$, operating at 300 MHz. Chemical shifts are reported in δ values (ppm) relative to TMS $\delta = 0$ (^{1}H), as an internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), brs (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), and m (multiplet). The mass spectra for compounds **3–30** were obtained on Waters ACQUITY™ TQD system with the TQ Detector (Waters, Milford, MA). The ACQUITY UPLC BEH C18, 1.7 µm, 2.1 × 50 mm column was used (Waters, Milford, MA). Preparative column chromatography was performed using silica gel 60 (particle size 0.063–0.200; 70–230 Mesh ATM) purchased from Merck (Darmstadt, Germany).

General procedure for the synthesis of 2-benzhydryl- and 2-isopropyl-succinic acids (1, 2)

The starting 2-benzhydryl- (**1**) and 2-isopropyl-succinic (**2**) acids were prepared according to the method described by Miller and Long [14].

General procedure for the synthesis of 3-benzhydryl- and 3-isopropyl-pyrrolidine-2,5-diones (3, 4)

A total of 0.05 mol of the 2-benzhydryl- (**1**) or 2-isopropyl- (**2**) succinic acid were dissolved in 50 ml of water and 0.05 mol of the 25% ammonia was gradually added. The mixture was heated in term-regulated sand bath (ST 72 Roth, Karlsruhe, Germany) with simultaneous distillation of water. After complete removal of water, the temperature of the reaction mixture was raised up to 180 °C and was maintained for 1 h. The crude product, 3-benzhydryl-pyrrolidine-2,5-dione (**3**), was crystallized from methanol, while 3-isopropyl-pyrrolidine-2,5-dione (**4**) was purified by column chromatography (dichloromethane:methanol, 9:0.5, *v/v*) to afford intermediates **4** as yellow oils.

3-Benzhydryl-pyrrolidine-2,5-dione (3)

White powdery crystals. Yield: 55%; m.p. 149–151°C; TLC: $R_f = 0.60$ (S_1); ^{1}H NMR ($CDCl_3$): δ 2.66 (dd, 1 H, imide, $J = 9.23$ Hz, $J = 5.13$ Hz), 2.92 (dd, 1 H, imide, $J = 18.46$ Hz, $J = 9.23$ Hz), 3.70–3.76 (m, 1H, imide), 4.83 (d, 1 H, CH, $J = 4.36$ Hz), 7.08–7.10 (m, 2 H, ArH), 7.17–7.21 (m, 2 H, ArH), 7.25–7.29 (m, 4 H, ArH), 7.31–7.38 (m, 2 H, ArH), 7.60 (brs, 1H, NH); ESI-MS: 266.14 ($C_{17}H_{15}O_2N[M+H]^+$); Anal. calcd for $C_{17}H_{15}O_2N$ (265.31) C: 76.96, H: 5.70, N: 5.28; Found C: 77.02, H: 5.79, N: 5.40.

3-Isopropyl-pyrrolidine-2,5-dione (4)

Yellow oil. Yield: 70%; TLC: $R_f = 0.53$ (S_1); ^{1}H NMR ($CDCl_3$): δ 0.93 (d, 3H, CH_3, $J = 6.67$ Hz), 1.01 (d, 3H, CH_3, $J = 6.67$ Hz), 2.24–2.36 (m, 1 H, CH), 2.50 (dd, 1 H, imide, $J = 18.35$ Hz, $J = 4.75$ Hz), 2.68 (dd, 1 H, imide, $J = 18.45$ Hz, $J = 9.25$ Hz), 2.83–2.89 (m, 1H, imide), 7.94 (brs, 1H, NH); ESI-MS: 142.02 ($C_7H_{11}O_2$ N $[M+H]^+$); Anal. calcd for $C_7H_{11}O_2N$ (142.04) C: 59.56, H: 7.85, N: 9.92; Found C: 59.53, H: 7.90, N: 9.95.

General procedure for the synthesis of compounds 5–30

The mixture of 3-benzhydryl-pyrrolidine-2,5-dione (**3**) (0.01 mol) or 3-isopropyl-pyrrolidine-2,5-dione (**4**) (0.01 mol), 40% formaldehyde solution (0.01 mol), and corresponding 4-substituted piperazines (0.01 mol) in 96% ethanol (40 ml) was left for *ca.* 12 h at room temperature (or was refluxed for 0.5 h additionally), and then refrigerated at *ca.* −10 °C for 24 h. The precipitated

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crude products were washed with cold ethanol, separated by filtration and recrystallized from 96% ethanol. Due to oily form of **30**, it was converted into hydrochloride salt in anhydrous ethanol saturated with HCl gas.

*N-[(4-Phenylpiperazin-1-yl)-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(5)***

White powdery crystals. Yield: 73%; m.p. 108–110 °C; TLC: $R_f = 0.46$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.51–2.62 (m, 4 H, piperazine), 2.68 (dd, 1 H, imide, $J = 18.33$ Hz, $J = 5.00$ Hz), 2.90 (dd, 1 H, imide, $J = 18.46$ Hz, $J = 9.23$ Hz), 3.08–3.12 (m, 4 H, piperazine), 3.68–3.75 (m, 1H, imide), 4.37 (d, 2H, CH_2, $J = 4.41$ Hz), 4.81 (d, 1H, CH, $J = 4.61$ Hz), 6.83–6.90 (m, 2 H, ArH), 7.09–7.13 (m, 2 H, ArH), 7.15–7.25 (m, 4 H, ArH), 7.27–7.29 (m, 4 H, ArH), 7.30–7.36 (m, 3 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.15, 44.12, 49.09, 50.35, 50.55, 59.97, 116.18, 119.73, 126.99, 127.43, 128.05, 128.70, 128.85, 129.13, 139.73, 141.43, 151.27, 176.99, 179.63; ESI-MS: 440.38 ($C_{28}H_{29}N_3O_3$ $[M+H]^+$); Anal. calcd for $C_{28}H_{29}O_2N_3$ (439.55): C: 76.51, H: 6.65, N: 9.56; Found C: 76.56, H: 6.71, N: 9.60.

*N-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(6)***

White powdery crystals. Yield: 82%; m.p. 154–156 °C; TLC: $R_f = 0.48$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.51–2.68 (m, 4 H, piperazine), 2.76 (dd, 1 H, imide, $J = 4.87$ Hz, $J = 4.9$ Hz), 2.91–3.02 (m, 5 H, imide, piperazyne), 3.69–3.76 (m, 1 H, piperazine), 4.39 (d, 2H, CH_2 $J = 7.44$ Hz), 4.85 (d, 1 H, CH, $J = 4.62$ Hz,), 6.88–6.97 (m, 2 H, ArH), 6.99–7.14 (m, 4 H, ArH), 7.15–7.25 (m, 4 H, ArH), 7.27–7.31 (m, 2 H, ArH), 7.31–7.39 (m, 2 H, ArH); ^{19}F NMR (282 MHz, $CDCl_3$) δ: −122.85; ESI-MS: 458.39 ($C_{28}H_{28}N_3O_3F$ $[M+H]^+$); Anal. calcd for $C_{28}H_{28}O_2N_3F$ (457.54): C: 73.50, H: 6.17, N: 9.18; Found C: 73.38, H: 6.20, N: 9.13.

*N-[{4-(4-Fluorophenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(7)***

White powdery crystals. Yield: 45%; m.p. 143–145 °C; TLC: $R_f = 0.43$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.48–2.65 (m, 4 H, piperazine), 2.72 (dd, 1 H, imide, $J = 4.90$ Hz, $J = 5.10$ Hz), 2.91–3.03 (m, 5 H, imide, piperazyne), 3.69–3.75 (m, 1 H, piperazine), 4.39 (d, 2H, CH_2, $J = 6.70$ Hz), 4.83 (d, 1 H, CH, $J = 4.62$ Hz), 6.81–6.86 (m, 2 H, ArH), 6.90–7.01 (m, 2 H, ArH), 7.08–7.12 (m, 2 H, ArH), 7.17–7.21 (m, 2 H, ArH), 7.22–7.25 (m, 3 H, ArH), 7.27–7.29 (m, 1 H, ArH), 7.31–7.39 (m, 2 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.17, 44.11, 50.11, 50.37, 50.57, 59.93, 115.37, 115.67, 117.89, 117.99, 126.99, 127.40, 128.05, 128.71, 128.84, 129.13, 139.77, 141.43, 147.96, 155.57, 158.74, 176.96, 179.60; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −124.51; ESI-MS: 458.32 ($C_{28}H_{28}O_2N_3F$ $[M+H]^+$); Anal. calcd for $C_{28}H_{28}O_2N_3F$ (457.54): C: 73.50, H: 6,17, N: 9.18; Found C: 73.45, H: 6.15, N: 9.20.

*N-[{4-(2-Chlorophenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(8)***

White powdery crystals. Yield: 79%; m.p. 155–157 °C; TLC: $R_f = 0.50$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.55–2.76 (m, 5 H, piperazine, imide), 2.92–3.02 (m, 5 H, imide, piperazyne), 3.69–3.75 (m, 1 H, piperazine), 4.41 (d, 2H, CH_2, $J = 7.44$ Hz), 4.88 (d, 1 H, CH, $J = 4.62$ Hz), 6.93–7.05 (m, 2 H, ArH), 7.07–7.14 (m, 2 H, ArH), 7.16–7.25 (m, 5 H, ArH), 7.27–7.30 (m, 3 H, ArH), 7.31–7.39 (m, 2 H, ArH); ESI-MS: 474.35 ($C_{28}H_{28}O_2N_3F$ $[M+H]^+$); Anal. calcd for $C_{28}H_{28}O_2N_3Cl$ (473.99): C: 70.95, H: 5.95, N: 8.87; Found C: 70.88, H: 5.90, N: 8.83.

*N-[{4-(3-Chlorophenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(9)***

White powdery crystals. Yield: 71%; m.p. 129–131 °C; TLC: $R_f = 0.50$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44–2.62 (m, 4 H, piperazine), 2.73 (dd, 1 H, imide, $J = 4.80$ Hz, $J = 4.90$ Hz), 2.96 (dd, 1 H, imide, $J = 9.50$ Hz, $J = 9.50$ Hz), 3.09 (t, 4H, piperazine, $J = 5.13$ Hz), 3.67–3.78 (m, 1 H, piperazine), 4.37 (d, $J = 6.70$ Hz, 2H, CH_2), 4.83 (d, $J = 4.62$ Hz, 1 H, CH), 6.70–6.77 (m, 1 H, ArH), 6.78–6.85 (m, 2 H, ArH), 7.07–7.18 (m, 4 H, ArH), 7.18–7.24 (m, 4 H, ArH), 7.27–7.38 (m, 3 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.11, 44.14, 48.57, 50.09, 50.50, 59.93, 113.94, 115.73, 119.22, 126.99, 127.43, 128.04, 128.71, 128.83, 129.18, 130.04, 134.95, 139.69, 141.38, 152.21, 176.97, 179.61; ESI-MS: 474.35 ($C_{28}H_{28}N_3O_2Cl$ $[M+H]^+$); Anal. calcd for $C_{28}H_{28}O_2N_3Cl$ (473.99): C: 70.95, H: 5.95, N: 8.87; Found C: 71.00, H: 5.99, N: 8.90.

*N-[{4-(4-Chlorophenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(10)***

White powdery crystals. Yield: 82%; m.p. 151–153 °C; TLC: $R_f = 0.46$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.44–2.62 (m, 4 H, piperazine), 2.73 (dd, 1 H, imide, $J = 4.90$ Hz, $J = 4.80$ Hz), 2.96 (dd, 1 H, imide, $J = 9.30$ Hz, $J = 9.20$ Hz), 3.06 (t, 4H, piperazine, $J = 5.00$ Hz) 3.69–3.75 (m, 1 H, piperazine), 4.37 (d, 2H, CH_2, $J = 6.60$ Hz,), 4.83 (d, 1 H, CH, $J = 4.62$ Hz), 6.78–6.81 (m, 2 H, ArH), 7.07–7.13 (m, 2 H, ArH), 7.13–7.18 (m, 1 H, ArH), 7.19–7.24 (m, 6 H, ArH), 7.27–7.38 (m, 3 H, ArH); ESI-MS: 474.28 ($C_{28}H_{29}N_3O_3$ $[M+H]^+$); Anal. calcd for $C_{28}H_{28}O_2N_3Cl$ (473.99): C: 70.95, H: 5.95, N: 8.87; Found C: 70.92, H: 5.89, N: 8.90.

*N-[{4-(2,3-Dichlorophenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(11)***

White powdery crystals. Yield: 85%; m.p. 157–159 °C; TLC: $R_f = 0.47$ (S_1);^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.54–2.81 (m, 5 H, piperazine, imide), 2.88–3.07 (m, 5H, piperazine, imide), 3.65–3.80 (m, 1 H, imide), 4.32 (d, 2H, CH_2, $J = 6.90$ Hz), 4.86 (d, 1 H, CH, $J = 4.36$ Hz), 6.87–6.98 (m, 1 H, ArH), 7.08–7.17 (m, 3 H, ArH), 7.18–7.26 (m, 5 H, ArH), 7.27–7.30 (m, 2 H, ArH), 7.31–7.39 (m, 2 H, ArH); ESI-MS: 508.25 ($C_{28}H_{27}N_3O_2Cl_2$ $[M+H]^+$); Anal. calcd for $C_{28}H_{27}N_3O_2Cl_2$ (508.44): C: 66.14, H: 5.35, N: 8.26; Found C: 66.20, H: 5.42, N: 8.32.

*N-[{4-(3,4-Dichlorophenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(12)***

White powdery crystals. Yield: 87%; m.p. 138–140 °C; TLC: $R_f = 0.47$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.41–2.60 (m, 4 H, piperazine), 2.74 (dd, 1 H, imide, $J = 18.45$ Hz, $J = 4.85$ Hz), 2.96 (dd, 1 H, imide, $J = 18.45$ Hz, $J = 9.25$ Hz), 3.06 (t, 4H, piperazine, $J = 5.13$ Hz), 3.69–3.75 (m, 1 H, piperazine), 4.37 (d, 2H, CH_2, $J = 6.70$ Hz,), 4.83 (d, 1 H, CH, $J = 4.62$ Hz), 6.69(dd, 1 H, ArH, $J = 8.85$ Hz, $J = 2.95$ Hz), 6.90 (d, 1 H, ArH, $J = 3.08$ Hz), 7.07–7.17 (m, 3 H, ArH), 7.18–7.24 (m, 4 H, ArH), 7.24–7.26 (m, 1 H, ArH) 7.27–7.38 (m, 3 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.10, 44.14, 48.53, 49.96, 50.47, 59.88, 115.32, 117.15, 122.03, 127.01, 127.41, 128.04, 128.72, 128.83, 129.21, 130.46, 132.79, 139.69, 141.35, 150.52, 176.97, 179.60; ESI-MS: 508.25 ($C_{28}H_{27}N_3O_2Cl_2$ $[M+H]^+$); Anal. calcd for $C_{28}H_{27}N_3O_2Cl_2$ (508.44): C: 66.14, H: 5.35, N: 8.26; Found C: 66.19, H: 5.42, N: 8.33.

*N-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione **(13)***

White powdery crystals. Yield: 64%; m.p. 84–86 °C; TLC**:** $R_f = 0.48$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.45–2.64 (m, 4

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H, piperazine), 2.74 (dd, 1 H, imide, $J = 18.50$ Hz, $J = 5.10$ Hz), 2.96 (dd, 1 H, imide, $J = 18.45$ Hz, $J = 9.25$ Hz), 3.06 (t, 4H, piperazine, $J = 5.00$ Hz), 3.66–3.78 (m, 1 H, piperazine), 4.38 (d, 2H, CH_2, $J = 6.67$ Hz), 4.84 (d, 1 H, CH, $J = 4.62$ Hz), 6.98–7.10 (m, 4 H, ArH), 7.10–7.17 (m, 2 H, ArH), 7.17–7.24 (m, 3 H, ArH), 7.24–7.30 (m, 2 H, ArH) 7.30–7.39 (m, 3 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 32.10, 44.15, 48.56, 50.11, 50.50, 59.92, 112.10, 112.16, 115.80, 118.73, 118.74, 127.00, 127.40, 128.05, 128.71, 128.83, 129.21, 129.58, 139.71, 141.38, 151.27, 176.97, 179.60; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −62.70; ESI-MS: 508.32 ($C_{29}H_{28}O_2N_3F_3$ $[M+H]^+$); Anal. calcd for $C_{29}H_{28}O_2N_3F_3$ (507.55): C: 68.63, H: 5.56, N: 8.28; Found C: 68.58, H: 5.63, N: 8.33.

N-[{4-(2-methylphenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione ***(14)***

White powdery crystals. Yield: 80%; m.p. 131–133 °C; TLC: $R_f = 0.50$ (S_1);^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.29 (s, 3H, CH_3), 2.51–2.68 (m, 4 H, piperazine), 2.74 (dd, 1 H, imide, $J = 18.35$ Hz, $J = 5.25$ Hz), 2.84 (t, 4H, piperazine, $J = 4.74$ Hz), 2.99 (dd, 1 H, imide, $J = 18.45$ Hz, $J = 9.25$ Hz), 3.65–3.79 (m, 1 H, piperazine), 4.41 (d, 2H, CH_2, $J = 4.36$ Hz,), 4.90 (d, 1 H, CH, $J = 4.36$ Hz), 6.99 (d, 2H, ArH, $J = 7.18$ Hz), 7.08–7.15 (m, 2 H, ArH), 7.15–7.20 (m, 3 H, ArH), 7.21–7.25 (m, 3 H, ArH) 7.27–7.39 (m, 4 H, ArH); ESI-MS: 454.40 ($C_{29}H_{31}O_2N_3$ $[M+H]^+$); Anal. calcd for $C_{29}H_{31}O_2N_3$ (453.58): C: 76.79, H: 6.89, N: 9.26; Found C: 76.84, H: 6.82, N: 9.30.

N-[{4-(3-methylphenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione ***(15)***

White powdery crystals. Yield: 83%; m.p. 137–139 °C; TLC: $R_f = 0.46$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.32 (s, 3H, CH_3), 2.49–2.64 (m, 4 H, piperazine), 2.74 (dd, 1 H, imide, $J = 18.30$ Hz, $J = 5.10$ Hz), 2.96 (dd, 1 H, imide, $J = 18.50$ Hz, $J = 9.25$ Hz), 3.09 (t, 4H, piperazine, $J = 5.13$ Hz), 3.65–3.78 (m, 3 H, piperazine), 4.38 (d, 2H, CH_2, $J = 6.41$ Hz), 4.82 (d, 1 H, CH, $J = 4.87$ Hz), 6.66–6.73 (m, 3 H, ArH), 7.07–7.13 (m, 2 H, ArH), 7.13–7.19 (m, 2 H, ArH), 7.19–7.25 (m, 4 H, ArH), 7.27–7.38 (m, 3 H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 21.79, 32.17, 44.11, 49.18, 50.39, 50.57, 59.99, 113.37, 116.96, 120.64, 126.99, 127.43, 128.06, 128.70, 128.85, 128.95, 129.11, 138.81, 139.75, 141.45, 151.34, 176.99, 179.63; ESI-MS: 454.40 ($C_{29}H_{31}O_2N_3$ $[M+H]^+$); Anal. calcd for $C_{29}H_{31}O_2N_3$ (453.58): C: 76.79, H: 6.89, N: 9.26; Found C: 76.84, H: 6.92, N: 9.24.

N-[{4-(2-methoxyphenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione ***(16)***

White powdery crystals. Yield: 45%; m.p. 90–92 °C; TLC: $R_f = 0.38$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.59–2.79 (m, 5H, imide, piperazine), 2.85–3.09 (m, 5H, imide, piperazine), 3.66–3.77 (m, 1H, imide), 3.86 (s, 3H, CH_3) 4.39 (d, 2H, CH_2, $J = 6.50$ Hz), 4.79 (d, 1H, CH, $J = 5.13$ Hz), 6.82–6.93 (m, 3H, ArH), 6.93–7.04 (m, 1H, ArH), 7.06–7.13 (m, 2H, ArH), 7.15–7.25 (m, 4H, ArH), 7.27–7.38 (m, 4H, ArH); ESI-MS: 470.36 ($C_{29}H_{31}O_3N_3$ $[M+H]^+$); Anal. calcd for $C_{29}H_{31}O_3N_3$ (469.57): C: 74.18, H: 6.65, N: 8.95; Found C: 74.25, H: 6.70, N: 8.89.

N-[{4-(3-methoxyphenyl)-piperazin-1-yl}-methyl]-3-benzhydryl-pyrrolidine-2,5-dione ***(17)***

White powdery crystals. Yield: 75%; m.p. 107–109 °C; TLC: $R_f = 0.40$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 2.46–2.63 (m, 4H, piperazine), 2.72 (dd, 1H, imide, $J = 18.50$ Hz, $J = 5.10$ Hz), 2.95 (dd, 1H, imide, $J = 18.35$ Hz, $J = 9.35$ Hz), 3.09 (t, 4H, piperazine, $J = 5.13$ Hz), 3.66–3.76 (m, 1H, imide), 3.79 (s, 3H, CH_3) 4.38 (d, 2H, CH_2, $J = 6.70$ Hz), 4.82 (d, 1H, CH, $J = 4.87$ Hz), 6.36–6.46 (m, 2H, ArH), 6.49–6.53 (m, 1H, ArH), 7.06–7.12 (m, 2H, ArH), 7.12–7.19 (m, 2H, ArH),7.19–7.25 (m, 4H, ArH), 7.27–7.38 (m, 3H, ArH); ESI-MS: 470.36 ($C_{29}H_{31}O_3N_3$ $[M+H]^+$); Anal. calcd for $C_{29}H_{31}O_3N_3$ (469.57): C: 74.18, H: 6.65, N: 8.95; Found C: 74.12, H: 6.72, N: 8.92.

N-[(4-Phenylpiperazin-1-yl)-methyl]-3-isopropyl-pyrrolidine-2,5-dione ***(18)***

White powdery crystals. Yield: 61%; m.p. 104–106 °C; TLC: $R_f = 0.44$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.91 (d, 3H, CH_3, $J = 6.92$ Hz), 1.01 (d, 3H, CH_3, $J = 6.92$ Hz), 2.26–2.39 (m, 1H, CH), 2.49 (dd, 1H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.64–2.77 (m, 5H, imide, piperazine) 2.78–2.87 (m, 1H, imide), 3.10–3.19 (m, 4H, piperazine), 4.52 (s, 2H, CH_2), 6.80–6.94 (m, 3H, ArH), 7.19–7.30 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.55, 20.08, 28.57, 30.20, 45.86, 49.20, 50.65, 59.74, 116.18, 119.85, 129.10, 151.17, 177.83, 180.34; ESI-MS: 316.26 ($C_{18}H_{25}N_3O_2$ $[M+H]^+$); Anal. calcd for $C_{18}H_{25}N_3O_2$ (315.19): C: 68.54, H: 7.99, N: 13.32; Found C: 68.58, H: 8.10, N: 13.35.

N-[{4-(2-Fluorophenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione ***(19)***

White powdery crystals. Yield: 82%; m.p. 88–90 °C; TLC: $R_f = 0.49$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.91 (d, 3H, CH_3, $J = 6.92$ Hz), 1.02 (d, 3H, CH_3, $J = 6.92$ Hz), 2.26–2.39 (m, 1H, CH), 2.44 (dd, 1H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.65–2.79 (m, 5H, imide, piperazine) 2.80–2.87 (m, 1H, imide), 3.01–3.10 (m, 4H, piperazine), 4.51 (s, 2H, CH_2), 6.87–6.97 (m, 2H, ArH), 6.98–7.08 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.57, 20.09, 28.58, 30.24, 45.85, 50.51, 59.76, 115.94, 118.97, 122.59, 124.44 140.05, 154.02, 157.27, 177.82, 180.34; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −122.85; ESI-MS: 334.28 ($C_{18}H_{24}N_3O_2F$ $[M+H]^+$); Anal. calcd for $C_{18}H_{24}N_3O_2F$ (333.40): C: 64.84, H: 7.26, N: 12.60; Found C: 64.80, H: 7.30, N: 12.68.

N-[{4-(4-Fluorophenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione ***(20)***

White powdery crystals. Yield: 80%; m.p. 121–123 °C; TLC: $R_f = 0.37$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.91 (d, 3H, CH_3, $J = 6.67$ Hz), 1.01 (d, 3H, CH_3, $J = 6.92$ Hz), 2.27–2.38 (m, 1H, CH), 2.49 (dd, 1H, imide, $J = 18.25$ Hz, $J = 4.65$ Hz), 2.64–2.77 (m, 5H, imide, piperazine) 2.78–2.87 (m, 1H, imide), 3.00–3.12 (m, 4H, piperazine), 4.51 (s, 2H, CH_2), 6.76–6.88 (m, 2H, ArH), 6.89–6.99 (m, 2H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.55, 20.07, 28.57, 30.21, 45.86, 50.21, 50.65, 59.69, 115.35, 115.64, 117.90, 118.00, 147.81 158.79, 177.82, 180.32; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −124.41; ESI-MS: 334.28 ($C_{18}H_{24}N_3O_2F$ $[M+H]^+$); Anal. calcd for $C_{18}H_{24}N_3O_2F$ (333.40): C: 64.84, H: 7.26, N: 12.60; Found C: 64.83, H: 7.24, N: 12.64.

N-[{4-(2-Chlorophenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione ***(21)***

White powdery crystals. Yield: 82%; m.p. 111–113 °C; TLC: $R_f = 0.46$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (d, 3H, CH_3, $J = 6.92$ Hz), 1.03 (d, 3H, CH_3, $J = 6.92$ Hz), 2.29–2.39 (m, 1 H, CH), 2.51 (dd, 1 H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.67–2.80 (m, 5H, imide, piperazine), 2.81–2.90 (m, 1H, imide), 2.99–3.07 (m, 4H, piperazine), 4.52 (s, 2H, CH_2), 6.91–7.04 (m, 2 H, ArH), 7.16–7.23 (m, 1 H, ArH), 7.33 (dd, 1 H, ArH, $J = 7.95$ Hz, $J = 1.54$ Hz); ESI-MS: 350.30 ($C_{18}H_{24}O_2N_3Cl$ $[M+H]^+$); Anal. calcd for $C_{18}H_{24}O_2N_3Cl$ (349.86): C: 61.79, H: 6.91, N: 12.01; Found C: 61.76, H: 7.02, N: 12.06.

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*N-[{4-(3-Chlorophenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione **(22)***

White powdery crystals. Yield: 73%; m.p. 98–100 °C; TLC: $R_f = 0.43$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (d, 3H, CH_3, $J = 6.67$ Hz), 1.01 (d, 3H, CH_3, $J = 6.92$ Hz), 2.26–2.38 (m, 1H, CH), 2.49 (dd, 1H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.64–2.76 (m, 5H, imide, piperazine) 2.79–2.87 (m, 1H, imide), 3.14 (dd, 4H, piperazine, $J = 5.90$ Hz, $J = 4.10$ Hz), 4.50 (s, 2H, CH_2), 6.71–6.76 (m, 1H, ArH), 6.77–6.82 (m, 1H, ArH), 6.82–6.85 (m, 1H, ArH), 7.14 (t, 1H, ArH, $J = 8.08$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.52, 20.05, 28.58, 30.20, 45.85, 48.73, 50.44, 59.68, 114.04, 115.88, 119.41, 130.00 134.91, 152.19, 177.78, 180.29; ESI-MS: 350.30 ($C_{18}H_{24}O_2N_3Cl$ $[M+H]^+$); Anal. calcd for $C_{18}H_{24}O_2N_3Cl$ (349.86): C: 61.79, H: 6.91, N: 12.01; Found C: 61.77, H: 7.01, N: 12.00.

*N-[{4-(4-Chlorophenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione **(23)***

White powdery crystals. Yield: 74%; m.p. 115–117 °C; TLC: $R_f = 0.41$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (d, 3H, CH_3, $J = 6.67$ Hz), 1.01 (d, 3H, CH_3, $J = 6.92$ Hz), 2.26–2.37 (m, 1 H, CH), 2.49 (dd, 1 H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.63–2.76 (m, 5H, imide, piperazine), 2.78–2.87 (m, 1H, imide), 3.04–3.16 (m, 4H, piperazine), 4.50 (s, 2H, CH_2), 6.74–6.84 (m, 2 H, ArH), 7.13–7.22 (m, 2 H, ArH); ESI-MS: 350.23 ($C_{18}H_{24}O_2N_3Cl$ $[M+H]^+$); Anal. calcd for $C_{18}H_{24}O_2N_3Cl$ (349.86): C: 61.79, H: 6.91, N: 12.01; Found C: 61.77, H: 6.95, N: 12.08.

*N-[{4-(2,3-Dichlorophenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione **(24)***

White powdery crystals. Yield: 56%; m.p. 105–107 °C; TLC: $R_f = 0.49$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.93 (d, 3H, CH_3, $J = 6.67$ Hz), 1.03 (d, 3H, CH_3, $J = 6.92$ Hz), 2.28–2.40 (m, 1H, CH), 2.51 (dd, 1H, imide, $J = 18.45$ Hz, $J = 4.65$ Hz), 2.67–2.81 (m, 5H, imide, piperazine) 2.83–2.91 (m, 1H, imide), 2.94–3.07 (m, 4H, piperazine), 4.51 (s, 2H, CH_2), 6.92 (dd, 1H, ArH, $J = 6.41$ Hz, $J = 3.08$ Hz), 7.08–7.17 (m, 2 H, ArH); ESI-MS: 384.27 ($C_{18}H_{24}N_3O_2Cl_2$ $[M+H]^+$); Anal. calcd for $C_{18}H_{23}N_3O_2Cl_2$ (384,30): C: 56.26, H: 6.03, N: 10.93; Found C: 56.28, H: 6.09, N: 10.98.

*N-[{4-(3,4-Dichlorophenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione **(25)***

White powdery crystals. Yield: 76%; m.p. 95–97 °C; TLC: $R_f = 0.46$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (d, 3H, CH_3, $J = 6.92$ Hz), 1.00 (d, 3H, CH_3, $J = 6.92$ Hz), 2.25–2.37 (m, 1H, CH), 2.48 (dd, 1H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.64–2.75 (m, 5H, imide, piperazine) 2.79–2.86 (m, 1H, imide), 3.08–3.14 (m, 4H, piperazine), 4.49 (s, 2H, CH_2), 6.66–6.72 (m, 1H, ArH), 6.90 (d, 1H, ArH, $J = 2.82$ Hz) 7.22–7.27 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.52, 20.04, 28.57, 30.20, 45.85, 48.71, 50.31, 59.64, 115.46, 117.33, 122.24, 130.39, 132.74, 150.52, 177.76, 180.28; ESI-MS: 384.27 ($C_{18}H_{23}N_3O_2Cl_2$ $[M+H]^+$); Anal. calcd for $C_{18}H_{24}N_3O_2Cl_2$ (384,30): C: 56.26, H: 6.03, N: 10.93; Found C: 56.28, H: 6.15, N: 10.99.

*N-[{4-(3-Trifluoromethylphenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione **(26)***

White powdery crystals. Yield: 48%; m.p. 65–67 °C; TLC**:** $R_f = 0.45$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (d, 3H, CH_3, $J = 6.67$ Hz), 1.01 (d, 3H, CH_3, $J = 6.92$ Hz), 2.26–2.38 (m, 1 H, CH), 2.49 (dd, 1 H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.65–2.77 (m, 5H, imide, piperazine), 2.79–2.88 (m, 1H, imide), 3.11–3.27 (m, 4H, piperazine), 4.51 (s, 2H, CH_2), 6.97–7.09 (m, 3 H, ArH), 7.29–7.36 (m, 1H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 17.52, 20.04, 28.58, 30.21, 45.85, 48.73, 50.44, 59.67, 112.28, 112.33, 115.92, 115.98, 118.84, 118.85, 129.52, 151.24, 177.77, 180.29; ^{19}F NMR (282 MHz, $CDCl_3$) δ: −62.78; ESI-MS: 384.27 ($C_{19}H_{24}O_2N_3F_3$ $[M+H]^+$); Anal. calcd for $C_{19}H_{24}O_2N_3F_3$ (383.41): C: 59.52, H: 6.31, N: 10.96; Found C: 59.58, H: 6.38, N: 10.93.

*N-[{4-(2-methylphenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione **(27)***

White powdery crystals. Yield: 75%; m.p. 91–93 °C; TLC: $R_f = 0.47$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.94 (d, 3H, CH_3, $J = 6.92$ Hz), 1.03 (d, 3H, CH_3, $J = 6.92$ Hz), 2.24 (s, 3 H, CH_3), 2.29–2.42 (m, 1 H, CH), 2.52 (dd, 1 H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.68–2.79 (m, 5H, imide, piperazine), 2.81–2.93 (m, 5H, imide, piperazine), 4.52 (s, 2H, CH_2), 6.91–7.03 (m, 2 H, ArH), 7.08–7.21 (m, 2H, ArH); ESI-MS: 330.29 ($C_{19}H_{27}O_2N_3$ $[M+H]^+$); Anal. calcd for $C_{19}H_{27}O_2N_3$ (329.44): C: 69.27, H: 8.26, N: 12.76; Found C: 69.24, H: 8.22, N: 12.85.

*N-[{4-(3-methylphenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione **(28)***

White powdery crystals. Yield: 76%; m.p. 71–73 °C; TLC: $R_f = 0.48$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.91 (d, 3H, CH_3, $J = 6.67$ Hz), 1.01 (d, 3H, CH_3, $J = 6.92$ Hz), 2.31 (s, 3H, CH_3), 2.32–2.39 (m, 1H, CH), 2.52 (dd, 1H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.64–2.77 (m, 5H, imide, piperazine), 2.79–2.88 (m, 1H, imide), 3.08–3.20 (m, 4H, piperazine), 4.51 (s, 2H, CH_2), 6.62–6.75 (m, 3H, ArH), 7.07–7.17 (m, 1H, ArH); ESI-MS: 330.29 ($C_{19}H_{27}O_2N_3$ $[M+H]^+$); Anal. calcd for $C_{19}H_{27}O_2N_3$ (329.44): C: 69.27, H: 8.26, N: 12.76; Found C: 69.30, H: 8.31, N: 12.80.

*N-[{4-(2-methoxyphenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione **(29)***

White powdery crystals. Yield: 77%; m.p. 97–99 °C; TLC: $R_f = 0.54$ (S_1); ^{1}H NMR (300 MHz, $CDCl_3$) δ: 0.90 (d, 3H, CH_3, $J = 6.67$ Hz), 1.02 (d, 3H, CH_3, $J = 6.92$ Hz), 2.24–2.38 (m, 1H, CH), 2.47 (dd, 1H, imide, $J = 18.20$ Hz, $J = 4.60$ Hz), 2.66 (dd, 1H, imide, $J = 18.10$ Hz, $J = 9.30$ Hz), 2.77–2.85 (m, 5H, piperazine, imide), 2.97–3.10 (m, 4H, piperazine), 3.83 (s, 3H, CH_3), 4.52 (s, 2H, CH_2), 6.80–6.86 (m, 1H, ArH), 6.87–6.93 (m, 2H, ArH), 6.94–7.02 (m, 1H, ArH); ESI-MS: 345.21 ($C_{19}H_{27}O_3N_3$ $[M+H]^+$); Anal. calcd for $C_{19}H_{27}O_3N_3$ (354.44): C: 66.06, H: 7.88, N: 12.16; Found C: 66.02, H: 7.82, N: 12.19.

*N-[{4-(3-methoxyphenyl)-piperazin-1-yl}-methyl]-3-isopropyl-pyrrolidine-2,5-dione monohydrochloride **(30)***

White powdery crystals. Yield: 75%; m.p. 147–149 °C; TLC: $R_f = 0.39$ (S_1); ^{1}H NMR (300 MHz, DMSO) δ: 0.84 (d, 3H, CH_3, $J = 6.90$ Hz), 0.96 (d, 3H, CH_3, $J = 6.92$ Hz), 2.05–2.17 (m, 1H, CH), 2.10 (dd, 1H, imide, $J = 18.10$ Hz, $J = 4.70$ Hz), 2.53–2.63 (m, 1H, imide), 2.92 (m, 1H, imide) 3.14 (brs, 4H, piperazine), 3.30–3.45 (m, 4H, piperazine), 3.70 (s, 3H, CH_3), 4.64 (s, 2H, CH_2), 6.39–6.47 (m, 1H, ArH), 6.52–6.63 (m, 2H, ArH), 7.09–7.16 (m, 1H, ArH), 11.07 (brs, 1H, +NH); ESI-MS: 345.21 ($C_{19}H_{27}O_3N_3$ $[M+H]^+$); Anal. calcd for $C_{19}H_{27}O_3N_3$ (354.44): C: 65.87, H: 8.15, N: 12.13; Found C: 65.82, H: 8.22, N: 12.23.

In vivo pharmacology

The initial anticonvulsant evaluations were performed at the Department of Pharmacodynamics, Faculty of Pharmacy,

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Jagiellonian University Medical College. Phase I studies involved three tests: maximal electroshock (MES), subcutaneous pentylenetetrazole (*sc*PTZ), and rotorod test for acute neurological toxicity (NT). The compounds were injected intraperitoneally into mice as a suspension in 0.5% methylcellulose/water mixture at doses of 100 and 300 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 and 4 h intervals after administration. For the most promising derivatives, the ED_{50} and TD_{50} values were designated in mice.

Chemicals

Pentylenetetrazole (PTZ) was purchased from Sigma-Aldrich (Szelagowska, Poland). PTZ was dissolved in saline solution and administered subcutaneously. All the compounds were administered intraperitoneally into mice in volumes 0.1 ml per 10 g body weight. Control animals were given appropriate amounts of vehicle (methylcellulose).

Animals

For the experiments, adult male albino CD-1 mice weighing 16–26 g were used. The animals were kept in cages at room temperature of $22 \pm 2\,^{\circ}C$, under a light/dark (12/12) cycle with access to standard laboratory food and tap water before experiments. The ambient temperature of the room and humidity were kept consistent throughout all tests. For the experiments, the animals were randomly selected. Each group consisted of four animals and each mouse was used only once. The experiments were performed between 8 a.m. and 3 p.m. All the procedures were approved by the Local Ethics Committee of the Jagiellonian University in Cracow.

Maximal electroshock seizure (MES) test

The MES test was performed according to procedure originally described by Toman et al.[15]. Briefly, the mice received a stimulus of sufficient intensity (25 mA, 500 V, 50 Hz) delivered by an electroshock generator (HugoSachs rodent shocker, Hugo Sachs Elektronik, Freiburg, Germany) to induce maximal seizures. Electroconvulsions were produced with the use of auricular electrodes and the stimulus duration was 0.2 s. The endpoint was the tonic extension of the hind limbs. Mice not displaying hindlimb tonic extension were considered to be protected from seizure.

Subcutaneous pentylenetetrazole seizure (scPTZ) test

Seizures were performed by subcutaneously injection of PTZ (85 mg/kg). This produced clonic convulsions lasting for at least 5 s in 97% of animal tested. The observation was carried out for 30 min. In the control groups, the first episode of clonic convulsions was observed between 5 and 14 min of observation. The absence of clonic convulsions in the observed time period of 0.5 and 4 h was interpreted as the compound's ability to protect against PTZ-induced seizure[16]. Moreover, the latency time to first clonus episodes was noted and compared between vehicle-treated and drug-treated groups.

Neurotoxicity screening

Minimal motor impairment was established in mice by standard rotarod procedure[17]. Mice were trained to balance on an accelerating rotarod (rotarod apparatus, May Commat RR0711, Turkey; rod diameter: 2 cm). Proper experimentation was conducted at least 24 h after the training trial. On the test day, mice were intraperitoneally pretreated with the test compound and after 0.5 and 4 h were tested on the rotarod revolving at 10 rpm. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for at least one minute.

Median effective dose (ED50), median toxic dose (TD50), and protective index (PI)

The ED_{50} is defined as the dose of a drug protecting 50% of animals against the MES and PTZ seizures. The neurotoxic effect was expressed as a TD_{50} value, representing the doses at which the compound resulted in minimal motor impairment in 50% of the animals in the rotarod test. To evaluate the ED_{50} and TD_{50} values, at least three groups of animals were injected with various doses of tested compounds. Both ED_{50} and TD_{50} values with 95% confidence limits were calculated by probit analysis[18]. The PI (protective index) value was calculated as the ratio of TD_{50} to ED_{50} ($TD_{50}/ED_{50} = PI$).

In vitro pharmacology

The radioligand binding studies were performed commercially by Cerep (Celle I'Evescault, France), using methodology described elsewhere[19,20].

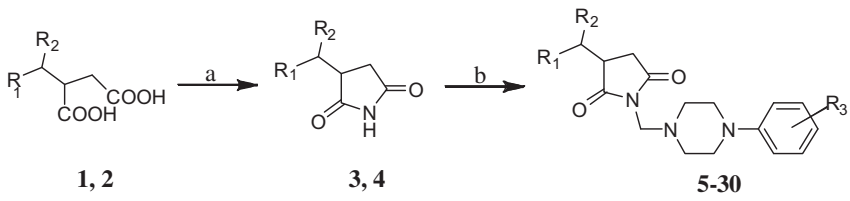
Results and discussion

Compounds **3**–**30** were synthesized according to Scheme 1. The cyclocondensation reaction of **1** or **2** with the 25% ammonia at 180°C for 1.5 h yielded in 3-benzhydryl- (**3**) or 3-isopropyl- (**4**) pyrrolidine-2,5-diones. The final compounds **5**–**30** were synthesized in the aminoalkylation (Mannich-type) reaction from the appropriately substituted 3-benzhydryl- or 3-isopropyl-pyrrolidine-2,5-dione (**3** or **4**), formaldehyde and corresponding 4-substituted piperazines. The reaction was carried out in 96% ethanol at room temperature for *ca.* 12 h. The crude products were crystallized from 96% ethanol giving the final compounds in yields ranging from 45% to 87%. Except compound (**30**), which was isolated as hydrochloride salts, the other derivatives were obtained as a free bases.

Pharmacology

Assessment of anticonvulsant activity of new compounds potentially useful for the treatment of epilepsy is based mainly on the use of animal seizure models. Nowadays, the two fundamental tests enable to examine initially an anticonvulsant activity: the maximal electroshock (MES) test – model of tonic-clonic seizures in humans, and subcutaneous pentylenetetrazole (scPTZ) test – model of absence epilepsy[21,22]. It should be noticed that in spite of significant advances that have been made in epilepsy research during last years, the MES and scPTZ screens are still recognized as the ''gold standards'' in the early stages of testing of new anticonvulsants.

The results of *in vivo* studies showed that among obtained compounds the highest anticonvulsant activity in the MES test revealed **9**, **12**, **13** and **26**, which showed protection at the dose of 100 mg/kg; however, this result was not as good as the result of phenytoin, which is still recognized as the model antiepileptic drug active in the MES test. Moreover, compound **8** was effective at the dose of 300 mg/kg after 0.5 h. The other active compounds in the MES test revealed protection after 4 h. Additionally, compounds **9**, **12**, and **13** were found to be active in the *sc*PTZ test. Likewise, nine substances **5**, **6**, **7**, **10**, **11**, **15**, **17**, **19**, and **28** were effective only in the *sc*PTZ test. Derivatives **5**, **7**, **9**, **11**, **12**, **13**, **15**, **17**, **19**, and **28** showed protection at the dose of 100 mg/kg after 4 h. It is a better protection at thse same time point than the result of ethosuximide, recognized as the model antiepileptic drug effective in the *sc*PTZ test. Especially interesting seem to be compounds **11** and **12**, which revealed activity at the dose of 100 mg/kg not only after 4 h, but also after 0.5 h. The other compounds have demonstrated protection at the dose of 300 mg/kg after 4 h (**6** and **10**), and 0.5 h (compound **9**).



$R_1 = R_2 = C_6H_5$ (**1**)
$R_1 = R_2 = CH_3$ (**2**)
R_3 = H; 2-F; 4-F; 2-Cl; 3-Cl; 4-Cl; 2,3-Cl; 3,4-Cl; 3-CF_3; 2-CH_3; 3-CH_3, 2-OCH_3; 3-OCH_3

Reagents and conditions: (a) 25% NH_4OH, 180°C, 1.5 h, (b) 4 phenylpiperazine derivatives, formaldehyde, 96% Et-OH, 12 h room temperature.

Scheme 1. Synthetic procedures of intermediates **3**, **4** and final compounds **5–30**.

The results of *in vivo* tests indicated higher toxicity (motor impairment) for 3-isopropyl-pyrrolidine-2,5-dione derivatives in comparison with 3-benzhydryl-pyrrolidine-2,5-dione analogs, in general. In the first series **(5–17),** only one showed neurotoxicity in the rotarod test (NT), namely compound **7**, which was neurotoxic at the dose of 100 mg/kg after 0.5 h. The other compounds did not show neurotoxicity. It was not possible to perform MES, *sc*PTZ, and NT tests for compounds **18**–**30** due to rapid death following administration at the dose of 300 mg/kg (Table 1).

Moreover, for the most active compounds in the *sc*PTZ test, the latency time to first clonus episodes was compared between vehicle-treated and drug-treated groups (Figure 3). Compounds **5**, **7**, **11**, **12**, **13**, **15**, and **17**, as well as ethosuximide and valproic acid compared with the vehicle-treated group, prolonged in a dose-dependent manner the latency time to first seizure episode. Compounds **5**, **7**, **11**, **12**, **13**, and **17** at doses 100 and 150 mg/kg significantly prolonged latency time to the first seizure episode by 99–142% and 119–176%, respectively. Compound **15** at the dose of 150 mg/kg lengthened latency time to the first seizure episode by 128%, in a statistically significant manner, whereas reference compounds significantly prolonged latency time to the first seizure episode starting at the dose of 120 mg/kg (ethosuximide) or 250 mg/kg (valproic acid).

The most effective molecules, namely **5**, **7**, **9**, **11**, **12**, **13**, **15**, and **17**, were selected for quantification of the pharmacological parameters (ED_{50} and TD_{50}). The quantitative data of the MES and *sc*PTZ median effective doses (ED_{50}) and neurotoxic dose (TD_{50}) were examined at previously estimated time to peak effect (TPE). The results are shown in Table 2.

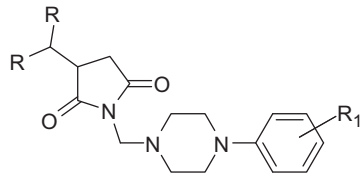
All compounds (**5**, **7**, **9**, **11**, **12**, **13**, **15**, and **17**) showed higher potency in the *sc*PTZ tests in comparison with the reference drugs – ethosuximide and valproic acid. Moreover, all these molecules demonstrated lower rotarod toxicity than phenytion, ethosuximide, and valproic acid, what resulted in more favorable protection indexes. Furthermore, for derivatives **9**, **12**, and **13**, quantitative parameters in the MES test were also estimated. All these compounds had better ED_{50} values in the MES seizures than valproic acid.

Structure–activity relationships

On the basis of the initial pharmacological investigation, it can be noticed that anticonvulsant activity has a close connection with kind of substituted at the position-3 of pyrrolidine-2,5-dione ring. From the obtained molecules, the most active compounds are those which possess aromatic groups at the position-3 of the imide ring. Replacement of two phenyl rings with the methyl group caused reduction of anticonvulsant activity.

Another factor influencing the anticonvulsant activity was the presence of substituent in the 4-phenylpiperazine moiety. In general, in both series of molecules effective in the MES test were

Table 1. The results after intraperitoneal injection in mice (**5–30**).



			MES*		*sc*PTZ†		NT‡	
Compd	R	R^1	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
5	C_6H_5	H	–	–	–	100	–	–
6	C_6H_5	2-F	–	–	–	300	–	–
7	C_6H_5	4-F	–	–	–	100	100	–
8	C_6H_5	2-Cl	–	300	–	–	–	–
9	C_6H_5	3-Cl	–	100	300	100	–	–
10	C_6H_5	4-Cl	–	–	–	300	–	–
11	C_6H_5	2,3-Cl	–	–	100	100	–	–
12	C_6H_5	3,4-Cl	–	100	100	100	–	–
13	C_6H_5	3-CF_3	–	100	–	100	–	–
14	C_6H_5	2-CH_3	–	–	–	–	–	–
15	C_6H_5	3-CH_3	–	–	–	100	–	–
16	C_6H_5	2-OCH_3	–	–	–	–	–	–
17	C_6H_5	3-OCH_3	–	–	–	100	–	–
18¶	CH_3	H	–	–	–	–	–	–
19¶	CH_3	2-F	–	–	–	100	–	–
20¶	CH_3	4-F	–	–	–	–	–	–
21¶	CH_3	2-Cl	–	–	–	–	–	–
22¶	CH_3	3-Cl	–	–	–	–	–	–
23¶	CH_3	4-Cl	–	–	–	–	–	–
24¶	CH_3	2,3-Cl	–	–	–	–	–	–
25¶	CH_3	3,4-Cl	–	–	–	–	–	–
26¶	CH_3	3-CF_3	100	–	–	–	–	–
27¶	CH_3	2-CH_3	–	–	–	–	–	–
28¶	CH_3	3-CH_3	–	–	–	100	–	–
29¶	CH_3	2-OCH_3	–	–	–	–	100	100
30¶	CH_3	3-OCH_3	–	–	–	–	–	–
Phenytoin§	–	–	30	30			100	100
Ethosuximide|	–	–	–	–	100	300	–	–
Valproic acid#	–	–	300	–	300	–	–	–

The figures in the table indicate the minimum dose whereby bioactivity or neurotoxicity was demonstrated in half or more animals. A dash indicates the absence of activity or neurotoxicity at the maximum dose administrated (300 mg/kg).
*Maximal electroshock test.
†Subcutaneous pentylenetetrazole test.
‡Neurotoxicity screening – rotorod test.
¶Rapid death of animals after compounds administration at dose of 300 mg/kg.
§Phenytoin, reference drug[29].
|Ethosuximide, reference drug[29].
#Valproic acid, reference drug[29].

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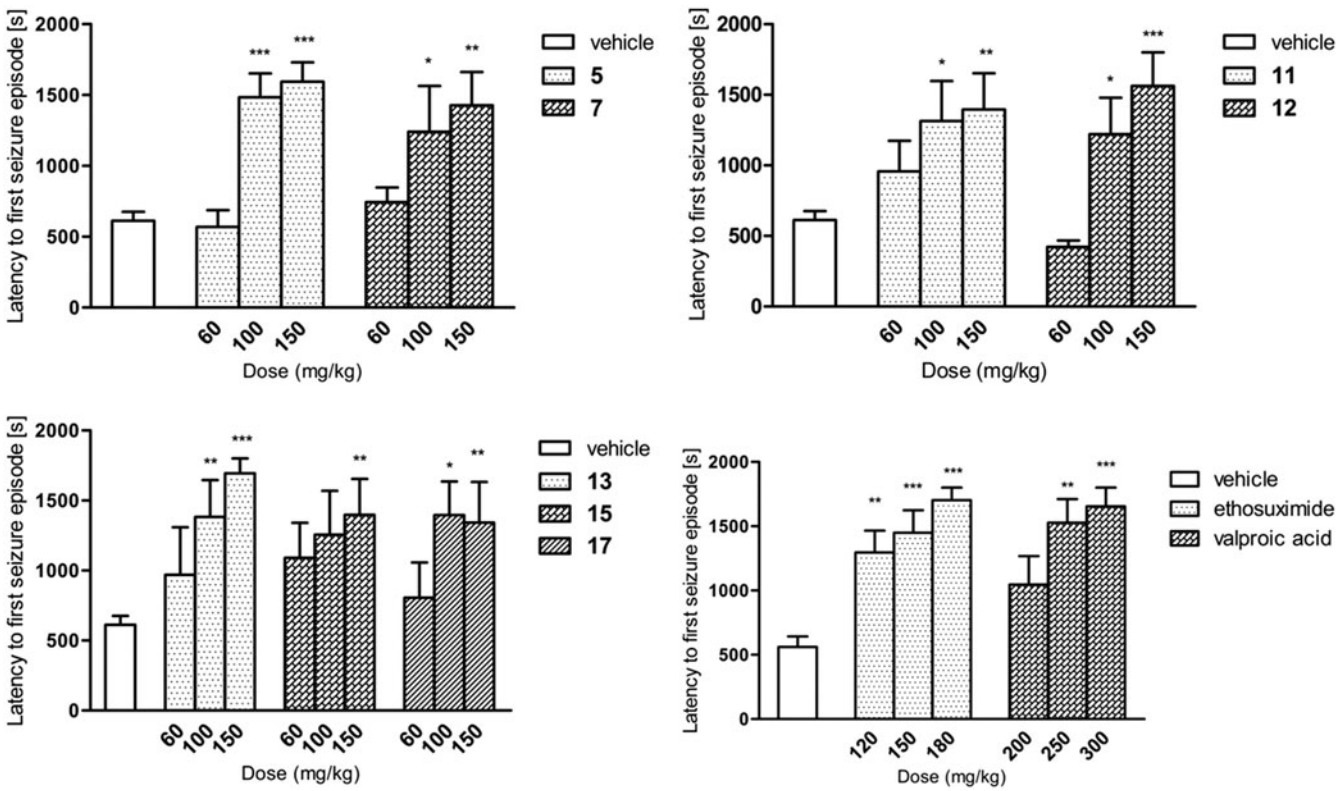

Figure 3. Anticonvulsant activity of compounds **5**, **7**, **11**, **12**, **13**, **15**, **17**, and reference AEDs: ethosuximide and valproic acid in the *sc*PTZ test. Each value represents the mean ± SEM obtained from 6 to 8 mice. Statistical analysis: one-way analysis of variance (ANOVA), followed by Dunnett's *post hoc* test: $F[3,27] = 24.19$, $p < 0.0001$ (**5**); $F[3,23] = 7.358$, $p < 0.01$ (**7**), $F[3,25] = 5.96$, $p < 0.01$ (**11**); $F[3,25] = 10.36$, $p < 0.001$ (**12**), $F[3,26] = 8.731$, $p < 0.001$ (**13**); $F[3,26] = 4.926$, $p < 0.01$ (**15**); $F[3,25] = 5.382$, $p < 0.01$ (**17**), $F[3,20] = 12.71$, $p < 0.0001$ (ethosuximide); $F[3,20] = 8.94$, $p < 0.001$ (valproic acid). The compounds were administered i.p. 0.25 h (ethosuximide), 0.5 h (valproic acid) or 4 h before the test. Significant difference compared with the control group: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

Table 2. The quantitative anticonvulsant data in mice after *i.p.* administration.

		ED_{50}			PI‡	
Compound	TPE (h)*	MES (mg/kg)†	*sc*PTZ(mg/kg)‡	TD_{50} (mg/kg)†	MES	*sc*PTZ
5	4	ND	118.38 (95.80–146.29)	>400	ND	>3.38
7	4	ND	122.63 (97.83–153.73)	>500	ND	>4.07
9	4	42.71 (32.61–55.93)	>150	>500	>11.70	>3.33
11	4	ND	106.17 (66.80–168.74)	>500	ND	>4.71
12	4	132.37 (111.54–157.09)	111.19 (92.29–133.97)	>500	>3.77	>5.49
13	4	101.46 (80.86–127.30)	72.59 (42.17–124.97)	>500	>4.95	>6.88
15	4	ND	85.86 (55.30–133.30)	>400	ND	>4.65
17	4	ND	106.17 (66.80–168.74)	>400	ND	>3.77
Phenytoin¶	1§	6.65 (5.42–8.16)	>500	56.9 (48.5–66.7)	8.56	>0.57
Etosuximide¶	0.25§	>500	140.40 (115.81–170.21)	318.01 (295.80–341.89)	>0.64	2.26
Valproic acid¶	0.5§	252.74 (220.10–290.22)	239.45 (209.18–274.10)	430.77 (407.92–454.90)	1.7	1.8

ND, no data.
*Time to peak effect.
†Results are represented as mean ± SEM at 95% confidence limit (MES, maximal electroshock test; *sc*PTZ, subcutaneous pentylenetetrazole test; neurotoxicity-rotarod screen).
‡Protection index (TD_{50}/ED_{50}).
¶Reference drug tested in the same conditions.
§TPEs taken from the literature[30].

these with 3-trifluoromethyl-piperazine derivatives (**13** and **26**), while in the *sc*PTZ test, activity showed compounds with 2-fluoro- (**6** and **19**) and 3-methyl-piperazine derivatives (**15** and **26**).

As far as 3-benzydryl-pyrrolidine-2,5-dione series of compounds are concerned, these molecules revealed significantly better protection in the scPTZ test. Yet it can noticed that compounds which had the substituent at the position-2 of phenylpiperazine were devoid of anticonvulsant activity in the scPTZ test, apart from the derivatives which possessed flour atom at this position (**6** and **19**).

Comparison of the pharmacological data for pyrrolidine-2,5-dione derivatives described in our former studies[9] and compounds obtained currently proved that connection of two aromatic rings through methine bridge with imide ring at the position-3 resulted in obtaining compounds which showed improved activity in the *sc*PTZ test, but at the same time, reduced activity in the MES test.

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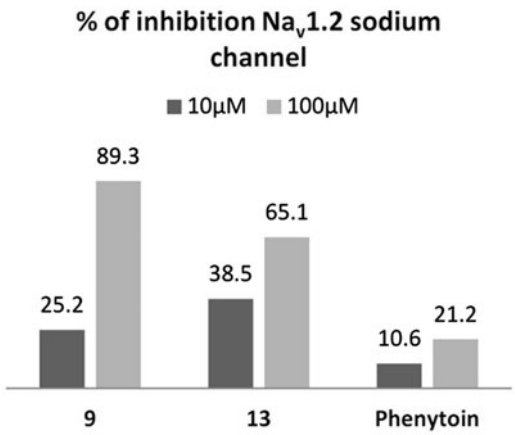

Figure 4. The data indicate the percentage of blocking $Na_v1.2$ sodium channel. The results are divided as follow: <25% inactive compounds, 25–50% compounds with marginal activity, >50% active compounds. Phentoin, reference antiepileptic drug, data from Ref.[30].

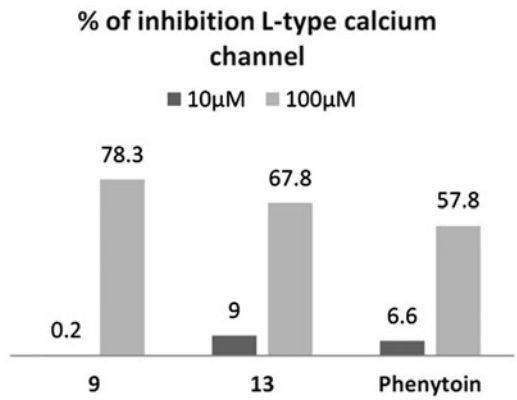

Figure 5. The data indicate the percentage of blocking l-type calcium channel. The results are divided as follow: <25% inactive compounds, 25–50% compounds with marginal activity, >50% active compounds. Phenytoin, reference antiepileptic drug. These studies were performed commercially by Cerep (Celle I'Evescault, France), using methodology described elsewhere [20].

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Moreover, introduction of isopropyl substituent at the position-3 of imide ring, not only reduced significantly anticonvulsant activity but also increased toxicity among this type of derivatives, consequently, these compounds will not be investigated in the future. Additionally, this modification gave us important information for further design of new anticonvulsant compounds, namely, in order to obtain active and safe derivatives, we should avoid introducing isopropyl group at position-3 of pyrrolidine-2,5-dione.

In vitro sodium and calcium channels binding assays

The voltage gated sodium and/or calcium channels have been the target of many antiepileptic drugs[23–28]. Therefore, for the two most active compounds (**9** and **13**), which demonstrated efficacy both in the MES and *sc*PTZ test, the influence on $Na_v1.2$ sodium channel and L-type calcium channels in concentrations of 10 μM and 100 μM were determined.

As it is indicated in Figure 4, both derivatives **9** and **13** efficiently blocked sodium channels in concentration of 100 μM, as they showed inhibition greater than 50% – compound **9** showed blocking in 89.30%, while compound **13** – 65.10%. Regarding concentration of 10 μM, these derivatives revealed inhibition in ranges from about 50% to 25% (compound **9 – 25.20%** and compound **13** – 38.50%). It should be highlighted that derivatives **9** and **13** were stronger $Na_v1.2$ channel blockers than phenytoin.

Moreover, both compounds **9** and **13** revealed also effective blocking of L-type calcium channels in concentration of 100 μM, as they showed inhibition higher than 50% – compound **9** blocked calcium channels in 78.30%, while compound **13** – in 67.80%. Reducing the concentration to 10 μM resulted in a significant decrease in calcium channel blocking. It was particularly surprising for compound **9**, which did not demonstrate inhibition in this concentration, whereas was highly active in concentration of 100 μM. Derivative **13** blocked this channel in 9% in concentration of 10 μM. Derivatives **9** and **13** were stronger L-type calcium channel blockers in concentration of 100 μM than phenytoin (Figure 5).

Conclusion

In current research, 26 new N-Mannich bases of *N*-[(4-phenylpiperazin-1-yl)methyl] derivatives of 3-benzhydryl- and 3-isopropyl-pyrrolidine-2,5-dione were synthesized and evaluated for their anticonvulsant activity in the maximum electroshock (MES) and subcutaneous pentylenetetrazole seizure tests (*sc*PTZ). The obtained results revealed that the majority of 3-bezhydryl-succinimide derivatives exhibited anticonvulsant activity, especially in the *sc*PTZ test. Moreover, several of them revealed activity in the MES test. The quantitative studies in mice after *i.p.* administration showed that seven compounds were more potent than ethosuximide in the *sc*PTZ test and also three of them revealed activity in the MES test higher than valproic acid. It was important that all of the active compounds demonstrated low neurotoxicity. For the two most active compounds, their probable mechanism of action *in vitro* was established. These selected active molecules inhibited sodium and calcium voltage-gated channels in more than 50%, what is a better result than for the reference drug – phenytoin.

Declaration of interest

The authors report that they have no conflicts of interest. The studies were supported by the Polish National Scientific Center grant no DEC-2013/11/N/NZ7/00426.

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