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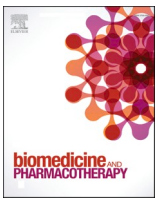

Mechanochemical synthesis and anticonvulsant activity of 3-aminopyrrolidine-2,5-dione derivatives

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ABSTRACT

A series of 3-aminopyrrolidine-2,5-dione derivatives was synthesized and tested for anticonvulsant activity. Succinimide derivatives were obtained from a simple solvent-based reaction and a mechanochemical aza-Michael reaction of maleimide or its *N*-substituted derivatives with selected amines. The structure of the compounds was confirmed by spectroscopic methods (NMR, FT-IR, HPLC, ESI-MS, EA and XRD for four compounds). The cytotoxic activity of the succinimide derivatives was evaluated using HepG2 cells for hepatocytotoxicity and SH-SY5Y cells for neurocytotoxicity. None of the studied compounds showed hepatocytotoxicity and two showed neurocytotoxicity. Initial anticonvulsant screening was performed in mice using the psychomotor seizure test (6 Hz, 32 mA). The selected compounds were evaluated in the following acute models of epilepsy: the maximal electroshock test, psychomotor seizure test (6 Hz, 44 mA), subcutaneous pentylenetetrazole seizure test, and acute neurotoxicity (rotarod test). The most active compound 3-((4-chlorophenyl)amino)pyrrolidine-2,5-dione revealed antiseizure activity in all seizure models (including pharmacoresistant seizures) and showed better median effective doses (ED_{50}) and protective index values than the reference compound, ethosuximide. Furthermore, 3-(benzylamino)pyrrolidine-2,5-dione and 3-(phenylamino)pyrrolidine-2,5-dione exhibited antiseizure activity in the 6 Hz and MES tests, and 3-(butylamino)−1-phenylpyrrolidine-2,5-dione and 3-(benzylamino)−1-phenylpyrrolidine-2,5-dione exhibited antiseizure activity in the 6 Hz test. All active compounds demonstrated low in vivo neurotoxicity in the rotarod test and yielded favourable protective indices.

1. Introduction

Epilepsy is a chronic neurological disorder characterized by repeated seizures that affects people of all ages. A seizure is caused by uncontrolled, abnormal electrical activity in the brain. There are three types of seizure, as defined by the International League Against Epilepsy: generalized onset seizures (motor or absence), focal onset seizures, and unknown onset seizures. Antiepileptic drugs (AEDs), which are the primary therapy for the treatment of epilepsy, selectively depress the central nervous system, however, the efficacy of these drugs is limited; as nearly 30% of patients on medication still suffer from uncontrolled seizures. [1,2] Although there are numerous AEDs available, new compounds are required to improve seizure control and reduce the many side effects, including sedation, ataxia, gastrointestinal disorders, hepatotoxicity, megaloblastic anaemia, and cancer risk. [3–5] Because phensuximide, methsuximide, and ethosuximide are AEDs (Fig. 1), many pyrrolidine-2,5-dione derivatives have been developed and biologically evaluated. [6–10] Succinimide itself does not show anticonvulsant activity; however, the substitution of the C3 position of succinimide by one or two alkyl or aryl groups affords potent agents for

Abbreviations: 6 Hz, six-Hertz seizure test; AcOEt, ethyl acetate; DBU, 1,8-Diazabicyclo[5.4.0]undec-7-ene; DCM, dichloromethane; DIPEA, *N*,*N*-diisopropylethylamine; ETX, ethosuximide; GABA, γ-aminobutyric acid; HepG2, hepatocellular cancer; MES, maximal electroshock seizure test; MTS, methsuximide; NT, neurotoxicity screening–rotarod test; PHT, phenytoin; PI, protective index (TD50/ED50); PT, pretreatment time; PTS, phensuximide; PTZ, pentylenetetrazole; scPTZ, subcutaneous pentylenetetrazole seizure test; SH-SY5Y, neuroblastoma cell lines; THF, tetrahydrofuran.

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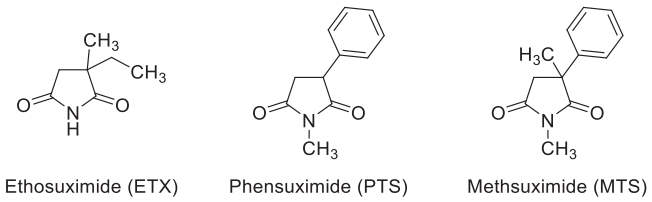


Fig. 1. Marketed succinimide-based antiepileptic drugs.

the management of absence seizures. [6,11].

There have been many structure-activity relationship studies on the anticonvulsant activity of succinimides, as summarized in the 1970 s by Vida, who reported that potent anticonvulsant succinimide derivatives must have an alkyl or aryl group bind to C3 and a substituent on the nitrogen atom. [12] Kornet *et al.* investigated the activity of several 3-phenyl- and/or alkyl-substituted succinimides and *N*-phenyl- or alkyl-substituted succinimides against maximal electroshock (MES) or metrazole-induced seizures and found that the alkylation of nitrogen has no substantial effect on anticonvulsant activity. [13] Furthermore, Magarian *et al.* showed that *N*-Mannich bases derived from succinimide and their 3-methyl, 3-isopropyl, and 3-benzhydryl derivatives are anticonvulsant agents. [14] *N*-Mannich bases derived from 3,3-disubstituted pyrrolidine-2,5-diones were also studied by Kaminski *et al.* One of the *N*-Mannich bases they synthesized, *N*-[(4-benzylpiperidin-1-yl)-methyl]−3-methyl-3-phenylpyrrolidine-2,5-dione, had protective effects in the MES and subcutaneous pentylenetetrazole (scPTZ) tests compared with ethosuximide. [15] Wolfe *et al.* investigated 2-benzylsuccinimides as anticonvulsant agents and found that the chloroderivatives, 2-(4-chlorobenzyl)succinimide and 2-(3,4-dichlorobenzyl)succinimide, were the most potent compounds and were more active than ethosuximide; however, the neurotoxicity of these compounds excluded them from further studies. [16].

Recently, hybrid compounds based on the succinimide scaffold have been found to act as potent, broad-spectrum anticonvulsant agents. [17–19] These hybrids consist of combinations of structural fragments of pharmacologically active substances. One of the hybrid derivatives, called KA11, integrates the structures of the drugs ethosuximide, levetiracetam, and lacosamide, and has been advanced to clinical trials. [20,21].

The aza-Michael reaction is a synthetic tool used to access aminopyrrolidine-2,5-dione derivatives. Much research has focused on developing highly efficient aza-Michael reactions. [22–24] However, the reactions often require harsh conditions and the use of organometallic catalysts in organic solvents, which could produce undesirable compounds, such as hydrolysis or polymerization products. [24] Therefore, new methods are required for synthesizing aza-Michael products with high yield and purity. Green chemistry requires the transformation of substrates into desired products by reducing or avoiding the use of catalysts and solvents. The development of aza-Michael additions under both catalyst- and solvent-free conditions remains challenging and this method is highly desirable due to its potential economic and environmental benefits. Interest has been increasing in mechanochemical synthesis, such as the ball milling method. Switching from organic solution to solid state synthesis allows reactions to be performed without harmful organic solvents, reduces reaction time, and opens new possible synthetic pathways that were unavailable in solution and classic glass laboratory equipment. [25–28] To the best of our knowledge, the synthesis of succinimide derivatives via aza-Michael addition using ball milling has not been described previously. Herein, we report the synthesis of 3-aminosuccinimides **3a**–**v** from the simple solvent-based or mechanochemical aza-Michael reaction between *N*-unsubstituted **1** and *N*-substituted maleimides **1a**–**c** and primary and secondary amines **2a**–**v**, together with their anticonvulsant properties in animal models.

2. Materials and methods

2.1. Materials

All chemicals and solvents were purchased from commercial suppliers and were used without further purification. Solvents were dried over sodium or calcium hydride, and distilled under inert atmosphere of argon prior to use. Reactions were carried out in air. Mechanochemical reactions were performed by using Retsch Mixer Mill MM400. Both jars (1.5 mL and 5 mL) and balls are made of stainless steel. Thin-layer chromatography (TLC) was performed on aluminum sheets precoated with Merck Silicagel 60 F_{254}. Products were purified by filtration through a short silica gel plug or by standard column chromatography (CC) on silica gel (230–400 mesh) deactivated with 1% Et_3N in hexane, by using freshly distilled solvents (hexane, ethyl acetate, dichloromethane) as eluents or by recrystallization from appropriate solvents. The ^1H, ^{13}C and ^{19}F NMR spectra were recorded on a Bruker AVIII (^1H NMR (600 MHz); ^{13}C NMR (151 MHz); ^{19}F NMR (565 MHz)). Chemical shifts are given relative to residual non deuterated solvent peaks ($CDCl_3$: $\delta = 7.26$ ppm for ^1H, $\delta = 77.16$ ppm for ^{13}C; DMSO-d_6: $\delta = 2.50$ ppm for ^1H, $\delta = 39.52$ ppm for ^{13}C). IR spectra were recorded in KBr on an FT-IR NEXUS (Thermo Nicolet) spectrometer. Mass spectra were performed with a Varian 500-MS LC IonTrap. Elemental analyses were obtained with a Vario EL III (Elementar Analysensysteme GmbH) instrument. Melting points were determined in capillaries with Stuart SMP30 apparatus and they are uncorrected.

3. Methods

3.1. Synthesis of 3-aminosuccinimides

3.1.1. General procedure for the synthesis of aminosuccinimides (Method A)

Maleimide (1.0 mmol) was dissolved in anhydrous ethyl acetate (10 mL) and purged with argon for 10 min, then corresponding amine (1.1 mmol) was added dropwise. The resulting solution was stirred for 10 min followed by the addition of Et_3N (1.0 mmol). The reaction mixture was stirred for 72–96 h at room temperature. After this time, solvent was removed under reduced pressure. The resulting residue was dissolved in CH_2Cl_2 (10 mL) and washed with saturated NH_4Cl (10 mL), H_2O (10 mL), $NaHCO_3$ (10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$, filtered and the solvent was evaporated under vacuum. The crude products were purified by flash column chromatography (silica gel, hexane/ethyl acetate as eluent) to afford the corresponding products.

3.1.2. General procedure for the synthesis of aminosuccinimides using a ball mill (Method B)

Maleimide (1.0 mmol), the appropriate amine or amine hydrochloride salt (1.1 mmol), Et_3N (1.0 mmol) were placed in the stainless steel jar (5 mL) loaded with one grinding ball (stainless steel, diameter: 5.0 mm). The vial was placed in the ball mill (Retsch Mixer Mill MM400) and the mixture was ball-milled for 6 h at 25 Hz. After this time, the resulting material was dissolved in EtOAc (15 mL) and filtered. The organic layer was washed with brine (2 ×10 mL), dried over $MgSO_4$, filtered and the solvent was evaporated under vacuum. The crude products were purified by flash column chromatography (silica gel, hexane/ethyl acetate as eluent) to afford the corresponding products. The crude products were purified by flash column chromatography (silica gel, hexane/ethyl acetate as eluent) to afford the corresponding products.

3-(benzylamino)pyrrolidine-2,5-dione (**3a**). Method A: 144 mg (71%), Method B: 181 mg (89%). Colorless crystalline solid, m.p. 75–77 °C. CC (SiO_2, hexane/EtOAc 1:1). **^1H NMR** (600 MHz, DMSO-d_6): δ 2.37 (dd, $J_{H\text{-}H} = 4.8$, 17.7 Hz, 1 H), 2.78–2.83 (m, 2 H), 3.63 (dd, $J_{H\text{-}H} = 4.8$, 8.4 Hz, 1H), 3.82 (dd, $J_{H\text{-}H} = 13.2$, 36.0 Hz, 2H), 7.22–7.26 (m,

1H), 7.28–7.34 (m, 4H), 11.12 (s_{br}, 1H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 37.1, 50.5, 56.2, 126.8, 128.1, 128.2, 140.2, 177.2, 180.2 ppm. **IR** (KBr) 3508, 3298, 3026, 1705, 1361, 1201, 923 cm^{-1}. **ESI-MS** (*m/z*): 205.2 (100, $[M+H]^+$). Anal. calcd for $C_{11}H_{12}N_2O_2{\cdot}H_2O$ (222.24): C 59.45, H 6.35, N 12.61; found: C 59.29, H 6.42, N 12.80. HPLC retention time: 13.197 min and 15.449 min (purity 96.6%).

3-(butylamino)pyrrolidine-2,5-dione (3b). Method A: 95 mg (56%), Method B: 140 mg (82%). Light yellow thick oil. CC (SiO_2, hexane/EtOAc 2:3). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 0.88 (t, $J_{H\text{-}H}$ = 7.4 Hz, 3H), 1.26–1.38 (m, 2H), 1.40–1.50 (m, 2H), 2.53–2.59 (m, 2H), 2.62–2.67 (m, 1H), 2.89 (dd, $J_{H\text{-}H}$ = 8.4, 18.0 Hz, 1H), 3.78 (dd, $J_{H\text{-}H}$ = 5.2, 8.4 Hz, 1H) ppm. **^{13}C NMR** (151 MHz, $CDCl_3$): δ 13.9, 20.3, 31.9, 37.1, 47.3, 57.6, 176.4, 179.1 ppm. **IR** (liquid film) 3292, 2958, 2931, 1716, 1194 cm^{-1}. **ESI-MS** (*m/z*): 171.2 (100, $[M+H]^+$). Anal. calcd for $C_8H_{14}N_2O_2$ (170.21): C 56.45, H 8.29, N 16.46; found: C 56.43, H 8.44, N 16.76. HPLC retention time: 10.532 min and 14.618 min (purity 98.5%).

3-(propylamino)pyrrolidine-2,5-dione (3c). Method A: 78 mg (50%), Method B: 136 mg (87%). Colorless solid, m.p. 61–63 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 0.85 (t, $J_{H\text{-}H}$ = 7.4 Hz, 3H), 1.32–1.44 (m, 2 H), 2.32 (dd, $J_{H\text{-}H}$ = 4.8, 17.6 Hz, 1H), 2.45 (dt, $J_{H\text{-}H}$ = 7.2, 11.2 Hz, 1H), 2.63 (dt, $J_{H\text{-}H}$ = 7.2, 11.2 Hz, 1H), 2.82 (dd, $J_{H\text{-}H}$ = 8.4, 17.6 Hz, 1H), 3.64 (dd, $J_{H\text{-}H}$ = 4.8, 8.4 Hz, 1H), 11.09 (s_{br}, 1H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 11.7, 22.8, 37.1, 48.7, 57.1, 177.2, 180.1 ppm. **IR** (KBr) 3292, 2966, 2934, 1708, 1193, 800 cm^{-1}. **ESI-MS** (*m/z*): 157.2 (100, $[M+H]^+$). Anal. calcd for $C_7H_{12}N_2O_2$ (156.19): C 53.83, H 7.74, N 17.94; found: C 53.73, H 7.78, N 18.07. HPLC retention time: 11.646 min and 21.715 min (purity 98.9%).

3-(*tert*-butylamino)pyrrolidine-2,5-dione (3d). Method A: 109 mg (64%), Method B: 157 mg (92%). Colorless solid, m.p. 73–75 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 1.02 (s, 9 H), 2.35 (dd, $J_{H\text{-}H}$ = 5.4, 17.4 Hz, 1H), 2.87 (dd, $J_{H\text{-}H}$ = 8.2, 17.4 Hz, 1H), 3.84 (dd, $J_{H\text{-}H}$ = 5.4, 8.2 Hz, 1H), 11.14 (s_{br}, 1H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 29.3, 41.2, 50.5, 52.9, 177.1, 180.9 ppm. **IR** (KBr) 3251, 2968, 1712, 1368, 1193 cm^{-1}. **ESI-MS** (*m/z*): 171.2 (100, $[M+H]^+$). Anal. calcd for $C_8H_{14}N_2O_2$ (170.21): C 56.45, H 8.29, N 16.46; found: C 56.26, H 8.11, N 16.34. HPLC retention time: 9.888 min and 12.198 min (purity 97.7%).

3-(isopropylamino)pyrrolidine-2,5-dione (3e). Method A: 83 mg (53%), Method B: 124 mg (79%). Colorless solid, m.p. 72–74 °C. CC (SiO_2, hexane/EtOAc 3:2). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 0.96 (d, $J_{H\text{-}H}$ = 2.4 Hz, 3H), 0.97 (d, $J_{H\text{-}H}$ = 2.4 Hz, 3H), 2.29 (dd, $J_{H\text{-}H}$ = 4.8, 17.6 Hz, 1H), 2.83 (dd, $J_{H\text{-}H}$ = 8.4, 17.6 Hz, 1H), 2.95–3.01 (m, 1H), 3.73 (dd, $J_{H\text{-}H}$ = 4.8, 8.4 Hz, 1H), 11.05 (s_{br}, 1H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 22.7, 22.7, 38.2, 46.2, 54.8, 177.2, 180.4 ppm. **IR** (KBr) 3283, 2985, 2965, 1708, 1351, 1184 cm^{-1}. **ESI-MS** (*m/z*): 157.2 (100, $[M+H]^+$). Anal. calcd for $C_7H_{12}N_2O_2$ (156.19): C 53.83, H 7.74, N 17.94; found: C 53.68, H 7.78, N 18.09. HPLC retention time: 7.932 min and 19.526 min (purity 98.4%).

3-(cyclohexylamino)pyrrolidine-2,5-dione (3f). Method A: 117 mg (60%), Method B: 165 mg (84%). Colorless crystalline solid, m.p. 159–161 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 0.94–1.00 (m, 2 H), 1.06–1.13 (m, 1 H), 1.15–1.23 (m, 2H), 1.52–1.55 (m, 1 H), 1.64–1.66 (m, 2H), 1.79–1.82 (m, 2 H), 2.11(s_{br}, 1H), 2.29 (dd, $J_{H\text{-}H}$ = 5.0, 17.6 Hz, 1H), 2.58–2.62 (m, 1H), 2.83 (dd, $J_{H\text{-}H}$ = 8.4, 17.6 Hz, 1H), 3.78 (dd, $J_{H\text{-}H}$ = 5.0, 8.4 Hz, 1H), 11.07 (s_{br}, 1H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 24.4, 24.5, 25.7, 32.9, 33.0, 38.4, 54.1, 54.4, 177.2, 180.5 ppm. **IR** (KBr) 3290, 2938, 2921, 1723, 1705, 1346, 1188 cm^{-1}. **ESI-MS** (*m/z*): 197.3 (100, $[M+H]^+$). Anal. calcd for $C_{10}H_{16}N_2O_2$ (196.25): C 61.20, H 8.22, N 14.27; found: C 61.08, H 8.23, N 14.42. HPLC retention time: 10.518 min and 14.550 min (purity 95.8%).

3-(cyclopropylamino)pyrrolidine-2,5-dione (3g). Method A: 75 mg (49%), Method B: 114 mg (74%). White solid, m.p. 66–68 °C. CC (SiO_2, hexane/EtOAc 3:2). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 0.21–0.26 (m, 2 H), 0.33–0.39 (m, 3 H), 2.28–2.31 (m, 1 H), 2.38 (dd, $J_{H\text{-}H}$ = 5.0, 17.8 Hz, 1 H), 2.81 (dd, $J_{H\text{-}H}$ = 8.6, 17.8 Hz, 1 H), 2.96 (s_{br}, 1 H), 3.71 (dd, $J_{H\text{-}H}$ = 5.0, 8.6 Hz, 1 H), 11.06 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 5.9, 6.7, 28.1, 36.9, 57.3, 177.2, 180.4 ppm. **IR** (KBr) 3307, 3210, 2944, 2921, 1720, 1699, 1361, 1182 cm^{-1}. **ESI-MS** (*m/z*): 155.2 (100, $[M+H]^+$). Anal. calcd for $C_7H_{10}N_2O_2$ (154.17): C 54.54, H 6.54, N 18.17; found: C 54.59, H 6.65, N 18.37. HPLC retention time: 12.933 min and 21.951 min (purity 96.3%).

3-(phenylamino)pyrrolidine-2,5-dione (3h). Method A: 104 mg (55%), Method B: 150 mg (79%). Light yellow crystalline solid, m.p. 176–178 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 2.43 (dd, $J_{H\text{-}H}$ = 5.6, 17.6 Hz, 1 H), 3.07 (dd, $J_{H\text{-}H}$ = 8.6, 17.6 Hz, 1 H), 4.56–4.60 (m, 1 H), 6.03 (d, $J_{H\text{-}H}$ = 8.0 Hz, 1 H), 6.56–6.68 (m, 3 H), 7.08–7.11 (m, 2 H), 11.30 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 37.3, 52.7, 112.7, 116.8, 129.0, 147.4, 176.5, 178.9 ppm. **IR** (KBr) 3389, 3080, 1603, 1197, 750 cm^{-1}. **ESI-MS** (*m/z*): 191.2 (100, $[M+H]^+$), 213.2 (100, $[M+Na]^+$). Anal. calcd for $C_{10}H_{10}N_2O_2$ (190.20): C 63.15, H 5.30, N 14.73; found: C 63.01, H 5.31, N 14.93. HPLC retention time: 18.590 min and 22.292 min (purity >99.0%).

3-((4-methoxyphenyl)amino)pyrrolidine-2,5-dione (3i). Method A: 103 mg (47%), Method B: 159 mg (72%). Yellow crystalline solid, m.p. 149–151 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 2.39 (dd, $J_{H\text{-}H}$ = 5.4, 17.6 Hz, 1 H), 3.05 (dd, $J_{H\text{-}H}$ = 8.6, 17.6 Hz, 1 H), 3.64 (s, 3 H), 4.45–4.48 (m, 1 H), 5.64 (d, $J_{H\text{-}H}$ = 8.0 Hz, 1 H), 6.58–6.61 (m, 2 H), 6.72–6.74 (m, 2 H), 11.26 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 37.4, 53.5, 55.3, 114.0, 114.6, 141.5, 151.4, 176.6, 179.1 ppm. **IR** (KBr) 3389, 3083, 1706, 1514, 1178, 833 cm^{-1}. **ESI-MS** (*m/z*): 219.0 (100, $[M-H]^-$). Anal. calcd for $C_{11}H_{12}N_2O_3$ (220.23): C 59.99, H 5.49, N 12.72; found: C 59.79, H 5.42, N 12.61. HPLC retention time: 22.107 min and 25.309 min (purity 98.0%).

3-(*p*-tolylamino)pyrrolidine-2,5-dione (3j). Method A: 101 mg (49%), Method B: 170 mg (83%). Light orange crystalline solid, m.p. 140–142 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 2.16 (s, 3 H), 2.41 (dd, $J_{H\text{-}H}$ = 5.6, 17.6 Hz, 1 H), 3.05 (dd, $J_{H\text{-}H}$ = 8.6, 17.6 Hz, 1 H), 4.50–4.53 (m, 1 H), 5.82 (d, $J_{H\text{-}H}$ = 8.0 Hz, 1 H), 6.55 (d, $J_{H\text{-}H}$ = 8.2 Hz, 2 H), 6.91 (d, $J_{H\text{-}H}$ = 8.2 Hz, 2 H), 11.28 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 20.1, 37.4, 53.0, 113.0, 125.3, 129.4, 145.1, 176.6, 179.0 ppm. **IR** (KBr) 3391, 3169, 3077, 1706, 1616, 1522, 1197, 809 cm^{-1}. **ESI-MS** (*m/z*): 227.2 (100, $[M+Na]^+$). Anal. calcd for $C_{11}H_{12}N_2O_2$ (204.23): C 64.69, H 5.92, N 13.72; found: C 64.53, H 5.90, N 13.92. HPLC retention time: 14.852 min and 19.145 min (purity >99.0%).

3-((4-chlorophenyl)amino)pyrrolidine-2,5-dione (3k). Method A: 52 mg (23%), Method B: 132 mg (59%). Light yellow crystalline solid, m.p. 162–164 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 2.43 (dd, $J_{H\text{-}H}$ = 5.5, 17.7 Hz, 1 H), 3.07 (dd, $J_{H\text{-}H}$ = 8.8, 17.7 Hz, 1 H), 4.55–4.59 (m, 1 H), 6.25 (d, $J_{H\text{-}H}$ = 8.0 Hz, 1 H), 6.64–6.66 (m, 2 H), 7.11–7.13 (m, 2 H), 11.31 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 37.1, 52.7, 114.2, 120.1, 128.6, 146.5, 176.3, 178.6 ppm. **IR** (KBr) 3389, 3080, 1706, 1598, 1170, 826 cm^{-1}. **ESI-MS** (*m/z*): 225.2 (100, $[M+H]^+$). Anal. calcd for $C_{10}H_9ClN_2O_2$ (224.64): C 53.47, H 4.04, N 12.47; found: C 53.45, H 4.13, N 12.66. HPLC retention time: 18.269 min and 29.491 min (purity 98.7%).

3-((4-fluorophenyl)amino)pyrrolidine-2,5-dione (3l). Method A: 67 mg (32%), Method B: 134 mg (64%). Colourless crystalline solid, m.p. 168–170 °C. CC (SiO_2, hexane/EtOAc 2:3). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 2.42 (dd, $J_{H\text{-}H}$ = 5.4, 17.6 Hz, 1 H), 3.07 (dd, $J_{H\text{-}H}$ = 8.7, 17.6 Hz, 1 H), 4.51–4.54 (m, 1 H), 5.99 (d, $J_{H\text{-}H}$ = 8.0 Hz, 1 H), 6.62–6.65 (m, 2 H), 6.92–6.96 (m, 2 H), 11.30 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 37.3, 53.2, 113.7, 113.7, 115.2, 115.4, 144.1, 144.1, 154.1, 155.6, 176.5, 178.9 ppm. **^{19}F NMR** (188 MHz, DMSO-d_6): − 128.5 (m, Ar-F) ppm. **IR** (KBr) 3389, 3173, 3081, 1706, 1515, 1196, 837 cm^{-1}. **ESI-MS** (*m/z*): 209.2 (100, $[M+H]^+$). Anal. calcd for $C_{10}H_9FN_2O_2$ (208.19): C 57.69, H 4.36, N 13.46; found: C 57.64, H 4.36, N 13.43. HPLC retention time: 25.719 min and 37.106 min (purity

99.0%).

3-(((1*S*,3*S*)-adamantan-1-yl)amino)pyrrolidine-2,5-dione (3m). Method A: 119 mg (48%), Method B: 197 mg (79%). Colourless crystalline solid, m.p. 183–185 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 1.46–1.48 (m, 3 H), 1.54–1.61 (m, 9 H), 1.79 (s_{br}, 1 H), 1.99–2.01 (m, 3 H), 2.32 (dd, $J_{H\text{-}H}$ = 5.4, 17.5 Hz, 1 H), 2.85 (dd, $J_{H\text{-}H}$ = 8.2, 17.5 Hz, 1 H), 3.92 (dd, $J_{H\text{-}H}$ = 5.4, 8.2 Hz, 1 H), 11.30 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 29.0, 36.1, 41.5, 42.7, 50.3, 51.0, 177.1, 181.1 ppm. **IR** (KBr) 3223, 2892, 1706, 1359, 1197, 855 cm^{-1}. **ESI-MS** (*m/z*): 249.3 (100, $[M+H]^+$). Anal. calcd for $C_{14}H_{20}N_2O_2$ (248.33): C 67.72, H 8.12, N 11.28; found: C 67.63, H 8.21, N 11.42. HPLC retention time: 17.335 min and 18.117 min (purity 98.1%).

[1,3′-bipyrrolidine] − 2′,5′-dione (3n). Method A: 0 mg (0%), Method B: 129 mg (77%). Colourless crystalline solid, m.p. 120–122 °C. CC (SiO_2, hexane/EtOAc 2:3). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 1.64–1.70 (m, 4 H), 2.49–2.52 (m, 2 H), 2.58 (dd, $J_{H\text{-}H}$ = 5.0, 18.0 Hz, 1 H), 2.74–2.79 (m, 3 H), 3.73 (dd, $J_{H\text{-}H}$ = 5.0, 8.5 Hz, 1 H), 11.19 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 23.1, 34.0, 49.8, 60.7, 176.8, 178.4 ppm. **IR** (KBr) 2965, 2880, 1719, 1325, 1192, 868, 627 cm^{-1}. **ESI-MS** (*m/z*): 169.2 (100, $[M+H]^+$). Anal. calcd for $C_8H_{12}N_2O_2$ (168.20): C 57.13, H 7.19, N 16.66; found: C 57.02, H 7.18, N 16.80. HPLC retention time: 11.218 min and 21.636 min (purity 96.2%).

3-(piperidin-1-yl)pyrrolidine-2,5-dione (3o). Method A: 0 mg (0%), Method B: 126 mg (69%). Colourless crystalline solid, m.p. 140–142 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 1.34–1.37 (m, 2 H), 1.44–1.48 (m, 4 H), 2.32–2.36 (m, 2 H) 2.54 (dd, $J_{H\text{-}H}$ = 5.0, 18.2 Hz, 1 H), 2.64–2.72 (m, 3 H), 3.78 (dd, $J_{H\text{-}H}$ = 5.0, 9.0 Hz, 1 H), 11.11 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 23.9, 25.7, 32.1, 49.4, 64.0, 176.9, 178.2 ppm. **IR** (KBr) 2936, 2857, 1709, 1330, 1187, 740, 665 cm^{-1}. **ESI-MS** (*m/z*): 183.2 (100, $[M+H]^+$). Anal. calcd for $C_9H_{14}N_2O_2$ (182.22): C 59.32, H 7.74, N 15.37; found: C 59.15, H 7.79, N 15.55. HPLC retention time: 12.083 min and 22.875 min (purity 96.2%).

3-morpholinopyrrolidine-2,5-dione (3p). Method A: 0 mg (0%), Method B: 151 mg (82%). Colourless crystalline solid, m.p. 152–154 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 2.38–2.41 (m, 2 H), 2.61 (dd, $J_{H\text{-}H}$ = 5.0, 18.0 Hz, 1 H), 2.70–2.76 (m, 3 H), 3.52–3.56 (m, 4 H), 3.79 (dd, $J_{H\text{-}H}$ = 5.0, 8.8 Hz, 1 H), 11.21 (s_{br}, 1 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 32.2, 48.9, 63.4, 66.3, 176.8, 178.0 ppm. **IR** (KBr) 2965, 2860, 1712, 1286, 1190, 1110, 954, 677 cm^{-1}. **ESI-MS** (*m/z*): 185.2 (100, $[M+H]^+$). Anal. calcd for $C_8H_{12}N_2O_3$ (184.20): C 52.17, H 6.57, N 15.21; found: C 52.09, H 6.60, N 15.40. HPLC retention time: 9.787 min and 22.203 min (purity 95.3%).

3-(butylamino)− 1-methylpyrrolidine-2,5-dione (3q). Method A: 103 mg (56%), Method B: 144 mg (78%). Yellow thick oil. CC (SiO_2, hexane/EtOAc 3:2). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 0.87 (t, $J_{H\text{-}H}$ = 7.4 Hz, 3 H), 1.28–1.34 (m, 2 H), 1.41–1.46 (m, 2 H), 1.85 (s_{br}, 1 H), 2.47 (dd, $J_{H\text{-}H}$ = 4.6, 17.8 Hz, 1 H), 2.51–2.56 (m, 1 H), 2.61–2.65 (m, 1 H), 2.87 (dd, $J_{H\text{-}H}$ = 8.2, 17.8 Hz, 1 H), 2.93 (s, 3 H), 3.71 (dd, $J_{H\text{-}H}$ = 4.6, 8.2 Hz, 1 H) ppm. **^{13}C NMR** (151 MHz, $CDCl_3$): δ 13.9, 20.3, 24.8, 32.1, 36.2, 47.5, 56.5, 175.5, 178.1 ppm. **IR** (neat) 2956, 2863, 1692, 1435, 1278, 1114, 950, 697 cm^{-1}. **ESI-MS** (*m/z*): 185.3 (100, $[M+H]^+$). Anal. calcd for $C_9H_{16}N_2O_2$ (184.24): C 58.67, H 8.75, N 15.21; found: C 58.85, H 8.82, N 15.11. HPLC retention time: 12.669 min and 14.432 min (purity 95.7%).

3-(benzylamino)− 1-methylpyrrolidine-2,5-dione (3r). Method A: 142 mg (65%), Method B: 197 mg (90%). Light yellow thick oil. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 1.85 (s_{br}, 1 H), 2.51 (dd, $J_{H\text{-}H}$ = 4.8, 18.0 Hz, 1 H), 2.86 (dd, $J_{H\text{-}H}$ = 8.2, 18.0 Hz, 1 H), 2.98 (s, 3 H), 3.75 (dd, $J_{H\text{-}H}$ = 4.8, 8.2 Hz, 1 H), 3.86 (AB system, J_{AB} = 13.1 Hz, 2 H), 7.26–7.29 (m, 1 H), 7.30–7.35 (m, 4 H) ppm. **^{13}C NMR** (151 MHz, $CDCl_3$): δ 24.9, 36.4, 52.0, 55.6, 127.6, 128.4, 128.7, 138.7, 175.4, 178.1 ppm. **IR** (liquid film) 3319, 2912, 2816, 1694, 1443, 1282, 1111, 969, 704 cm^{-1}. **ESI-MS** (*m/z*): 219.3 (100, $[M+H]^+$). Anal. calcd for $C_{12}H_{14}N_2O_2$ (218.26): C 66.04, H 6.47, N 12.84; found: C 65.93, H 6.54, N 12.82. HPLC retention time: 13.964 min and 16.651 min (purity 98.8%).

3-(butylamino)− 1-phenylpyrrolidine-2,5-dione (3s). Method A: 151 mg (61%), Method B: 217 mg (88%). Light yellow solid, m.p. 94–96 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 0.89 (t, $J_{H\text{-}H}$ = 7.2 Hz, 3 H), 1.30–1.36 (m, 2 H), 1.39–1.43 (m, 2 H), 2.43 (s_{br}, 1 H), 2.53–2.60 (m, 2 H), 2.77–2.81 (m, 1 H), 3.06 (dd, $J_{H\text{-}H}$ = 8.6, 17.6 Hz, 1 H), 3.86 (dd, $J_{H\text{-}H}$ = 4.8, 8.6 Hz, 1 H), 7.25–7.28 (m, 2 H), 7.39–7.42 (m, 1 H), 7.47–7.51 (m, 2 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 13.9, 19.9, 31.9, 36.2, 46.4, 56.2, 127.0, 128.2, 128.8, 132.5, 175.0, 177.7 ppm. **IR** (KBr) 3300, 2953, 2856, 1698, 1403, 1185, 825, 701 cm^{-1}. **ESI-MS** (*m/z*): 247.3 (100, $[M+H]^+$). Anal. calcd for $C_{14}H_{18}N_2O_2$ (246.31): C 68.27, H 7.37, N 11.37; found: C 68.08, H 7.44, N 11.50. HPLC retention time: 17.959 min and 22.496 min (purity 96.1%).

3-(benzylamino)− 1-phenylpyrrolidine-2,5-dione (3t). Method A: 202 mg (72%), Method B: 263 mg (94%). Colourless solid, m.p. 101–103 °C. CC (SiO_2, hexane/EtOAc 1:1). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 2.62 (dd, $J_{H\text{-}H}$ = 4.8, 17.8 Hz, 1 H), 3.05 (dd, $J_{H\text{-}H}$ = 8.6, 17.8 Hz, 1 H), 3.87 (dd, $J_{H\text{-}H}$ = 4.8, 8.6 Hz, 1 H), 3.95 (AB system, J_{AB} = 13.4 Hz, 2 H), 7.25–7.30 (m, 3 H), 7.33–7.36 (m, 2 H), 7.39–7.43 (m, 3 H), 7.50–7.51 (m, 2 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 36.2, 50.4, 55.2, 126.8, 127.1, 128.17, 128.19, 128.21, 128.8, 132.5, 140.1, 174.9, 177.7 ppm. **IR** (KBr) 3298, 3060, 2871, 1705, 1493, 1399, 1197, 735, 699 cm^{-1}. **ESI-MS** (*m/z*): 281.3 (100, $[M+H]^+$). Anal. calcd for $C_{17}H_{16}N_2O_2$ (280.33): C 72.84, H 5.75, N 9.99; found: C 72.75, H 5.83, N 10.02. HPLC retention time: 17.304 min and 20.033 min (purity >99.0%).

1-benzyl-3-(butylamino)pyrrolidine-2,5-dione (3u). Method A: 154 mg (59%), Method B: 209 mg (80%). Light pink thick oil. CC (SiO_2, hexane/EtOAc 3:2). **^{1}H NMR** (600 MHz, $CDCl_3$): δ 0.91 (t, $J_{H\text{-}H}$ = 7.2 Hz, 3 H), 1.33–1.38 (m, 2 H), 1.44–1.49 (m, 2 H), 1.70 (s_{br}, 1 H), 2.52 (dd, $J_{H\text{-}H}$ = 5.0, 18.0 Hz, 1 H), 2.55–2.59 (m, 1 H), 2.64–2.68 (m, 1 H), 2.92 (dd, $J_{H\text{-}H}$ = 8.2, 18.0 Hz, 1 H), 3.75 (dd, $J_{H\text{-}H}$ = 5.0, 8.2 Hz, 1 H), 4.65 (AB system, J_{AB} = 14.2 Hz, 2 H), 7.27–7.31 (m, 3 H), 7.36–7.37 (m, 2 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 13.8, 19.8, 31.7, 35.8, 41.1, 46.2, 55.9, 127.3, 127.4, 128.3, 136.1, 175.5, 178.3 ppm. **IR** (neat) 2956, 2863, 1700, 1398, 1342, 1155, 928, 697 cm^{-1}. **ESI-MS** (*m/z*): 261.3 (100, $[M+H]^+$). Anal. calcd for $C_{15}H_{20}N_2O_2$ (260.34): C 69.20, H 7.74, N 10.76; found: C 69.08, H 7.78, N 10.73. HPLC retention time: 17.288 min and 20.566 min (purity 96.5%).

1-benzyl-3-(benzylamino)pyrrolidine-2,5-dione (3v). Method A: 200 mg (68%), Method B: 251 mg (85%). Light purple thick oil. CC (SiO_2, hexane/EtOAc 3:2). **^{1}H NMR** (600 MHz, DMSO-d_6): δ 2.50 (dd, $J_{H\text{-}H}$ = 4.6, 17.8 Hz, 1 H), 2.93–2.98 (m, 2 H), 3.77 (dd, $J_{H\text{-}H}$ = 4.6, 8.2 Hz, 1 H), 3.87 (AB system, J_{AB} = 13.5 Hz, 2 H), 4.57 (s, 2 H), 7.23–7.29 (m, 4 H), 7.31–7.35 (m, 6 H) ppm. **^{13}C NMR** (151 MHz, DMSO-d_6): δ 35.9, 41.2, 50.4, 126.8, 127.3, 127.5, 128.1, 128.4, 136.2, 140.1, 175.6, 178.4 ppm. **IR** (liquid film) 3306, 3030, 2842, 1701, 1431, 1328, 1176, 720, 692 cm^{-1}. **ESI-MS** (*m/z*): 295.3 (100, $[M+H]^+$). Anal. calcd for $C_{18}H_{18}N_2O_2$ (294.35): C 73.45, H 6.16, N 9.52; found: C 73.32, H 6.09, N 9.40. HPLC retention time: 13.031 min and 17.293 min (purity >99.0%).

All compounds are > 95% pure by HPLC analysis.

3.2. Crystallographic data

Single crystals of four pyrrolidine-2,5-dione derivatives **3a**, **3n**, **3o** and **3p** were obtained after slow evaporation of the acetone/heptane solution at room temperature. Suitable mono crystals were selected and sequentially mounted on a fiber loop and used for X-ray measurement. X-ray data were collected on the Oxford Diffraction SuperNova Dual Source diffractometer with use of the monochromated CuKα X-ray source (λ = 1.54184). The crystals were kept at 100 K during data

collection. Data reduction and analytical absorption correction were performed with CrysAlis PRO [29]. Using Olex2 [30], the structures were solved with the ShelXS [31] structure solution program using Direct Methods and refined with the ShelXL [31] refinement package using Least Squares minimisation. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were introduced in calculated positions with idealized geometry and constrained using a rigid body model with isotropic displacement parameters equal to 1.2 and 1.5 of the equivalent displacement parameter of the parent atoms. A summary of relevant crystallographic data is given in Table 3.

The **CCDC 2207522**, **CCDC 2207523**, **CCDC 2207524** and **CCDC 2207525** contain the supplementary crystallographic data for this paper. The data can be obtained free of charge from The Cambridge Crystallographic Data Centre via http://www.ccdc.cam.ac.uk/conts/retrieving.html.

3.3. Cell culture and MTT cytotoxicity test

HepG2 (HB-8065™, ATCC) hepatocellular cancer and SH-SY5Y (CRL-2266™, ATCC) neuroblastoma cell lines were grown in culture flasks under standard conditions of temperature (37 °C) and CO_2 concentration (5%). Cells were cultured in EMEM medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS; Gibco) and antibiotics (Lonza). Daunorubicin hydrochloride (95% purity) was purchased from Enzo Life Sciences, while ethosuximide (99% purity) was purchased from Sigma Aldrich. Cells were seeded on 96-well plates at a density of 10 000/well for the HepG2 cell line or 20 000/well for the SH-SY5Y cell line. After 24 h, cells were incubated with test compounds at a final concentration of 1–50 μM, and plates were gently mixed. Vehicle control (DMSO) was included. After 48 h of incubation, the MTT solution (5 mg/mL) was added to the medium. After 3 h, when black formazan crystals appeared at the bottom of the wells, the medium was removed and formazan was dissolved in DMSO. Absorbance was read on a microplate reader (Spectra Max iD3, Molecular Devices, USA) at 570 nm. Cell viability was determined by dividing the absorbance of the experimental wells by the absorbance of the vehicle control wells × 100%. Three separate repeats of the experiment were performed. Data from cytotoxicity tests were subjected to one-way analysis of variance, followed by the Dunnett's test using GraphPad Prism 9.0 software (GraphPad Software Inc., San Diego, CA, USA). Values of $p < 0.05$ were considered statistically significant.

3.4. Pharmacology in vivo

3.4.1. Animals

Male CD-1 mice weighing 20–26 g were used in the in vivo experiment. The animals were housed in an environmentally controlled room (temperature of 22 ± 2 °C, humidity 55 ± 10%) on 12-h light/dark cycles (light on at 7:00 AM and off at 7:00 PM) and had free access to food (standard laboratory pellets) and water. The experimental groups consisted of 4 (initial pharmacology screening) or 6 (anticonvulsant and neurotoxic studies) mice. The experiments were performed between 9 am and 3 pm. For the experiments the animals were selected in a random way and trained observers performed all measurements. Immediately following acute seizure tests mice were euthanized by cervical dislocation.

All procedures involving animals and their care were performed in accordance with the current European Community and Polish legislation on animal experimentation. The studies were carried out under experimental protocol that was approved by the First Local Ethics Committee on Animal Testing at Jagiellonian University in Kraków (Krakow, Poland, No 560/2021), and in accordance with the European Communities Council Directive of 22 September 2010 (2010/63/EU). Care was taken to minimize animal suffering and reduce the number of animals used (3 R policy).

3.4.2. Drug administration

For the in vivo studies, the investigated compounds and ethosuximide (Sigma-Aldrich) were suspended in a 1% aqueous solution of Tween 80 (Baxter). All drug solutions/suspensions were freshly prepared and administered intraperitoneally (*i.p.*) at a volume of 0.1 mL per 10 g body weight. Control animals were administered an equivalent volume of vehicle (1% Tween 80) via the same route as the test compound. The experiments were carried out 0.5 h after *i.p.* injection.

3.4.3. Maximal electroshock seizure test (MES)

In the MES test, an electrical stimulus of sufficient intensity (25 mA, 500 V, 50 Hz, 0.2 s) was delivered via auricular electrodes by the electroshock generator (Rodent Shocker, Type 221, Hugo Sachs, Germany) to induce maximal seizures. The endpoint was the tonic extension of the hind limbs. Mice not displaying hind-limb tonic extension were considered to be protected from seizures. [32].

3.4.4. Six Hertz (6 Hz) psychomotor seizure test

In the 6 Hz test, psychomotor seizures were induced via corneal stimulation (6 Hz, 32 mA or 44 mA, 0.2 ms rectangular pulse width, 3 s duration) using a constant-current device (ECT Unit 57800, Ugo Basile, Italy). A drop of 1% solution of lidocaine hydrochloride was applied to the mouse corneas before stimulation to provide local anesthesia and ensure optimal current conductivity. After electrical stimulation, mice were gently released and observed for the presence or absence of seizure activity, being characterized by immobility associated with rearing, forelimb clonus, twitching of the vibrissae, stun, and Straub-tail. [33,34] Mice resuming normal behavior within 10 s from the stimulation were considered as protected. [35,36].

3.4.5. Subcutaneous pentylenetetrazole seizure test (scPTZ)

In this test, PTZ (100 mg/kg) was administered subcutaneously 30 min after injections of tested compounds and observations were carried out for the next 30 min. The latency time (in seconds) to the first seizure episode (clonic convulsions lasting for at least 3 s, with accompanying loss of the righting reflex) was measures and compared to the vehicle-treated (1% Tween 80) group. [37].

3.4.6. Neurotoxicity screening (NT) - rotarod test

Before the experiment, mice were trained to balance on an accelerating rod that rotated at 10 rotations per minute (Rotarod apparatus, May Commat RR0711, Turkey; rod diameter: 3 cm). During the training session, the animals were placed on a rotating rod for 3 min with an unlimited number of trials. Proper experiment was conducted at least 24 h after the training trial. On the day of the test, trained mice were pretreated intraperitoneally with the test compounds and were evaluated in the rotarod test. Neurotoxicity was indicated by the inability of the animal to maintain equilibration on the rod for 1 min.

3.4.7. Median effective dose (ED_{50}), median toxic dose (TD_{50}) and protective index (PI)

The ED_{50} is defined as the dose of a drug that protect 50% of animals against seizure episodes. The neurotoxic effect was expressed as a TD_{50} value, representing the dose at which motor impairments were observed in 50% of the animals in the rotarod test. To evaluate the ED_{50} or TD_{50}, 3–4 groups of animals were injected with various doses of tested compounds. Each group consisted of six animals. Both ED_{50} and TD_{50} values with 95% confidence limits were calculated by probit analysis. [38] The PI value was calculated as the ratio of TD_{50} to the respective ED_{50} value, as determined in the 6 Hz or MES tests. The PI is considered as an index of the margin of safety and tolerability between anticonvulsant dose and a dose of the compound exerting acute adverse effects. [29].

3.4.8. Binding assays

The radioligand binding studies were performed commercially by Eurofins Cerep SA (France) using testing procedures described

elsewhere - sodium channels (site 2), L-type calcium channels (dihydropyridine site) (commercial tests).

4. Results and discussion

The first objective of the present study was to compare methods for the preparation of *N*-unsubstituted and *N*-substituted succinimides based on the aza-Michael reaction of maleimides and primary and secondary amines performed either in ethyl acetate as a solvent or via a mechanochemical approach.

We started our investigation by reacting maleimide **1** and benzylamine **2a** in the presence of triethylamine in ethyl acetate at room temperature (Scheme 1). Expected product **3a** was obtained with a yield of 33% (Table S1, entry 1, for detailed information, see Supporting Information). Increasing the reaction time to 72 h improved the yield to 62% (entry 3). However, increasing the temperature to 80 °C resulted in a dramatic reduction in product yield (entry 4). Switching the solvent to either toluene, chloroform, dichloromethane, or THF decreased the yield (entries 7–10). Extended screening of the bases revealed that triethylamine was the best base under these conditions, whereas K_2CO_3, Cs_2CO_3, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), *N*,*N*-diisopropylethylamine (DIPEA), and KF were generally much less effective (entries 11 −15 vs. entry 3). After optimization, we identified a combination of maleimide (1.0 equiv), benzylamine (1.1 equiv), and triethylamine (1.0 equiv) as the optimal system in AcOEt at 25 °C. After 72 h, the yield of compound **3a** reached 63% (entry 6).

Next, we developed a mechanochemical reaction to obtain succinimide derivative **3a**. To test and verify the feasibility of the reaction, we reacted maleimide **1** and benzylamine **2a** using Et_3N as a base. Reactions were carried out in stainless steel jars (5 mL) loaded with two stainless steel grinding balls in a mixer mill (MM400, Retsch) at a frequency of 25 Hz. First, a mixture of **1** (1.0 equiv), **2a** (1.0 equiv), and Et_3N (1.0 equiv) in a stainless-steel jar (5 mL) was ground at room temperature for 3 h and the desired product **3a** was isolated with a yield of 70% (Table S2, entry 1, for detailed information, see Supporting Information). Varying the amount of benzylamine **2a** (Table S2, entries 2 and 3) substantially increased the yield of **3a**, with the highest yield obtained with 1.1 equivalent. Increasing the grinding time improved the reaction and **3a** was obtained with yields of 83% and 91% after 4.5 and 6 h, respectively (Table S2, entries 4 and 5). Et_3N was the most effective base, and Cs_2CO_3 and K_2CO_3 gave a lower yield (Table S2, entry 5 vs. entries 6 and 8). Only a trace amount of **3a** was formed when DBU was used as the base (Table S2, entry 9).

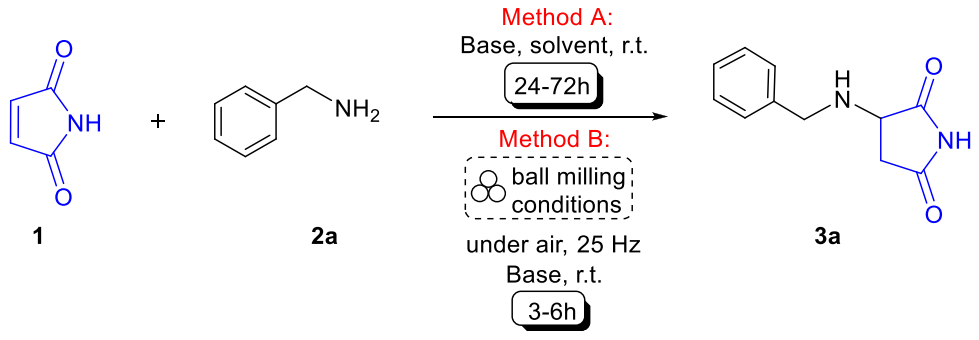
After optimizing the conditions, we examined the scope of the solvent and solvent-free aza-Michael reactions. Experiments to probe the scope of the amine component are summarized in Schemes 2 and 3. The mechanochemical method was applicable for both primary and secondary amines, bearing various electronically and sterically differentiated substrates. Various aliphatic amines, such as primary and secondary amines, can be used in the mechanochemical solid-state aza-Michael reaction (Scheme 2). Notably, these reactions include simple long-chain, benzyl and α-branched primary amines, such as *n*-butylamine **3b**, *n*-propylamine **3c**, *tert*-butylamine **3d**, and isopropylamine **3e**. Moreover, reactions with aliphatic cyclic amines, such as cyclohexylamine and cyclopropylamine, afforded aminosuccinimides **3f** and **3g** with yields of 84% and 74%, respectively.

A broad range of aromatic amines bearing electron-donating and electron-withdrawing substituents proved to be excellent substrates. Aniline provided aminosuccinimide **3h** at a yield of 79%. The electron-rich methyl substituent at the *para*-position gave **3j** with a high yield (83%), whereas the electron-poor substrates 4-Cl- and 4-F-anilines provided **3k** and **3l** with yields of 59% and 64%, respectively. Generally, arylamines with electron-donating substituents gave yields that were higher than those with electron-withdrawing substituents, probably because of the higher nucleophilicity of the nitrogen atom. The bulky nucleophile with a 1-adamantyl substituent formed the desired addition product **3m** in good yield (79%). Secondary amines were also used as nucleophiles under the optimized mechanochemical conditions. Various cyclic secondary amines were good substrates, and pyrrolidine and morpholine afforded products **3n** and **3p** with yields of 77% and 82%, respectively. In the reactions with secondary amines **2n**–**p**, the products were only obtained with the mechanochemical method; the analogous reactions in organic solutions did not generate the desired compounds **3n**–**p**.

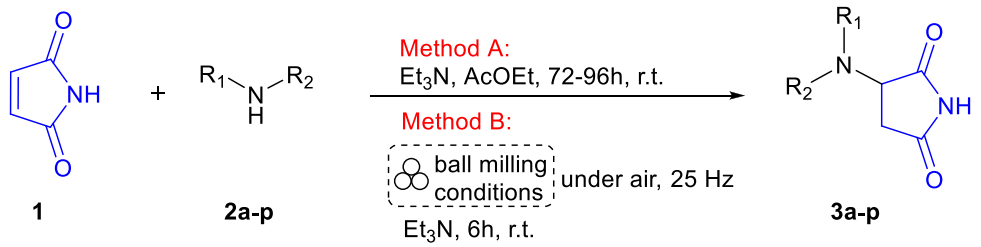
Next, we expanded the scope of the method with *N*-substituted maleimide derivatives (Scheme 3). Maleimides with a methyl, benzyl, or phenyl substituent on the nitrogen atom were used. Again, the mechanochemical method was better, resulting in desired compounds **3q**–**v** with good yields (78–94%). The aza-Michael addition products **3a**–**v** were extensively characterized by NMR (^1H, ^{13}C, ^{19}F), FT-IR, HPLC, MS, and EA, and the molecular structures of **3a**, **3n**, **3o**, and **3p** were confirmed by XRD.

4.1. Crystal structure description

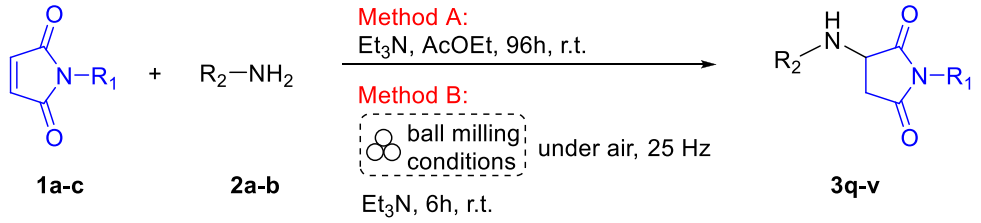
Compounds were crystallized in the orthorhombic *Pbca* (**3a** and **3o**) or $P2_12_12_1$ space groups (**3n**), and the monoclinic $P2_1/c$ space group (**3p**). The molecular structures of our pyrrolidine-2,5-dione derivatives are presented in Fig. 2. Succinimides **3o**, **3n** and **3p** crystallized in a pure form whereas 3-(benzylamino)pyrrolidine-2,5-dione (**3a**) did not; the crystal structure of **3a** revealed the monohydrate form of the compound (Fig. 4. **3a**). Two conformations of the [1,3′-bipyrrolidine]−2′,5′-dione molecule were observed in the crystal structure of **3n**, because its asymmetric unit contained two molecules. The packing in crystal structures of non-hydrated derivatives was arranged through the N-H…N hydrogen bonds, where the N-H bond of the pyrrolidine-2,5-dione ring functioned as the hydrogen bond donor and the N8 nitrogen atom with sp^3 geometry was the hydrogen bond acceptor. These interactions were reinforced by neighbouring C-H…O contacts. In the crystal structure of **3a**, the water molecule determined the molecular packing with four hydrogen bonds, where it simultaneously acted as a double donor and double acceptor of hydrogen bonds. In this structure, an additional fifth



Scheme 1. Optimization of the aza-Michael addition reaction.



Scheme 2. Aza-Michael addition of amines **2a–p** to maleimide **1**.



Scheme 3. Aza-Michael addition of benzylamine **2a** and butylamine **2b** to *N*-substituted maleimides **1a–c**.

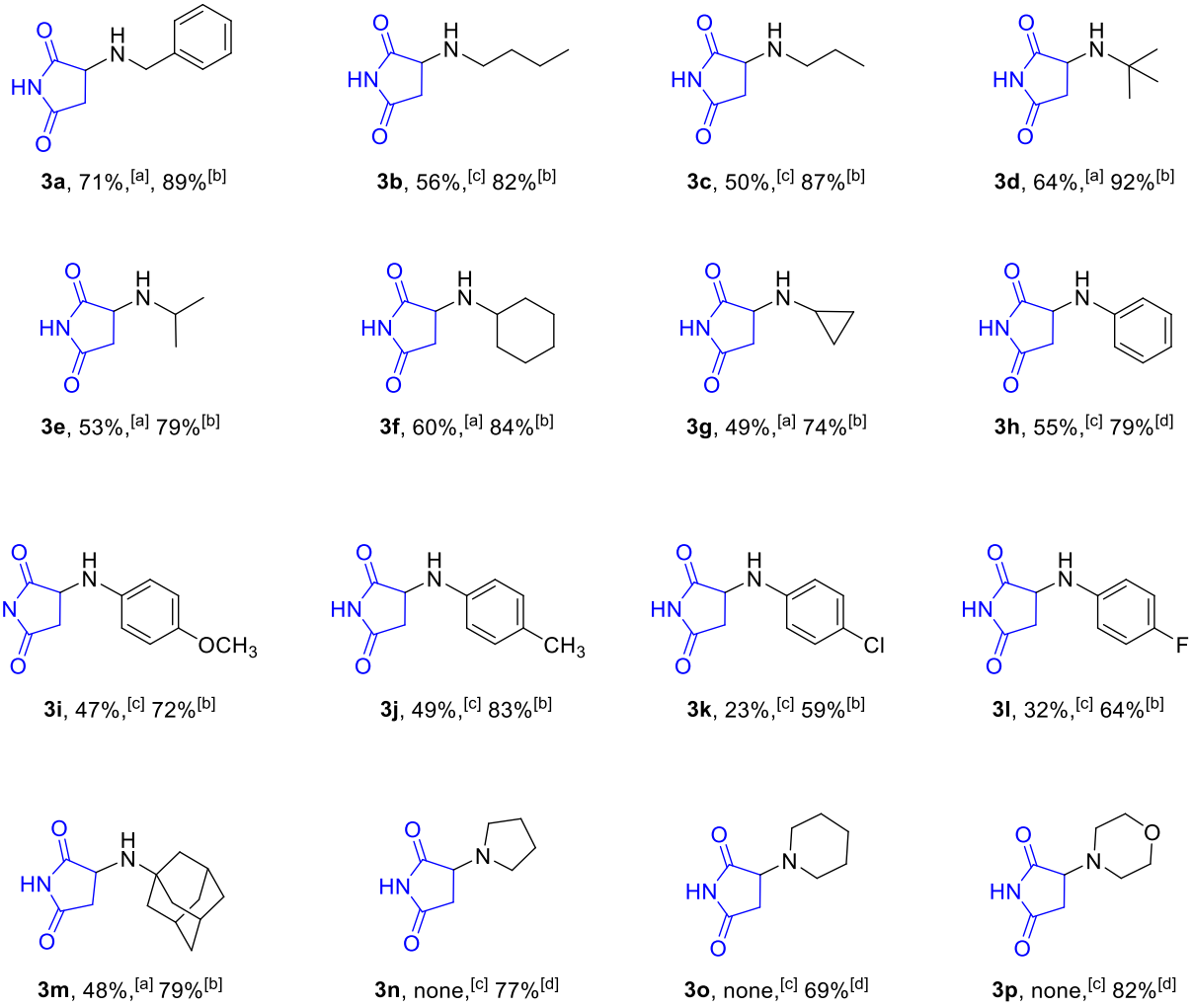


Fig. 2. Scope of 3-aminopyrrolidine-2,5-dione derivatives. Reaction conditions: [a] - Method A: maleimide (1.0 mmol), amine (1.1 mmol), triethylamine (1.0 mmol), AcOEt (5 mL), r.t., 72 h; [b] - Method B: maleimide (1.0 mmol), amine (1.1 mmol), triethylamine (1.0 mmol), ball mill (25 Hz), r.t., 6 h; [c] - Method A: maleimide (1.0 mmol), amine (1.1 mmol), triethylamine (1.0 mmol), AcOEt (5 mL), r.t., 96 h; [d] - Method B: maleimide (1.0 mmol), amine hydrochloride salt (1.1 mmol), triethylamine (1.0 mmol), ball mill (25 Hz), r.t., 6 h.

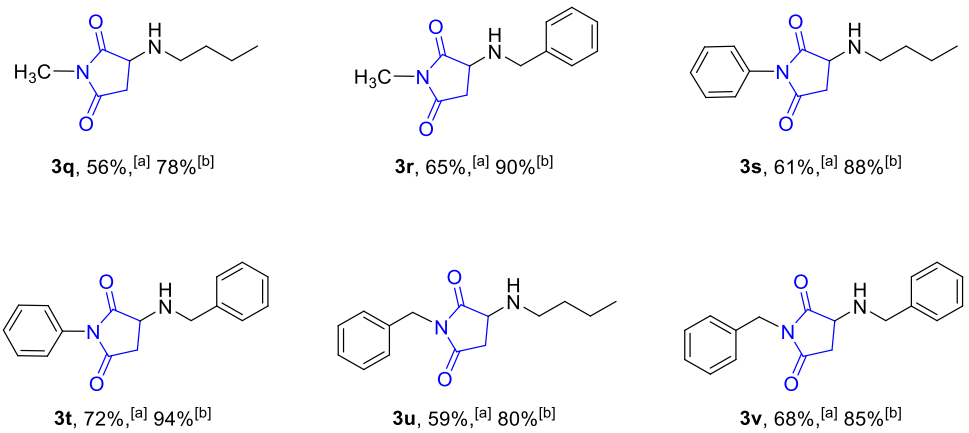


Fig. 3. Scope of *N*-substituted 3-aminopyrrolidine-2,5-dione derivatives. Reaction conditions: [a] - Method A: maleimide (1.0 mmol), amine (1.1 mmol), triethylamine (1.0 mmol), AcOEt (5 mL), r.t., 96 h; [b] - Method B: maleimide (1.0 mmol), amine (1.1 mmol), triethylamine (1.0 mmol), ball mill (25 Hz), r.t., 6 h.

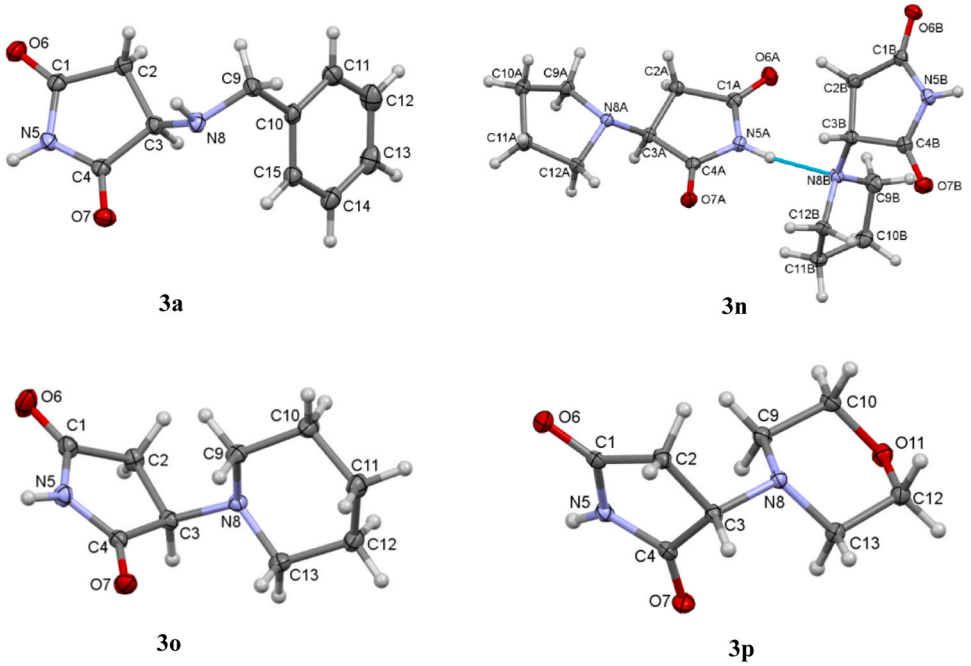


Fig. 4. Molecular structures of **3a**, **3n**, **3o**, **3p** with atom labeling scheme.

N-H…O hydrogen bond was observed. The hydrogen bond donor was the N8-H8 bond and the hydrogen bond acceptor was the oxygen atom O7 of the pyrrolidine-2,5-dione ring. The donor N8-H8 bond participated in another hydrogen bond, in which the hydrogen bond acceptor was the oxygen atom of the water molecule. Thus, a bifurcated donor hydrogen bond was observed. The geometry of the hydrogen bonds was calculated by Mercury 4.10 [39] and is summarized in Table S3 along with the symmetry codes of the non-covalent intermolecular interactions.

4.2. Cytotoxicity studies

Antiepileptic drugs exhibit several serious adverse drug reactions. For instance, phenytoin or valproic acid have hepatotoxic effects. Therefore, the hepatocytotoxicity of **3a**–**v** was investigated in HepG2 cells. Due to the action of antiepileptics on the central nervous system, the neurocytotoxicity of **3a**–**v** was also tested in SH-SY5Y cells to investigate the safety profile of these compounds. In both assays, ethosuximide was used as the reference drug. Most of the compounds studied exhibited good safety profiles, with tiny effects on the viability of HepG2 and SH-SY5Y cells (Figs. 5 and 6). However, compounds **3n** and **3o** displayed significant cytotoxicity against SH-SY5Y cells at concentrations greater than 25 μM, and decreased cell viability to 15.4% and 15.1% at 50 μM, respectively. At the highest concentration tested, **3c**, **3h,** and **3i** also decreased the viability of SH-SY5Y cells to 79.8%, 82.3%, and 78.1%, respectively.

4.3. Anticonvulsant activity

The preclinical development of new chemical agents for treating epilepsy is based on predictable animal seizure models, which correspond to different types of human epilepsy. The psychomotor seizure test (6 Hz), a model of focal seizures, and the MES test, a model of generalized tonic-clonic seizures, are widely used to screen compounds, distinguish lead analogues, and characterize established anticonvulsant drugs. [40–42] In these tests, seizures are induced by electrical impulses. In the 6 Hz test, stimulus intensities of 32 or 44 mA, which are 1.5 and 2 times the convulsive current for inducing seizures in 97% of mice (CC_{97}),

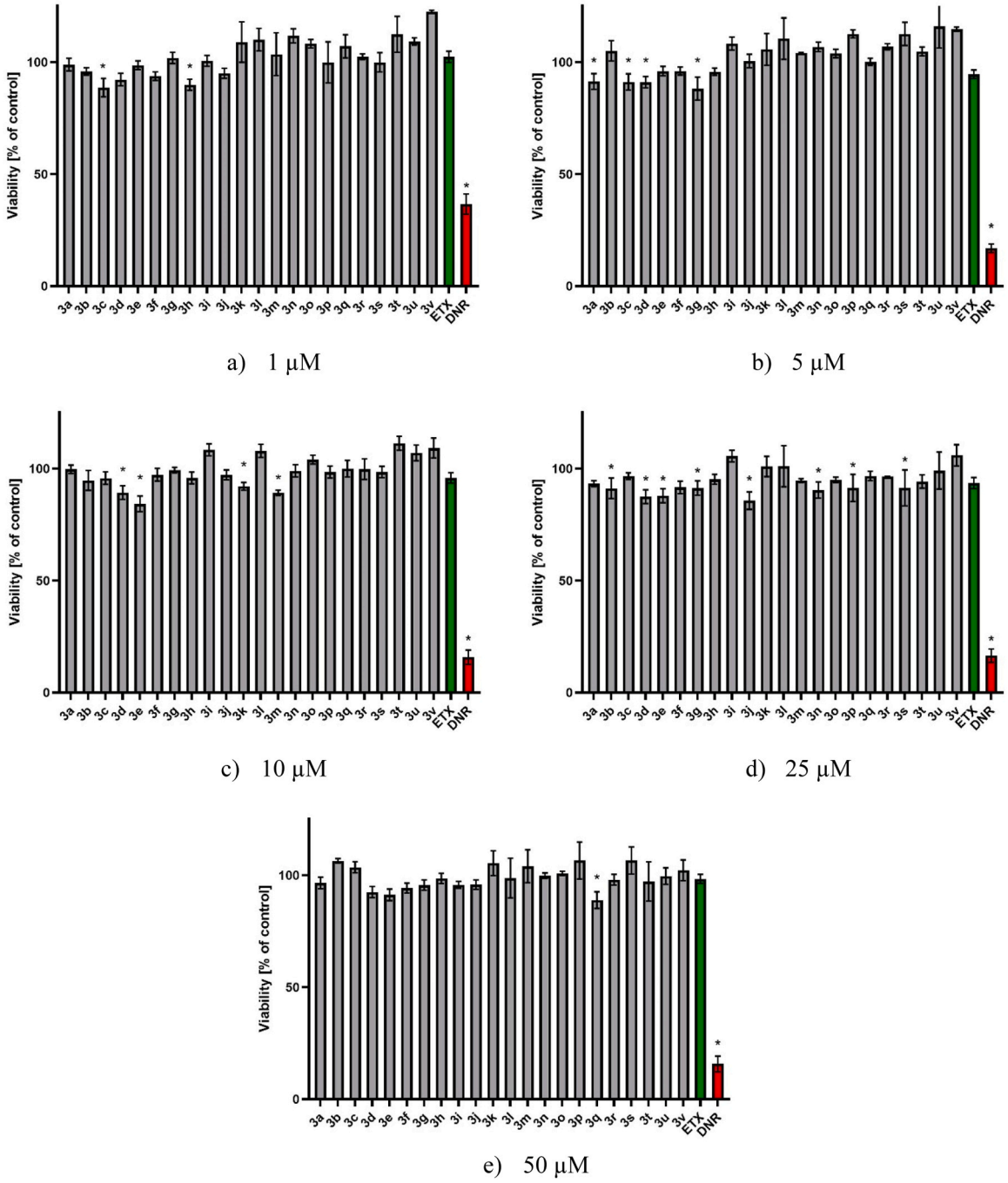


Fig. 5. Viability of HepG2 cells incubated with compounds **3a–v** at 1–50 μM for 48 h. Cell viability was determined using an MTT assay. Each experiment was repeated in triplicate. The graphs represent the number of viable cells expressed as a percent of the control (untreated cells) ± SD. ETX – ethosuximide, DNR – daunorubicin, *– data significantly different from control, $p < 0.05$.

are usually applied. [33] Some compounds that effectively block seizures at a lower stimulus intensity of 32 mA lose their efficacy at 44 mA. [42] For the third-generation anticonvulsant drug, levetiracetam, the median effective dose (ED_{50}) is at least 50 times higher at 44 mA than at 32 mA. Thus, the 6 Hz model with 44 mA stimulus intensity is a useful tool for identifying compounds with potential efficacy against pharmacoresistant seizures. [33,43] It should be emphasized that focal-onset seizures account for about 60% of all epilepsies. Unfortunately, despite the advent of new treatments, approximately 40% of people with this type of epilepsy are drug-resistant, which further increasing the risks associated with epilepsy, including premature death. [44] Bearing in mind the aforementioned facts and the structural similarities of new compounds with ethosuximide (which is active in preclinical studies in 6 Hz and scPTZ tests), in the present work, we screened all compounds (without in vitro cytotoxicity) using the 6 Hz (32 mA) test as a model of focal seizures.

The initial anticonvulsant screening of all the compounds was performed at a dose of 100 mg/kg using the psychomotor seizure test (6 Hz, 32 mA) as a model of focal seizures. The experiment was performed 0.5 h after intraperitoneal (i.p.) injection of compounds into groups of mice, with four mice per group. Compounds **3a**, **3k**, **3s**, and **3t** showed significant anticonvulsant activity by protecting a minimum of two of the four mice from seizures. Importantly, none of the compounds showed neuro-toxicity in the rotarod test at a dose of 100 mg/kg.

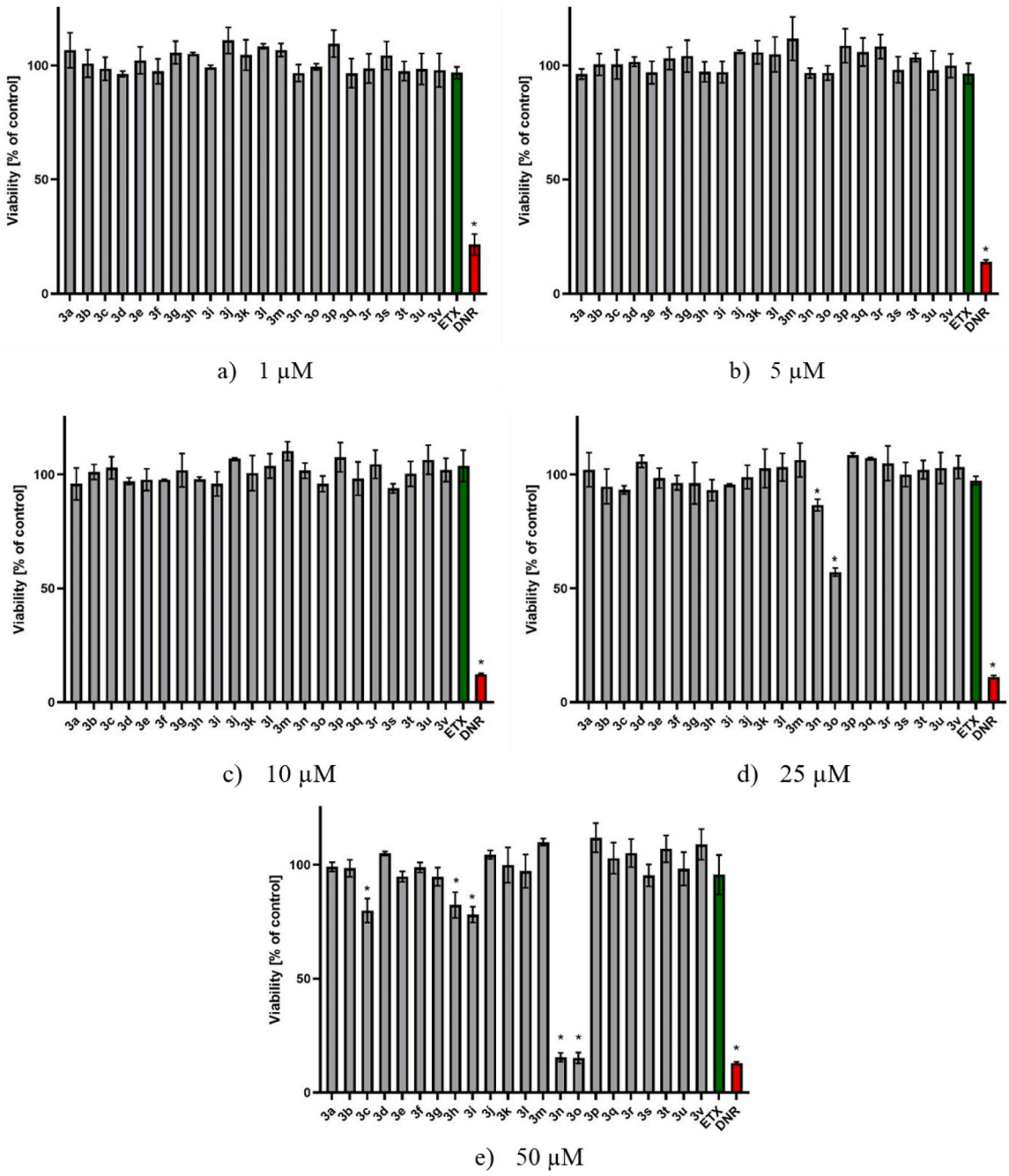


Fig. 6. Viability of SH-SY5Y cells incubated with compounds **3a–v** at 1–50 μM for 48 h. Cell viability was determined using an MTT assay. Each experiment was repeated in triplicate. The graphs represent the number of viable cells expressed as a percent of the control (untreated cells) ± SD. ETX – ethosuximide, DNR – daunorubicin, *– data significantly different from control, $p < 0.05$.

Compounds with no antiseizure activity at a dose of 100 mg/kg were also tested at a dose of 200 mg/kg. At this dose, **3h** protected all tested mice and **3i** protected two of four tested mice against seizure. After administration of the compounds at a higher dose of 200 mg/kg, only **3a**, **3d**, and **3k** caused impaired motor coordination in more than one mouse in the rotarod test. Next, **3a**, **3h**, **3k**, **3s**, and **3t** were tested at a dose of 200 mg/kg in the MES test, a model of generalized tonic-clonic seizures. All compounds, except **3s**, showed anticonvulsant activity in this test by protecting two (**3h** and **3t**), three (**3k**), or all (**3a**) of the four mice from tonic seizures. The detailed results of the preliminary pharmacological screening are presented in Table 1.

Based on the preliminary results from the 6 Hz and MES tests, the ED_{50} values for the active compounds (**3a**, **3h**, **3k**, **3s**, and **3t**) were determined and are presented in Table 2. In the 6 Hz test (32 mA current), the ED_{50} was lowest for compounds **3a** and **3k**, with values of 87.93 and 100.04 mg/kg, respectively. Thus, these two compounds were also tested in the 6 Hz test (44 mA current), which is a model of pharmacoresistant seizures. Of note, ED_{50} was 143.70 mg/kg for **3a** and 217.75 mg/kg for **3k**, whereas ethosuximide showed no activity at a dose of 300 mg/kg. These results were better than those obtained for the reference drug, ethosuximide (Table 2). Moreover, the protective indices (PIs), which measure the benefit-to-risk ratio of the therapeutic agent, were similar to or higher than the PI for ethosuximide. Additionally, in contrast to ethosuximide, compounds **3a**, **3h**, and **3k** were active in the MES test, a model of generalized tonic-clonic seizures, with ED_{50} values between 128.53 and 157.51 mg/kg. In our experiments, racemic

Table 1
Anticonvulsant activity (6 Hz (32 mA), MES) and acute neurotoxicity (rotarod test) 0.5 h after i.p. administration in mice.

Compound	Intraperitoneal administration in mice				
	6 Hz (32 mA)[a]		MES[b]	NT[c]	
	dose 100 mg/kg	dose 200 mg/kg	dose 200 mg/kg	dose 100 mg/kg	dose 200 mg/kg
3a	**3/4**	-	**4/4**	0/4	**2/4**
3c	0/4	0/4	-	0/4	1/4
3d	0/4	0/4	-	0/4	**2/4**
3e	0/4	0/4	-	¼	0/4
3f	0/4	1/4	-	¼	0/4
3g	0/4	0/4	-	¼	0/4
3h	1/4	**4/4**	**2/4**	¼	0/4
3i	0/4	**2/4**	-	¼	0/4
3j	0/4	1/4	-	0/4	0/4
3k	**2/4**	-	**3/4**	0/4	**3/4**
3l	1/4	1/4	-	0/4	1/4
3m	1/4	1/4	-	0/4	0/4
3p	1/4	1/4	-	0/4	0/4
3s	**2/4**	-	1/4	0/4	1/4
3t	**2/4**	-	**2/4**	0/4	0/4

Ratios where at least one animal was protected or with motor impairment are shown in bold for easier data interpretation. Data indicate the number of mice protected or with motor impairment/number of mice tested. The animals were examined 0.5 h after administration of the compounds at doses of 100 and 200 mg/kg. A dash indicates not tested.
[a] 6 Hz (32 mA): psychomotor seizure test.
[b] MES: maximal electroshock seizure test.
[c] NT: neurotoxicity screening–rotarod test.

ethosuximide ((*RS*)−3-ethyl-3-methyl-pyrrolidine-2,5-dione), was used, and HPLC analysis proved that succinimide derivatives **3a**–**v** were also racemic.

Compounds **3a**, **3k**, and **3s**, which were active in previous tests and had different substituents, were selected for evaluation in the *sc*PTZ test, which is a model of non-convulsive seizures (absence, myoclonic) and is now usually used to screen selected compounds. Pentylenetetrazole (PTZ) is a convulsive agent, that antagonizes the inhibitory function of γ-aminobutyric acid (GABA) by binding to the picrotoxin-binding site of the postsynaptic $GABA_A$ receptor. [46] The results of the *sc*PTZ test showed statistically significant antiseizure activity for compound **3k**, only, at doses of 150 and 180 mg/kg, but not at the dose of 120 mg/kg. **3k** significantly prolonged the latency time to first seizure episode compared with the vehicle group, from 701.2 ± 113.3 s to 1372 ± 192.2 s (by 95%, $p < 0.05$) and to 1494 ± 203.1 s (by 113%, $p < 0.001$), respectively (Fig. 7). Conversely, no significant effect was observed for compounds **3a** and **3s**. For the reference drug, statistically significant results were obtained at doses of 120, 150, and 180 mg/kg. It is worth mentioning that the most active compound **3k** possesses the *p*-chlorophenyl group in its structure. There were many findings reported where the presence of a terminal phenyl ring *p*-substituted with electron withdrawing groups (such as chlorine atom) was beneficial for anticonvulsant activity. [47–50] For example, Siddiqui *et al.* studied the pyridazinone-thiazole derivatives, among 18 reported compounds with similar structure, the derivative with *p*-chlorophenyl substituent was the most potent and safer anticonvulsant agent. [51].

As part of the safety profiling, we determined the in vivo neurotoxicity of **3a**, **3h**, **3k**, **3s,** and **3t** by examining their effects on the motor coordination of mice in the rotarod test. No neurotoxicity was observed for compound **3h** at a dose of 300 mg/kg, or for compound **3k** at a dose of 250 mg/kg. For compounds **3a**, **3t**, and ethosuximide at a dose of 300 mg/kg, impaired motor coordination was observed in 16.7% (1/6) of the animals. Only compound **3s** at doses of 220, 250, and 300 mg/kg resulted in impaired motor coordination, in 16.7% (1/6), 66.7% (4/6), and 83.3% (5/6) of the animals, respectively; thus, the neurotoxic dose (TD_{50}) for this compound was 248.55 mg/kg. For comparison, the values of ED_{50} (in the MES test) and TD_{50} for the classic drug phenytoin were 6.65 and 56.91 mg/kg, respectively. Furthermore, to investigate plausible molecular mechanisms of action, we assessed whether compounds **3a**, **3h**, and **3k** inhibit voltage-gated Na^+(site 2) and Ca^{2+} (L-type, dihydropyridine site) channels; however, none of the compounds tested showed significant binding activity to Na^+ and Ca^{2+} channels at concentrations of 10 and 100 μM (data not shown).

5. Conclusion

Mechanochemical activation is a suitable method for obtaining succinimide derivatives by reacting the corresponding *N*-unsubstituted

Table 2
Quantitative pharmacological parameters ED_{50}, TD_{50}, and PI in mice (i.p.).

Compound	Time (h)	ED_{50} 6 Hz (32 mA) (mg/kg)	ED_{50} 6 Hz (44 mA) (mg/kg)	ED_{50} MES (mg/kg)	TD_{50} (mg/kg)	PI (TD_{50}/ED_{50})
3a	0.5	87.93 (72.98 – 105.94)	143.70 (113.48 – 181.96)	128.53 (106.94 – 154.48)	> 300 (1/6) *	> 2.33 (MES) > 3.41 (6 Hz, 32 mA) > 2.09 (6 Hz, 44 mA)
3h	0.5	164.54 (154.71 – 175.01)	-	152.59 (132.72 – 175.45)	> 300 (0/6) *	> 1.97 (MES) > 1.82 (6 Hz)
3k	0.5	100.04 (76.07 – 131.57)	217.75 (184.13 – 257.50)	157.51 (103.12 – 240.60)	> 250 (0/6) *	> 1.59 (MES) > 2.50 (6 Hz, 32 mA) > 1.15 (6 Hz, 44 mA)
3s	0.5	152.59 (132.72 – 175.45)	-	-	248.55 (222.95 – 277.10)	1.63 (6 Hz, 32 mA)
3t	0.5	175.82 (161.25 – 191.71)	-	-	> 300 (1/6) *	> 1.71 (6 Hz, 32 mA)
ETX	0.5	191.34 (166.44 – 221.12)	> 300	-	> 300 (1/6) *	1.57 (6 Hz, 32 mA)
PHT [45]	1	6.65 (5.42–8.16)			56.91 (48.53–66.74)	8.56 (MES)

Values in parentheses are 95% confidence intervals determined by probit analysis according to the Litchfield and Wilcoxon method.
*Data in brackets indicate the number of mice with motor impairment/number of mice tested.
ED_{50}: median effective dose.
TD_{50}: median neurotoxic dose.
PI: protective index (TD_{50}/ED_{50}).

Table 3
Crystal data and structure refinement.

Identification code	3a	3o	3n	3p
Empirical formula	$C_{11}H_{12}N_2O_2$, H_2O	$C_9H_{14}N_2O_2$	$C_8H_{12}N_2O_2$	$C_8H_{12}N_2O_3$
Formula weight	222.24	182.22	168.2	184.2
Crystal system	orthorhombic	orthorhombic	orthorhombic	monoclinic
Space group	*Pbca*	*Pbca*	$P2_12_12_1$	$P2_1/c$
a/Å	9.44435(16)	10.28754(15)	5.95704(9)	12.4275(4)
b/Å	9.78104(16)	10.5445(2)	11.57889(17)	6.49886(13)
c/Å	23.4866(4)	17.1171(3)	23.7585(3)	10.7742(3)
α/°	90.0	90.0	90.0	90.0
β/°	90.0	90.0	90.0	107.636(3)
γ/°	90.0	90.0	90.0	90.0
Volume/Å^3	2169.59(6)	1856.81(5)	1638.76(4)	829.27(4)
Z	8	8	8	4
ρ_{calc}g/cm^3	1.361	1.304	1.363	1.475
μ/mm^{-1}	0.832	0.764	0.821	0.957
F(000)	944	784	720	392
Crystal size/mm^3	0.222 × 0.180 × 0.079	0.171 × 0.131 × 0.056	0.324 × 0.176 × 0.087	0.219 × 0.145 × 0.069
2Θ range for data collection/°	7.528 to 153.582	10.336 to 152.858	7.442 to 153.132	7.464 to 152.858
Index ranges	-9 ≤ h ≤ 11, -12 ≤ k ≤ 11, -29 ≤ l ≤ 29	-7 ≤ h ≤ 12, -12 ≤ k ≤ 13, -21 ≤ l ≤ 21	-7 ≤ h ≤ 7, -14 ≤ k ≤ 14, -23 ≤ l ≤ 29	-15 ≤ h ≤ 14, -5 ≤ k ≤ 8, -13 ≤ l ≤ 13
Reflections collected	21184	7070	17480	7259
Independent reflections	2273 [R_{int} = 0.0324, R_{sigma} = 0.0149]	1942 [R_{int} = 0.0244, R_{sigma} = 0.0212]	3454 [R_{int} = 0.0292, R_{sigma} = 0.0195]	1734 [R_{int} = 0.0214, R_{sigma} = 0.0171]
Data/restraints/parameters	2273/0/151	1942/0/119	3454/0/217	1734/0/119
Goodness-of-fit on F^2	1.048	1.049	1.058	1.067
Final R indexes [I> =2σ (I)]	R_1 = 0.0349, wR_2 = 0.0888	R_1 = 0.0323, wR_2 = 0.0783	R_1 = 0.0283, wR_2 = 0.0693	R_1 = 0.0316, wR_2 = 0.0790
Final R indexes [all data]	R_1 = 0.0383, wR_2 = 0.0917	R_1 = 0.0379, wR_2 = 0.0831	R_1 = 0.0301, wR_2 = 0.0711	R_1 = 0.0361, wR_2 = 0.0829
Largest diff. peak/hole / eÅ^{-3}	0.25/−0.23	0.32/−0.17	0.15/−0.19	0.33/−0.22
Flack parameter	-	-	0.01(6)	-
CCDC no.	2207522	2207523	2207524	2207525

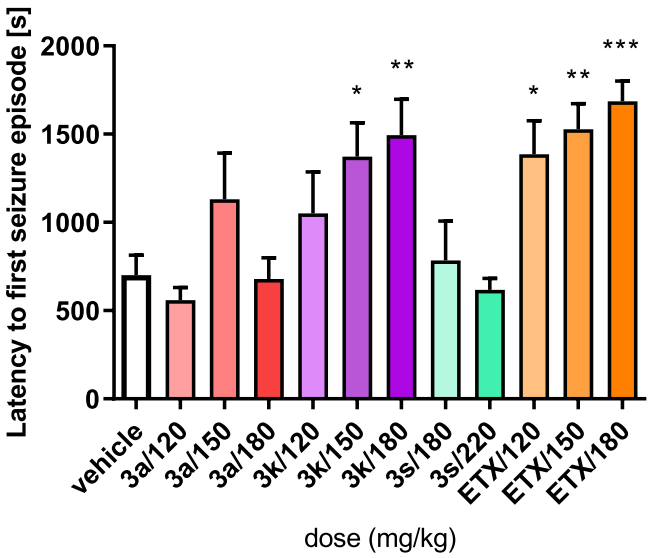


Fig. 7. Anticonvulsant activity of compounds 3a, 3k, and 3s, and ethosuximide (ETX) in the *sc*PTZ test. Each value represents the mean ± standard error of the mean obtained from 4 to 6 mice. Statistical analysis: one-way analysis of variance, followed by Dunnett's post hoc test. Significant difference compared with the control group: $^{**}p < 0.01$, $^{***}p < 0.001$.

and *N*-substituted maleimides with primary and secondary amines. The yields were higher (59–94%) compared with the reactions in organic solvents (0–72%), and the reaction times were much shorter (6 h vs. 72–96 h).

The anticonvulsant activity of each compound was tested using the psychomotor seizure test (6 Hz, 32 mA) in mice. The most active compounds (**3a**, **3h**, **3k**, **3s,** and **3t**) were also tested in the MES test and three of the *N*-unsubstituted succinimide derivatives (**3a**, **3h**, and **3k**) were active with ED_{50} values between 128.53 and 157.51 mg/kg. Three succinimide derivatives (**3a**, **3k**, and **3s**) were evaluated for their activity in the *sc*PTZ test but only compound **3k** showed significant activity. Furthermore, the compounds **3a** and **3k**, but not ethosuximide, showed antiseizure activity in the 6 Hz test with 44 mA stimulus intensity (a model of pharmacoresistant seizures). Of note, **3k** exhibited antiseizure activity in the 6 Hz (32 and 44 mA), MES, and *sc*PTZ tests. Therefore, **3k** could be a broad-spectrum anticonvulsant agent with possible activities against different types of seizures, including pharmacoresistant seizures. Moreover, **3a** and **3h** exhibited antiseizure activity in the 6 Hz and MES tests, and **3s** and **3t** exhibited antiseizure activity in the 6 Hz test. Importantly, all of the active compounds demonstrated low in vivo neurotoxicity in the rotarod test, as well as favourable protective indices. Cytotoxicity in vitro experiments using HepG2 and SH-SY5Y cells indicated that none of the compounds showed hepatocytotoxicity and none (except **3n** and **3o**) showed neurocytotoxicity. Our succinimide derivatives have interesting antiseizure activity and we believe that they merit further study.

Author contributions

S.J. Methodology, organic synthesis, manuscript writing; A.R. Biological part, investigation, supervision, manuscript writing; A.D. Biological part, investigation; E.P. Biological part, supervision, J.P. Biological part, investigation; K.P. Biological part, investigation; S.W. Crystallography, investigation. B.R. Conceptualization; supervision, manuscript writing.

CRediT authorship contribution statement

Szymon Jarzyński: Methodology, organic synthesis, manuscript writing; **Anna Rapacz**: Biological part, investigation, supervision, manuscript writing; **Anna Dziubina**: Biological part, investigation; **Elżbieta Pękala**: Biological part, supervision; **Justyna Popiół**: Biological part, investigation; **Kamil Piska**: Biological part, investigation; **Sławomir Wojtulewski**: Crystallography, investigation; **Bogna Rudolf**: Conceptualization; supervision, manuscript writing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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The animal study protocol was approved by the First Local Ethical Committee in Krakow, Poland, No 560/2021 (22 September 2021).

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2023.115749.

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