Arch. Pharm. Chem. Life Sci. 2017, *350*, e1600368

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Full Paper

Synthesis and Anticonvulsant Properties of New 3,3-Diphenyl-2,5-dioxo-pyrrolidin-1-yl-acetamides and 3,3-Diphenyl-propionamides

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The focused library of new amides derived from 3,3-diphenyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid (**2a–t**) and 3,3-diphenyl-propionic acid (**3a–t**) as potential anticonvulsant agents was synthesized. The final products were obtained in the amidation reaction of the given carboxylic acid (**2**, **3**) with appropriate secondary amines in the presence of carbonyldiimidazole (CDI) as a coupling reagent. The initial anticonvulsant screening was performed in mice intraperitoneally (*i.p.*) using the "classical" maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) seizure models, whereas the acute neurological toxicity was determined applying the rotarod test. Additionally, several compounds were studied also in the 6-Hz seizures recognized as the animal model of human pharmacoresistant epilepsy. In this series, compound **3q** displayed a broad spectrum of activity across the preclinical seizure models (ED_{50} MES = 31.64 mg/kg; ED_{50} *sc*PTZ = 75.41 mg/kg, ED_{50} 6-Hz (32 mA) = 38.15 mg/kg). Consequently, compound **3q** revealed a wider spectrum of protection, higher activity or/and a better safety profile than the commonly used antiepileptic drugs such as phenytoin, ethosuximide, valproic acid, or/and levetiracetam. Notably, the *in vitro* studies showed that the most possible mechanism of action of **3q** may be connected to the interaction with neuronal voltage-sensitive sodium channels (site 2). Other substances were active predominantly in the chemically induced seizures. The results of the current studies indicate that the presence of the pyrrolidine-2,5-dione ring is important but not indispensable for anticonvulsant activity.

Keywords: Anticonvulsant activity / *In vitro* studies / *In vivo* studies / Pyrrolidine-2,5-dione / Succinimide

Received: December 7, 2016; Revised: February 21, 2017; Accepted: February 22, 2017

DOI 10.1002/ardp.201600368

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Introduction

Approximately, 50 million people worldwide are affected by epilepsy, making it the second most common neurological disorder after stroke [1, 2]. As many as 20–40% of patients with epilepsy are likely to have drug-resistant epilepsy despite the significant advancements made in epilepsy research [3]. In case of pharmacotherapy of seizures, compliance is often limited by adverse side effects most notably related to the central nervous system (CNS) exposure, such as diminished attention, executive function, intelligence, language skills, memory, and processing speed [4]. During the last 20 years, several new AEDs such as levetiracetam, felbamate, lamotrigine, gabapentin, and topiramate have been implemented for the treatment of epilepsy. Although these medicines have been shown to be effective in many patients with epileptic syndromes, their efficacy does not appear to be superior to that of the

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established AEDs. Therefore, the ideal AED should prevent different types of seizures without producing side effects that adversely affect patients' quality of life. Therefore, continued research on safer and more effective AEDs is urgently necessary.

The variety of molecular targets, not always precisely definite, makes it difficult to establish an unequivocal mechanism of action of AEDs. This fact is also reflected in the limited possibilities of designing new drugs based on the structure of a given biological target [5]. In the past decades, there have been many demonstrations attempting to identify the structural features of compounds crucial for anticonvulsant activity. Thus, the amide bond is one of the important core fragments of anticonvulsants that may appear in the structure in chain form or as a part of the heterocyclic ring usually as lactam or imide (Fig. 1) [6]. Therefore, the previous research in our group has identified the pyrrolidine-2,5-dione system differently substituted at position-1 and -3 as targets for new AEDs. Many of these compounds were effective in the MES and/or *sc*PTZ screens that are known as the most popular seizure models in the early stages of search for the new anticonvulsants. Notably, the most potent activity was observed for compounds containing differently substituted piperazines as amine function [7–12].

Bearing in mind the aforementioned facts, in the current study we have focused on the synthesis of two series of compounds; one is the acetamide derivatives with the 3,3-diphenyl-pyrrolidine-2,5-dione fragment (series 1) and the other is without the imide ring, that is, benzhydryl derivatives (series 2). Thus, the main structural modification relied on the presence or lack of succinimide core; hence, the aim of the current study was to elucidate how the structure of imide/amide fragment affects the anticonvulsant activity. The general structures for compounds representing both series are shown in Fig. 2.

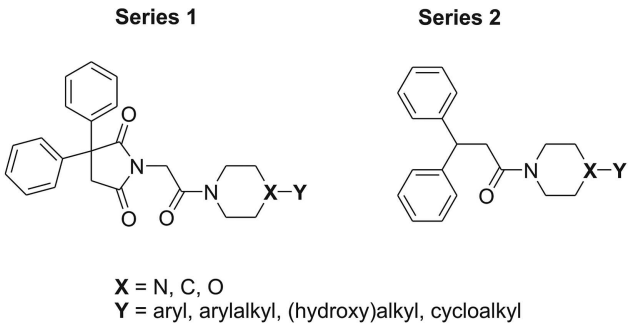


Figure 2. The general structures for compounds from series 1 and 2.

Results and discussion

Chemistry

The intermediate **2** and the final compounds **2a–t**, **3a–t** were synthesized according to Scheme 1. In the first step, the condensation reaction of the 2,2-diphenyl-succinic acid (**1**) (prepared as previously reported) [13], with amionoacetic acid, yielded corresponding intermediate **2**. 3,3-Diphenyl-propionic acid (**3**) was commercially purchased from Sigma–Aldrich (St. Louis, USA). In the next step, final substances (**2a–t** and **3a–t**) were obtained in the coupling reaction of intermediate **2** or 3,3-diphenyl-propionic acid (**3**) with equimolar amounts of appropriate 4-substituted piperazines, benzyl-piperidine, or morpholine in the presence of CDI as coupling reagent. The reaction was carried out at room temperature in dry DMF for 24 h. The oily residues were crystallized from 2-propanol after DMF's evaporation. Their purity and homogeneity were assessed by TLC and gradient UPLC chromatography. The purity of all final compounds determined by use of UPLC method was >99%. The final

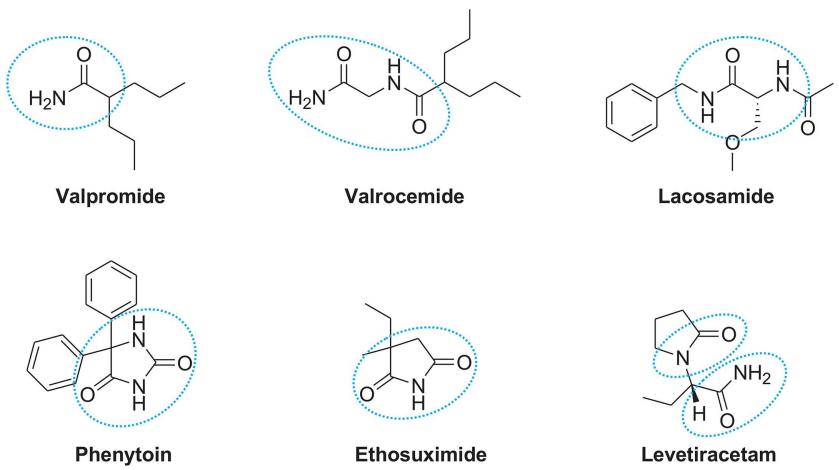
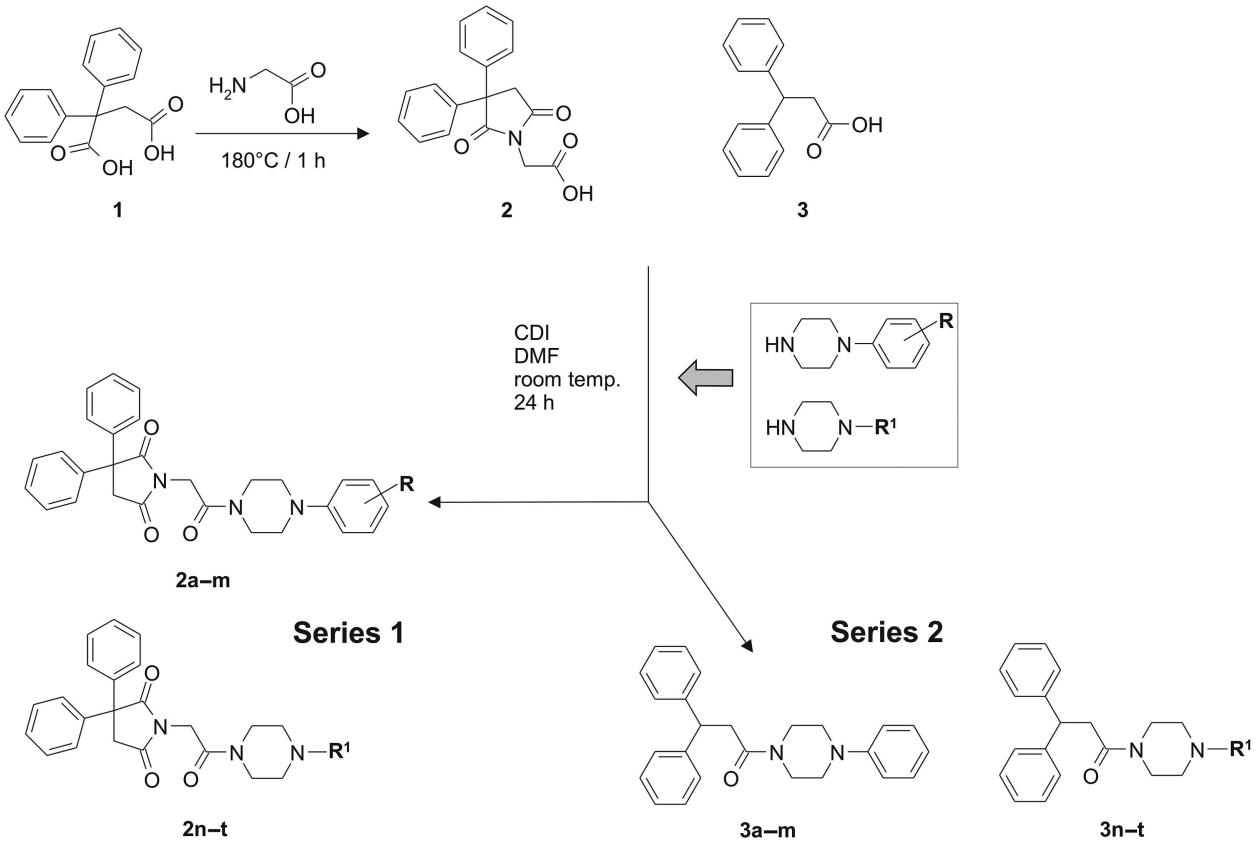


Figure 1. Structures of model amide/imide/lactam derived AEDs.



R = 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
R¹ = benzyl, 2-pyrimidinyl, cylcohexyl, 4-methyl, 2-hydroxyethyl
X = N,C,O

Scheme 1. Synthetic procedures for compounds **2a–t** (series 1) and **3a–t** (series 2).

substances were characterized by ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and LC-MS spectra.

Anticonvulsant activity

For the treatment of epilepsy, the pre-clinical development of new chemical agents is based on the use of animal models that imitate the given type of human seizures. Thus, empirical screening has successfully led to the identification of all clinically relevant AEDs. Despite the diversity of models that could potentially be used to screen for anticonvulsant activity, the MES model and the *sc*PTZ model remain the "gold standards" in the early stages of testing. The MES test is the mechanism-independent animal seizure model that enables to identify the compounds preventing seizure spread. This test uses an electrical stimulus to produce generalized tonic–clonic seizures, and thus it is thought to be an experimental model of tonic–clonic epilepsy and of partial convulsions with or without the secondary generalization in humans. The *sc*PTZ test uses chemically induced myoclonic seizures and is proposed to identify the agents raising the seizure threshold. This test is related to the absence seizures in human generalized epilepsies [14–18]. All the final compounds were tested *in vivo* for anticonvulsant activity using the MES and *sc*PTZ models.

The anticonvulsant activity was connected closely with chemical structures of compounds. Except for MES active **2c**, the other 3,3-diphenyl-2,5-dioxo-pyrrolidin-1-yl-acetamides (series 1) showed protection exclusively it the *sc*PTZ test or were inactive (Table 1). In this series, the most potent activity revealed the 4-phenylpiperazine derivatives with chlorine atom(s) at the aromatic ring (**2e**–**g**). These substances were effective in both time intervals 0.5 and 2 h, indicating the quick onset and long in duration anticonvulsant protection. The other 4-phenylpiperazin-amides active at dose of 100 mg/kg

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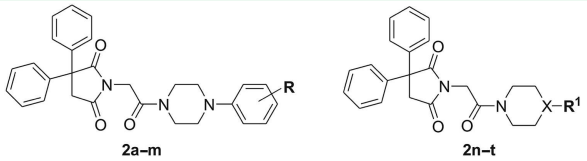
Table 1. Anticonvulsant activity (MES, *sc*PTZ) and acute neurotoxicity (rotarod test) following *i.p.* administration in mice (2a–t).



Cmpd	R	X	R^1	MES[a] 0.5 h	MES[a] 2 h	scPTZ[b] 0.5 h	scPTZ[b] 2 h	NT[c] 0.5 h	NT[c] 2 h	clog *P*[d]
2b	2-F	–	–	–	–	–	100	–	–	3.45
2c	4-F	–	–	–	100	–	–	–	–	3.45
2e	3-Cl	–	–	–	–	100	100	–	–	4.02
2f	4-Cl	–	–	–	–	100	100	–	–	4.02
2g	2,3-Cl	–	–	–	–	100	100	–	–	4.67
2i	3-CF_3	–	–	–	–	–	–	100	–	4.32
2j	2-CH_3	–	–	–	–	100	–	–	–	3.64
2l	2-OCH_3	–	–	–	–	100	–	–	–	3.16
2t	–	O	–	–	–	–	100	–	–	2.20

Dose of 100 mg/kg was administered. The data indicate the minimum dose whereby anticonvulsant activity was demonstrated. The animals were examined at 0.5 and 2 h. A dash indicates the absence of anticonvulsant activity or neurotoxicity at the maximum dose administered (100 mg/kg).
[a]Maximal electroshock test. [b] Subcutaneous pentylenetetrazole test. [c] Neurotoxicity screening – rotorod test. [d] clog *P* values calculated using a log *P* module of ChemDraw Ultra program, version 7.0.1 (Cambridge Soft Corporation, Cambridge, MA, USA).

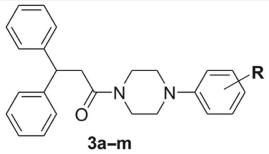
include 2-fluorine (**2b**), 2-methyl (**2j**), and 2-methoxy (**2l**) derivatives. The aforementioned substances protected mice at time point of 0.5 h (**2j**, **2l**) or 2 h (**2b**). In series 1, only one unaromatic compound **2t** containing the morpholine ring revealed the activity in the chemically induced seizures 2 h after *i.p.* administration. In the rotarod test for acute neurological toxicity inactive compound **2i** impaired the motor coordination in mice, whereas other substances were non-neurotoxic at the dose tested – 100 mg/kg.

More diversified anticonvulsant activity was observed for 3,3-diphenyl-propanamides (Table 2). In series 2, six compounds protected the mice in the MES test (**3f**, **3l**, **3p**, **3q**, **3r**, **3t**) and five substances were effective in *sc*PTZ seizures (**3h**, **3i**, **3m**, **3p**, **3q**). The widest spectrum of anticonvulsant activity (MES/*sc*PTZ) was observed for cyclohexyl (**3p**) and hydroxyethyl (**3q**) derivatives. Thus, these substances may be potentially effective in different types of epilepsy in humans, including tonic–clonic epilepsy, partial convulsions with or without secondary generalization, and generalized absence seizures. The other molecules were active in one seizure model. In this series, only two compounds **3i** and **3r** showed acute neurological toxicity at dose of 100 mg/kg. The comparison of the *in vivo* data for series 1 and 2 revealed that the removal of the pyrrolidine-2,5-dione ring decreased the activity in case of 4-phenylpiperazin-amides, while it increased the protection for (hydroxyl)alkyl derivatives.

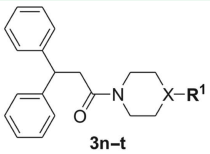
In the next step of the pharmacological investigations, the median effective doses (ED_{50}) were determined for compounds showing minimum 50% protection at the dose of 100 mg/kg in the preliminary studies (MES, *sc*PTZ tests). Moreover, at the same time the median neurotoxic doses (TD_{50}) were estimated in the rotarod test. These data were used to calculate the protective indexes (PIs), which are the measure of the benefit-to-risk ratio of the therapeutic agent. Notably, with the aim of estimating the anticonvulsant potency of compounds obtained, the same studies were performed for model and clinically relevant AEDs such as ethosuximide, phenytoin, and valproic acid. These studies were carried out in mice after *i.p.* injection at the time of peak activity taken from the screening data or literature in case of model AEDs. Table 3 summarizes the obtained results.

The quantitative *i.p.* data in mice confirmed the broad-spectrum and potent anticonvulsant activity for **3p** and **3q**. These substances revealed approximately fivefold (**3p**) and eightfold (**3q**) better activity in the MES test as well as 3.2-fold (both compounds) more potent protection in the *sc*PTZ seizures compared to wide-spectrum AED – valproic acid. Unfortunately, compared to the aforementioned AED, **3q** showed worse safety profile in the rotarod test that yielded more beneficial PI value in case of MES test, and less favorable PI for PTZ seizures. The cyclohexyl derivative **3p** provided better PIs in both seizure models. The rest of the derivatives

Table 2. Anticonvulsant activity (MES, *sc*PTZ) and acute neurotoxicity (rotarod test) following *i.p.* administration in mice (3a–t).



3a–m



3n–t

Cmpd	R	X	R^1	MES[a] 0.5 h	MES[a] 2 h	scPTZ[b] 0.5 h	scPTZ[b] 2 h	NT[c] 0.5 h	NT[c] 2 h	clog *P*[d]
3f	4-Cl	–	–	100	100	–	–	–	–	4.54
3h	3,4-Cl	–	–	–	–	100	100	–	–	5.20
3i	3-CF_3	–	–	–	–	100	–	100	–	4.84
3l	2-OCH_3	–	–	–	100	–	–	–	–	3.68
3m	3-OCH_3	–	–	–	–	100	–	–	–	3.68
3p	–	N	C_6H_{11}	100	–	100	100	–	–	5.10
3q	–	N	$(CH_2)_2OH$	100	–	100	100	–	–	2.63
3r	–	N	CH_3	100	–	–	–	100	–	3.28
3t	–	O	–	100	–	–	–	–	–	2.72

Dose of 100 mg/kg was administered. The data indicate the minimum dose whereby anticonvulsant activity was demonstrated. The animals were examined at 0.5 and 2 h. A dash indicates the absence of anticonvulsant activity or neurotoxicity at the maximum dose administered (100 mg/kg).
[a] Maximal electroshock test. [b] Subcutaneous pentylenetetrazole test. [c] Neurotoxicity screening – rotorod test. [d] clog *P* values calculated using a log *P* module of ChemDraw Ultra program, version 7.0.1 (Cambridge Soft Corporation).

(except for **2c** effective in MES test) showed satisfying protection exclusively in the *sc*PTZ test. Except for relatively neurotoxic compound **3r** and aforementioned **3q**, other substances were more potent and provided also more favorable PI values than ethosuximide and valproic acid. Surprisingly enough, despite of structural similarities to phenytoin, namely the presence of biphenyl fragment, all new compounds revealed weak activity in the MES test or were devoid of protection in electrically induced seizures (see Tables 1–3).

Traditionally, most screening programs assess efficacy of AED candidates against either electrically (e.g., MES) or chemically (e.g., *sc*PTZ) induced seizures. The number of new AEDs currently available, or in development, for the management of epilepsy certainly attest to the success of this approach. However, this method may overlook novel compounds that would be uniquely effective in the therapy-resistant population. One example supporting this hypothesis is provided by levetiracetam, which has demonstrated efficacy in refractory human partial epilepsies. It was found to be inactive against MES and *sc*PTZ seizures even at high doses, whereas it showed high efficacy in the 6-Hz model of pharmacoresistant limbic seizures [19]. Bearing in mind the aforementioned facts with the aim of finding substances active in the latter test, eight structurally diversified molecules (phenylpiperazine or (hydroxyl)alkylpiperazine derivatives) were screened in the 6-Hz (32-mA) model. The results after *i.p.* administration are summarized in Table 4.

As is shown in Table 4, five compounds tested displayed satisfactory activity in the 6-Hz seizure model. It should be emphasized that except for **3i**, the most beneficial anticonvulsant properties were observed for amides containing the aliphatic amines; cyclohexylpiperazine (**2p**) and especially 2-hydroxyethylpiperazine (**2q**, **3q**). The quantitative pharmacological parameters for **2p**, **2q**, **3i**, and **3q** revealed lower activity and worse PIs compared to 6-Hz active AED: levetiracetam. Nevertheless, **2p**, **2q**, and **3i** were more potent and safer than valproic acid (Table 5).

Intensive studies into the physiological and biochemical events occurring during epileptic seizures have provided insight into the molecular mechanisms by which these might be controlled. The fundamental role in establishing and regulating the excitability of CNS neurons as well as suppression of seizures is ascribed to voltage sodium and calcium channels [20, 21]. Bearing in mind the above-mentioned facts for the most promising compound (**3q**) the binding properties for sodium channel (site 2) and L-type calcium channel (dihydropyridine site) were conducted. The results obtained are presented in Table 6.

It can be noticed that **3q** revealed the high affinity to sodium channel at the lowest concentration of 1 μM (54.2% inhibition of control specific binding). Notably, the affinity for this molecular target increased distinctly with the concentration

Table 3. The quantitative pharmacological parameters ED_{50} (MES, *sc*PTZ), TD_{50} (rotarod test), and PI in mice ***i.p.***

Cmpd	TPE (h)[a)]	ED_{50} MES (mg/kg)[b)]	ED_{50} *sc*PTZ (mg/kg)[b)]	TD_{50} (mg/kg)[b)]	PI (TD_{50}/ED_{50})[c)]
2b	2.0	NT	70.32 (41.83–118.20)	>400	>5.70
2c	2.0	85.55 (57.23–127.87)	NT	>300	>3.50
2e	2.0	NT	81.41 (68.21–97.16)	>400	>4.90
2f	0.5	NT	85.55 (57.23–127.87)	>400	>4.70
2g	0.5	NT	76.86 (55.64–106.18)	>300	>3.90
2j	0.5	NT	87.53 (56.11–136.55)	>300	>3.43
2l	0.5	NT	84.07 (63.04–112.13)	>300	3.57
2t	2.0	NT	108.04 (75.27–155.18)	>400	>3.70
3f	0.5 2.0	>120	NT	NT	–
3h	2.0	NT	42.83 (24.47–74.99)	>400	>9.34
3i	0.5	NT	72.12 (61.06–85.19)	>400	>5.54
3l	2.0	>120	NT	NT	–
3m	0.5	NT	86.51 (62.81–119.16)	339.95 (292.49–383.69)	3.93
3p	0.5 (MES) 2.0 (PTZ)	50.91 (36.61–70.79)	73.26 (57.41–93.48)	268.38 (251.54–286.35)	5.27 (MES) 3.66 (*sc*PTZ)
3q	0.5	31.64 (27.21–36.80)	75.41 (63.60–89.42)	116.82 (102.21–133.51)	3.70 (MES) 1.50 (*sc*PTZ)
3r	0.5	38.15 (33.97–42.84)	NT	78.30 (65.82–93.13)	2.05 (MES)
3t	0.5	>120	NT	NT	–
PHT[d)]	1.0	6.65 (5.42–8.16)	NT	56.91 (48.53–66.74)	8.56 (MES)
ETX[d)]	0.25	NT	140.40 (115.81–170.21)	318.01 (295.80–341.89)	2.26 (*sc*PTZ)
VPA[d)]	0.5	252.74 (220.10–290.22)	239.45 (209.18–274.10)	430.77 (407.92–454.90)	1.70 (MES) 1.80 (*sc*PTZ)

NT, not tested.
[a)]Time to peak effect. [b)] Results are represented as mean ± SEM at 95% confidence limit (MES, maximal electroshock test; *sc*PTZ, subcutaneous pentylenetetrazole; TD_{50}, acute neurological toxicity determined in the rotarod test). [c)] Protection index (TD_{50}/ED_{50}). [d)] Reference AEDs: PHT (phenytoin), ETX (ethosuximide), and VPA (valproic acid) tested in the same conditions.

tested. As far as binding to L-type calcium channel is concerned, compound **3q** also significantly interacted with this channel, nevertheless at the highest concentration of 100 μM (97.0%). On the whole, these results suggest that the mechanism of action of compound **3q** may be primarily related to interaction with the sodium channel (site 2).

In summary, the results of the current study indicate that the presence of the pyrrolidine-2,5-dione ring is important, however, not indispensable for anticonvulsant activity. Furthermore, the bioactivity found in the series of 3,3-diphenyl-propanamides and importantly the wide spectrum of protection in the MES, *sc*PTZ, and 6-Hz seizures models for

Table 4. **Anticonvulsant activity, 6-Hz (32 mA) test in mice *i.p.***

Cmpd	R	X	R_1	6 Hz	
				0.5 h	2 h
2h	3,4-Cl	–	–	–	–
2i	3-CF_3	–	–	–	–
2p	–	N	C_6H_{11}	100	–
2q	–	N	$(CH_2)_2OH$	100	–
3h	3,4-Cl	–	–	–	–
3i	3-CF_3	–	–	100	–
3p	–	N	C_6H_{11}	100	–
3q	–	N	$(CH_2)_2OH$	100	–

Dose of 100 mg/kg was administered. The data indicate the minimum dose whereby anticonvulsant activity was demonstrated. The animals were examined at 0.5 and 2 h. A dash indicates the absence of anticonvulsant activity or at the maximum dose administered (100 mg/kg).

3q, provides an opportunity for the development of highly effective substances in this chemical group and will be examined more precisely in the next studies.

Conclusions

The library of 40 3,3-diphenyl-2,5-dioxo-pyrrolidin-1-yl-acetamides and 3,3-diphenyl-propanamides as potential new anticonvulsant agents was synthesized. The initial anticonvulsant screening was performed in mice (*i.p.*) using the "classical" MES and *sc*PTZ tests. Several compounds were studied also in the six-Hertz (6-Hz) seizures recognized as the animal model of human pharmacoresistant epilepsy. The acute neurological toxicity was determined applying the rotarod test. The pharmacological data revealed predominant activity in the pentylenetetrazole seizures. The broadest spectrum of efficacy across the preclinical seizure models displayed **3q**. This substance revealed wider spectrum of protection, higher activity or/and better safety profile than the commonly used antiepileptic drugs (AEDs) such as phenytoin, ethosuximide, valproic acid, or/and levetiracetam. The *in vitro* studies showed that the most possible mechanism of action of compound **3q** may be connected to the interaction with neuronal voltage-sensitive sodium channel (site 2).7

Experimental

Chemistry

General

The reagents and solvents were purchased from Sigma–Aldrich. Melting points (mp) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland). The purity and homogeneity of the compounds were confirmed by use of the thin-layer chromatography (TLC) and high performance liquid chromatography (HPLC). The TLC was performed on Merck silica gel 60 F_{254} aluminum sheets (Merck; Darmstadt, Germany), using subsequent developing systems: S_1 = chloroform/acetone (9:1 v/v), S_2 = methylene chloride/methanol (9:0.2 v/v), S_3 = methylene chloride/methanol (9:1 v/v), S_4 = ethyl acetate/hexane (8:5 v/v). Spots were detected by their absorption under UV light ($\lambda = 254$ nm). ^{1}H NMR and

Table 5. **The quantitative pharmacological parameters ED_{50} (6 Hz, 32 mA), TD_{50} (rotarod test) and PI in mice *i.p.***

Cmpd	TPE (h)[a)]	ED_{50} 6 Hz (mg/kg)[b)]	TD_{50} (mg/kg)[b)]	PI (TD_{50}/ED_{50})[c)]
2p	0.5	88.24 (69.25–112.43)	>300	>3.40
2q	0.5	39.23 (31.14–49.41)	>300	>7.65
3i	0.5	42.83 (24.47–74.99)	>400	>9.34
3q	0.5	38.15 (33.97–42.84)	116.82 (102.21–133.51)	3.06
VPA[d)]	0.5	130.64 (117.61–145.19)	430.77 (407.92–454.90)	3.30
LEV[d)]	0.5	15.73 (10.45–23.69)	>500	>31.79

[a)] Time to peak effect. [b)] Results are represented as mean ± SEM at 95% confidence limit (6 Hz, psychomotor seizure model; TD_{50}, acute neurological toxicity determined in the rotarod test). [c)] Protection index (TD_{50}/ED_{50}). [d)] Reference AEDs: VPA (valproic acid), levetiracetam (LEV) tested in the same conditions.

Table 6. *In vitro* binding assays for compound 3q.

Cmpd	Concn (μM)	% Inhibition of control specific binding[a)]	
		Voltage-sensitive Na^+ channel (site 2)[b)]	Voltage-sensitive Ca^{2+} channel L-type[c)]
3q	1	54.2	–
	10	80.4	19.0
	100	99.6	97.4

[a)] Results showing an inhibition higher than 50% are considered to represent significant effects of the test compounds; results showing an inhibition between 25 and 50% are indicative of moderate effect; results showing an inhibition lower than 25% are not considered significant and mostly attributable to variability of the signal around the control level. [b)] Compound was evaluated in synaptoneurosomal preparations from rat cerebral cortex as inhibitors of the specific binding of [^{3}H]BTX to the voltage-sensitive sodium channel [28]. [c)] Compound was evaluated in synaptoneurosomal preparations from rat cerebral cortex as inhibitors of the specific binding of [^{3}H]nitrendipine to the voltage-sensitive Ca^{2+} channel [29].

^{13}C NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$ operating at 300 MHz (^{1}H NMR) or 75 MHz (^{13}C NMR). Chemical shifts are reported in δ values (ppm) relative to TMS $\delta = 0$ (^{1}H), as internal standard. The *J* values are expressed in Hertz (Hz). Signal multiplicities are represented by the following abbreviations: s (singlet), br s (broad singlet), d (doublet), dd (doublet of doublets), t (triplet), td (triplet of doublets), m (multiplet). The UPLC analyses and mass spectra (LC–MS) were obtained on a Waters ACQUITY^{TM} TQD system (Waters, Milford, USA) with the MS–TQ detector and UV-Vis–DAD eλ detector. The ACQUITY UPLC BEH C18, 1.7 μm (2.1 × 100 mm) column was used with the VanGuard Acquity UPLC BEH C18, 1.7 μm (2.1 × 5 mm) (Waters, Milford, USA). Standard solutions (1 mg/mL) of each compound were prepared in analytical grade acetonitrile/water mixture (1:1 v/v). Conditions applied were as follow: eluent A (water/0.1% HCOOH), eluent B (acetonitrile/0.1% HCOOH); flow rate of 0.3 mL/min, gradient of 5–100% B over 10 min were used, injection volume was 10 μL. Preparative column chromatography was performed using silica gel 60 (particle size 0.063–0.200; 70–230 Mesh ATM) purchased from Merck (Darmstadt, Germany).

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

*General procedure for the synthesis of 3,3-diphenyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid (**2**)*

2,2-Diphenyl-succinic acid (5.0 g, 0.018 mol, 1 eq) was suspended in 20 mL of water and aminoacetic acid (1.39 g, 0.018, 1 eq) was gradually added. The mixture was heated in a thermoregulated sand bath (ST 72 Roth, Karlsruhe, Germany), with simultaneous distillation of water. After the water was completely removed, the temperature of the reaction was maintained at 180°C for 1 h. The crude products were purified by crystallization from 2-propanole.

White solid (yield 68%), mp 86–88°C; TLC: $R_f = 0.58$ (S_2); UPLC: $t_R = 5.86$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.54 (s, 2H, imide), 4.36 (s, 2H, CH_2), 7.29–7.40 (m, 10H, ArH); $C_{18}H_{15}NO_4$ (309.32) monoisotopic mass 309.10; $[M-H]^- = 308.1$; $[M+H]^+ = 309.1$.

*General procedure for the synthesis of compounds **2a–t** and **3a–t***

Carbonyldiimidazole (0.97 g, 0.006 mol, 1.2 eq) in 5 mL of dry DMF was added under stirring to a solution of 3,3-diphenyl-2,5-dioxo-pyrrolidin-1-yl-acetic acid (**2**) (1.55 g, 0.005 mol, 1 eq) or 3,3-diphenyl-propionic acid (**3**) (1.13 g, 0.005 mol, 1 eq) dissolved in 10 mL of anhydrous DMF. After the end of gaseous (carbon dioxide) evolution (ca. 0.5 h), the respective secondary amine derivative (0.006 mol, 1.2 eq) in 5 mL of anhydrous DMF was added dropwise in 5 min. The mixture was stirred overnight at room temperature. After concentration of organic solvent under reduced pressure the oil residue was crystallized from 2-propanole. The final amides were obtained as solid substances. Compounds **3i**, **3q**, and **3r** due to oily form were converted into solid hydrochloride salts by stirring in anhydrous ethanol saturated with gaseous HCl.

*3,3-Diphenyl-1-[2-oxo-2-(4-phenyl-piperazin-1-yl)-ethyl]-pyrrolidine-2,5-dione (**2a**)*

White powdery crystals. Yield: 58%; mp 123–125°C; TLC: $R_f = 0.61$ (S_1), $R_f = 0.36$ (S_2); UPLC: $t_R = 7.63$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.15–3.23 (m, 4H, piperazine), 3.57 (s, 2H, imide), 3.61–3.68 (m, 2H, piperazine), 3.74–3.83 (m, 2H, piperazine), 4.42 (s, 2H, CH_2), 6.93 (d, 3H, ArH, $J = 7.2$ Hz), 7.26–7.40 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.1, 42.2, 44.7, 45.5, 49.4, 57.3, 116.8, 120.8, 127.6, 128.6, 129.3, 141.5, 163.2, 174.8, 178.4; $C_{28}H_{27}N_3O_3$ (453.53); monoisotopic mass 453.20; $[M+H]^+ = 454.1$.

*1-{2-[4-(2-Fluorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione (**2b**)*

White powdery crystals. Yield: 65%; mp 140–143°C; TLC: $R_f = 0.69$ (S_1), $R_f = 0.47$ (S_2); UPLC: $t_R = 7.84$ min; ^{1}H NMR

(300 MHz, $CDCl_3$): δ 3.06–3.17 (m, 4H, piperazine), 3.57 (s, 2H, imide), 3.62–3.69 (m, 2H, piperazine), 3.76–3.84 (m, 2H, piperazine), 4.42 (s, 2H, CH_2), 6.92–7.09 (m, 4H, ArH), 7.26–7.39 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.1, 42.4, 44.9, 45.5, 50.1, 50.6, 57.3, 116.2, 119.3, 123.3, 124.6, 127.6, 128.8, 141.5, 163.1, 174.8, 178.4; $C_{28}H_{26}N_3O_3F$ (471.52); monoisotopic mass 471.20; $[M+H]^+ = 472.1$.

1-{2-[4-(4-Fluorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2c)***
White powdery crystals. Yield: 62%; mp 82–85°C; TLC: $R_f = 0.91$ (S_1), $R_f = 0.82$ (S_2); UPLC: $t_R = 7.62$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.11 (t, 4H, piperazine, $J = 5.3$ Hz), 3.56 (s, 2H, imide), 3.77 (d, 4H, piperazine, $J = 5.4$ Hz), 4.41 (s, 2H, CH_2), 6.86–7.01 (m, 4H, ArH), 7.26–7.37 (m, 10H, ArH); $C_{28}H_{26}N_3O_3F$ (471.52); monoisotopic mass 471.20; $[M+H]^+ = 473.1$.

1-{2-[4-(2-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2d)***
White powdery crystals. Yield: 68%; mp 174–176°C; TLC: $R_f = 0.83$ (S_1), $R_f = 0.87$ (S_2); UPLC: $t_R = 8.19$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01–3.14 (m, 4H, piperazine), 3.57 (s, 2H, imide), 3.62–3.68 (m, 2H, piperazine), 3.77–3.84 (m, 2H, piperazine), 4.42 (s, 2H, CH_2), 6.97–7.07 (m, 2H, ArH), 7.20–7.25 (m, 1H, ArH), 7.31–7.37 (m, 11H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.1, 42.6, 45.1, 45.5, 50.8, 51.2, 57.3, 120.6, 124.4, 127.6, 128.7, 130.7, 141.6, 148.4, 163.2, 174.8, 178.4; $C_{28}H_{26}N_3O_3Cl$ (487.98); monoisotopic mass 487.17; $[M+H]^+ = 488.1$.

1-{2-[4-(3-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2e)***
White powdery crystals. Yield: 70%; mp 154–156°C; TLC: $R_f = 0.73$ (S_1), $R_f = 0.87$ (S_2); UPLC: $t_R = 8.16$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.20 (t, 4H, piperazine, $J = 4.8$ Hz), 3.57 (s, 2H, imide), 3.63 (t, 2H, piperazine, $J = 4.7$ Hz), 3.77 (t, 2H, piperazine, $J = 5.0$ Hz), 4.41 (s, 2H, CH_2), 6.76–6.88 (m, 3H, ArH), 7.16–7.37 (m, 11H, ArH); $C_{28}H_{26}N_3O_3Cl$ (487.98); monoisotopic mass 487.17; $[M+H]^+ = 488.1$.

1-{2-[4-(4-Chlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2f)***
White powdery crystals. Yield: 62%; mp 145–147°C; TLC: $R_f = 0.66$ (S_1), $R_f = 0.82$ (S_2); UPLC: $t_R = 8.11$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.12–3.23 (m, 4H, piperazine), 3.56 (s, 2H, imide), 3.60–3.67 (m, 2H, piperazine), 3.74–3.81 (m, 2H, piperazine), 4.41 (s, 2H, CH_2), 6.84 (d, 2H, ArH, $J = 9.2$ Hz), 7.23 (d, 2H, ArH, $J = 8.9$ Hz), 7.31–7.37 (m, 10H, ArH); $C_{28}H_{26}N_3O_3Cl$ (487.98); monoisotopic mass 487.17; $[M+H]^+ = 488.1$.

1-{2-[4-(2,3-Dichlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2g)***
White powdery crystals. Yield: 49%; mp 203–205°C; TLC: $R_f = 0.83$ (S_1), $R_f = 0.91$ (S_2); UPLC: $t_R = 8.61$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.01–3.11 (m, 4H, piperazine), 3.57 (s, 2H, imide), 3.63–3.68 (m, 2H, piperazine), 3.77–3.85 (m, 2H piperazine), 4.42 (s, 2H CH_2), 7.13–7.24 (m, 3H, ArH), 7.27–7.39 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.1, 42.5, 45.0, 45.5, 50.9, 51.3, 57.3, 118.8, 125.3, 127.6, 128.8, 141.5, 150.3, 163.2, 174.8, 178.4; $C_{28}H_{26}N_3O_3Cl_2$ (522.42); monoisotopic mass 521.13; $[M+H]^+ = 522.0$.

1-{2-[4-(3,4-Dichlorophenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2h)***
White powdery crystals. Yield: 57%; mp 198–200°C; TLC: $R_f = 0.72$ (S_1), $R_f = 0.84$ (S_2); UPLC: $t_R = 8.53$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.15–3.26 (m, 4H, piperazine), 3.57 (s, 2H, imide), 3.59–3.66 (m, 2H, piperazine), 3.74–3.81 (m, 2H, piperazine), 4.41 (s, 2H, CH_2), 6.71–6.77 (m, 1H, ArH), 6.95–6.98 (m, 1H, ArH), 7.26–7.40 (m, 11H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.0, 41.9, 44.4, 45.5, 48.7, 57.3, 115.9, 118.0, 123.6, 127.6, 128.8, 130.6, 141.5, 150.1, 163.2, 174.7, 178.4; $C_{28}H_{26}N_3O_3Cl_2$ (522.42); monoisotopic mass 521.13; $[M+H]^+ = 522.0$.

1-{2-[4-(3-Trifluoromethylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2i)***
White powdery crystals. Yield: 57%; mp 188–190°C; TLC: $R_f = 0.70$ (S_1), $R_f = 0.82$ (S_2); UPLC: $t_R = 8.38$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.21–3.32 (m, 4H, piperazine), 3.57 (s, 2H, imide), 3.62–3.69 (m, 2H, piperazine), 3.77–3.84 (m, 2H, piperazine), 4.42 (s, 2H, CH_2), 7.02–7.19 (m, 4H, ArH), 7.26–7.39 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 40.08, 42.0, 44.5, 45.5, 48.8, 57.3, 112.9, 116.9, 119.4, 127.8, 128.6, 129.7, 141.5, 150.8, 163.3, 174.7, 178.4; $C_{29}H_{26}N_3O_3F_3$ (521.53); monoisotopic mass 521.20; $[M+H]^+ = 522.1$.

1-{2-[4-(2-Methylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2j)***
White powdery crystals. Yield: 85%; mp 175–177°C; TLC: $R_f = 0.85$ (S_1), $R_f = 0.87$ (S_2); UPLC: $t_R = 8.29$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 3H, CH_3), 2.88–2.98 (m, 4H, piperazine), 3.57 (s, 2H, imide), 3.59–3.65 (m, 2H, piperazine), 3.73–3.81 (m, 2H, piperazine), 4.42 (s, 2H, CH_2), 6.97–7.06 (m, 2H, ArH), 7.14–7.23 (m, 2H, ArH), 7.25–7.39 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.8, 40.1, 42.91, 45.4, 51.5, 57.3, 119.3, 123.9, 126.7, 127.6, 128.8, 131.1, 132.7, 141.6, 150.6, 163.2, 174.8, 178.4; $C_{29}H_{29}N_3O_3$ (467.56); monoisotopic mass 467.22; $[M+H]^+ = 468.2$.

1-{2-[4-(3-Methylphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione ***(2k)***
White powdery crystals. Yield: 66%; mp 123–125°C; TLC: $R_f = 0.64$ (S_1), $R_f = 0.87$ (S_2); UPLC: $t_R = 7.93$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 3H, CH_3), 3.14–3.27 (m, 4H, piperazine), 3.57 (s, 2H, imide), 3.59–3.67 (m, 2H, piperazine), 3.74–3.82 (m, 2H, piperazine), 4.42 (s, 2H, CH_2), 6.75 (s, 3H, ArH), 7.14–7.21 (m, 1H, ArH), 7.27–7.39 (m, 10H, ArH); $C_{29}H_{29}N_3O_3$ (467.56); monoisotopic mass 467.22; $[M+H]^+ = 468.1$.

*1-{2-[4-(2-Methoxyphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione (**2l**)*
White powdery crystals. Yield: 55%; mp 173–176°C; TLC: $R_f = 0.56$ (S_1), $R_f = 0.24$ (S_2); UPLC: $t_R = 7.41$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.27–3.46 (m, 2H, piperazine), 3.57 (s, 2H, imide), 3.64–3.77 (m, 1H, piperazine), 3.79–3.88 (m, 1H, piperazine), 3.91 (s, 3H, OCH_3), 3.95–4.22 (m, 4H, piperazine), 4.42 (s, 2H, CH_2), 6.93–7.05 (m, 2H, ArH), 7.14–7.43 (m, 12H, ArH); $C_{29}H_{29}N_3O_4$ (483.56); monoisotopic mass 483.22; $[M+H]^+ = 484.2$.

*1-{2-[4-(3-Methoxyphenyl)-piperazin-1-yl]-2-oxo-ethyl}-3,3-diphenyl-pyrrolidine-2,5-dione (**2m**)*
White powdery crystals. Yield: 65%; mp 71–73°C; TLC: $R_f = 0.22$ (S_1), $R_f = 0.83$ (S_2); UPLC: $t_R = 7.56$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.15–3.26 (m, 4H, piperazine), 3.56 (s, 2H, imide), 3.58–3.65 (m, 2H, piperazine), 3.76 (d, 2H, piperazine, $J = 5.6$ Hz), 3.79 (s, 3H, OCH_3), 4.41 (s, 2H, CH_2), 6.44–6.56 (m, 3H, ArH), 7.16–7.37 (m, 11H, ArH); $C_{29}H_{29}N_3O_4$ (483.56); monoisotopic mass 483.22; $[M+H]^+ = 484.2$.

*1-[2-(4-Benzyl-piperazin-1-yl)-2-oxo-ethyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**2n**)*
White powdery crystals. Yield: 52%; mp 133–135°C; TLC: $R_f = 0.22$ (S_1), $R_f = 0.16$ (S_2); UPLC: $t_R = 5.40$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.44–2.50 (m, 4H, piperazine), 3.43–3.48 (m, 2H, piperazine), 3.53 (s, 2H, imide), 3.55 (s, 2H, CH_2), 3.60–3.67 (m, 2H, piperazine), 4.35 (s, 2H, CH_2), 7.23–7.39 (m, 15H, ArH); $C_{29}H_{29}N_3O_3$ (467.56); monoisotopic mass 467.22; $[M+H]^+ = 468.1$.

*1-[2-(4-Pyrimidin-piperazin-1-yl)-2-oxo-ethyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**2o**)*
White powdery crystals. Yield: 72%; mp 182–184°C; TLC: $R_f = 0.44$ (S_1), $R_f = 0.22$ (S_2); UPLC: $t_R = 6.61$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.53–3.55 (m, 2H, piperazine), 3.57 (s, 2H, imide), 3.70–3.73 (m, 2H, piperazine), 3.90 (dt, 4H, piperazine, $J = 5.2$ Hz, $J = 19.9$ Hz), 4.42 (s, 2H, CH_2), 6.55 (t, 1H, pyrimidine, $J = 4.7$ Hz), 7.26–7.37 (m, 10H, ArH), 8.33 (d, 2H, pyrimidine, $J = 4.6$ Hz); $C_{26}H_{25}N_5O_3$ (455.52); monoisotopic mass 455.19; $[M+H]^+ = 456.1$.

*1-[2-(4-Cyclohexyl-piperazin-1-yl)-2-oxo-ethyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**2p**)*
White powdery crystals. Yield: 52%; mp 80–82°C; TLC: $R_f = 0.23$ (S_1), $R_f = 0.36$ (S_2); UPLC: $t_R = 5.32$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.19–1.26 (m, 6H, cyclohexane), 1.78–1.83 (m, 4H, cyclohexane), 2.54–2.62 (m, 4H piperazine, 1H cyclohexane), 3.45 (d, 2H, piperazine, $J = 4.6$ Hz), 3.55 (s, 2H, imide), 3.59–3.67 (m, 2H, piperazine), 4.36 (s, 2H, CH_2), 7.29–7.36 (m, 10H, ArH); $C_{28}H_{33}N_3O_3$ (459.58); monoisotopic mass 459.25; $[M+H]^+ = 460.2$.

*1-[2-(2-Hydroxyethyl-piperazin-1-yl)-2-oxo-ethyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**2q**)*
White powdery crystals. Yield: 50%; mp 113–115°C; TLC: $R_f = 0.47$ (S_1), $R_f = 0.54$ (S_3); UPLC: $t_R = 4.51$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.52 (t, 2H, CH_2, $J = 5.0$ Hz), 2.57–2.60 (m, 4H, piperazine), 3.48–3.51 (m, 2H, CH_2), 3.55 (s, 2H, imide), 3.64 (t, 4H, piperazine, $J = 5.2$ Hz), 4.36 (s, 2H, CH_2), 7.26–7.36 (m, 10H, ArH); $C_{24}H_{27}N_3O_4$ (421.49); monoisotopic mass 421.20; $[M+H]^+ = 422.2$.

*1-[2-(4-Methyl-piperazin-1-yl)-2-oxo-ethyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**2r**)*
White powdery crystals. Yield: 57%; mp 140–142°C; TLC: $R_f = 0.52$ (S_1), $R_f = 0.56$ (S_3); UPLC: $t_R = 4.59$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.32 (s, 3H, CH_3), 2.43 (dt, 4H, piperazine, $J = 15.1$ Hz, $J = 5.0$ Hz), 3.46–3.50 (m, 2H, piperazine), 3.55 (s, 2H, imide), 3.64 (d, 2H, piperazine, $J = 4.9$ Hz), 4.36 (s, 2H, CH_2), 7.25–7.39 (m, 10H, ArH); $C_{23}H_{25}N_3O_3$ (391.46); monoisotopic mass 391.20; $[M+H]^+ = 392.1$.

*1-(2-Morpholine-2-oxyethyl)-3,3-diphenyl-pyrrolidine-2,5-dione (**2s**)*
White powdery crystals. Yield: 58%; mp 168–170°C; TLC: $R_f = 0.82$ (S_1), $R_f = 0.37$ (S_3); UPLC: $t_R = 6.05$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.56 (s, 2H, imide), 3.64 (t, 4H, morpholine, $J = 5.2$ Hz), 3.70–3.74 (m, 4H, morpholine), 4.36 (s, 2H, CH_2), 7.26–7.37 (m, 10H, ArH); $C_{22}H_{22}N_2O_4$ (378.42); monoisotopic mass 378.16; $[M+H]^+ = 379.1$.

*1-[2-(4-Benzyl-piperidin-1-yl)-2-oxoethyl]-3,3-diphenyl-pyrrolidine-2,5-dione (**2t**)*
White powdery crystals. Yield: 68%; mp 126–128°C; TLC: $R_f = 0.80$ (S_1), $R_f = 0.84$ (S_3); UPLC: $t_R = 8.46$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10–1.37 (m, 2H, piperidine), 1.64–1.86 (m, 4H, piperidine), 2.47–2.66 (m, 2H, CH_2), 2.94–3.10 (m, 2H, piperidine), 3.55 (s, 2H, imide), 3.66–3.80 (m, 1H, piperidine), 4.35 (s, 2H, CH_2), 7.09–7.42 (m, 15H, ArH); $C_{30}H_{30}N_2O_3$ (466.57); monoisotopic mass 466.23; $[M+H]^+ = 467.1$.

*3,3-Diphenyl-1-(4-phenylpiperazin-1-yl)-propan-1-one (**3a**)*
White powdery crystals. Yield: 55%; mp 130–132°C; TLC: $R_f = 0.72$ (S_1), $R_f = 0.62$ (S_4); UPLC: $t_R = 7.69$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.86 (t, 2H, piperazine, $J = 5.2$ Hz), 3.03 (t, 2H, piperazine, $J = 5.2$ Hz), 3.10 (d, 2H, CH_2, $J = 7.7$ Hz), 3.48 (t, 2H, piperazine, $J = 5.0$ Hz), 3.70 (t, 2H, piperazine, $J = 5.3$ Hz), 4.69 (t, 1H, CH, $J = 7.4$ Hz), 6.84–6.92 (m, 3H, ArH), 7.14–7.31 (m, 12H, ArH); $C_{25}H_{26}N_2O$ (370.49); monoisotopic mass 370.20; $[M+H]^+ = 371.4$.

*1-[4-(2-Fluorophenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3b**)*
White powdery crystals. Yield: 62%; mp 111–113°C; TLC: $R_f = 0.87$ (S_1), $R_f = 0.71$ (S_4); UPLC: $t_R = 7.92$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.73 (t, 2H, piperazine, $J = 5.0$ Hz), 2.91 (t, 2H, piperazine, $J = 5.1$ Hz), 3.10 (d, 2H, CH_2, $J = 7.4$ Hz), 3.50 (t, 2H, piperazine, $J = 5.1$ Hz), 3.72 (t, 2H, piperazine, $J = 5.0$ Hz), 4.69 (t, 1H, CH, $J = 7.6$ Hz), 6.99 (m, 1H, ArH), 7.02–7.06 (m, 3H, ArH), 7.24–7.32 (m, 10H, ArH); $C_{25}H_{25}N_2OF$ (388.48); monoisotopic mass 388.20; $[M+H]^+ = 389.4$.

*1-[4-(4-Fluorophenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3c**)*
White powdery crystals. Yield: 72%; mp 140–142°C; TLC: $R_f = 0.62$ (S_1), $R_f = 0.76$ (S_2); UPLC: $t_R = 7.72$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.74 (t, 2H, piperazine, $J = 5.1$ Hz), 2.93 (t, 2H, piperazine, $J = 5.1$ Hz), 3.09 (d, 2H, CH_2, $J = 7.4$ Hz), 3.47 (t, 2H, piperazine, $J = 5.1$ Hz), 3.69 (t, 2H, piperazine, $J = 5.1$ Hz), 4.68 (t, 1H, CH, $J = 7.6$ Hz), 6.78–6.84 (m, 2H, ArH), 6.92–6.99 (m, 2H, ArH), 7.18–7.31 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.7, 41.6, 45.7, 47.4, 50.4, 115.5, 115.8, 118.5, 126.3, 126.5, 127.8, 128.5, 144.0, 169.7; $C_{25}H_{25}N_2OF$ (388.48); monoisotopic mass 388.20; $[M+H]^+ = 389.4$.

*1-[4-(2-Chlorophenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3d**)*
White powdery crystals. Yield: 62%; mp 130–132°C; TLC: $R_f = 0.43$ (S_1), $R_f = 0.57$ (S_2); UPLC: $t_R = 8.30$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.68 (t, 2H, piperazine, $J = 4.9$ Hz), 2.87 (t, 2H, piperazine, $J = 5.0$ Hz), 3.11 (d, 2H, CH_2, $J = 7.7$ Hz), 3.52 (t, 2H, piperazine, $J = 5.0$ Hz), 3.73 (t, 2H, piperazine, $J = 5.0$ Hz), 4.69 (t, 1H, CH, $J = 7.6$ Hz), 6.88–6.91 (dd, 1H, ArH, $J = 1.5$ Hz, $J = 6.4$ Hz), 6.96–7.02 (td, 1H, ArH, $J = 1.5$ Hz, $J = 6.1$ Hz), 7.16–7.34 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.2, 41.9, 46.1, 47.4, 51.1, 120.4, 124.2, 126.4, 127.6, 127.9, 128.5, 128.9, 130.6, 144.0, 169.8; $C_{25}H_{25}N_2OCl$ (404.94); monoisotopic mass 404.17; $[M+H]^+ = 405.3$.

*1-[4-(3-Chlorophenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3e**)*
White powdery crystals. Yield: 58%; mp 107–108°C; TLC: $R_f = 0.80$ (S_1), $R_f = 0.64$ (S_4); UPLC: $t_R = 8.33$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.83 (t, 2H, piperazine, $J = 5.1$ Hz), 3.03 (t, 2H, piperazine, $J = 5.1$ Hz), 3.09 (d, 2H, CH_2, $J = 7.7$ Hz), 3.45 (t, 2H, piperazine, $J = 5.0$ Hz), 3.68 (t, 2H, piperazine, $J = 5.1$ Hz), 4.68 (t, 1H, CH, $J = 7.6$ Hz), 6.69–6.73 (m, 1H, ArH), 6.79–6.86 (m, 3H, ArH), 7.13–7.31 (m, 10H, ArH); $C_{25}H_{25}N_2OCl$ (404.94); monoisotopic mass 404.17; $[M+H]^+ = 405.3$.

*1-[4-(4-Chlorophenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3f**)*
White powdery crystals. Yield: 78%; mp 157–159°C; TLC: $R_f = 0.74$ (S_1), $R_f = 0.58$ (S_4); UPLC: $t_R = 8.28$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.79 (t, 2H, piperazine, $J = 5.0$ Hz), 2.98 (t, 2H, piperazine, $J = 5.1$ Hz), 3.09 (d, 2H, CH_2, $J = 7.4$ Hz), 3.46 (t, 2H, piperazine, $J = 5.0$ Hz), 3.69 (t, 2H, piperazine, $J = 5.1$ Hz), 4.68 (t, 1H, CH, $J = 7.6$ Hz), 6.74–6.79 (m, 2H, ArH), 7.15–7.31 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.7, 41.4, 45.5, 47.4, 49.3, 117.7, 126.5, 127.8, 128.3, 129.0, 143.9, 169.8; $C_{25}H_{25}N_2OCl$ (404.94); monoisotopic mass 404.17; $[M+H]^+ = 405.3$.

*1-[4-(2,3-Dichlorophenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3g**)*
White powdery crystals. Yield: 57%; mp 119–121°C; TLC: $R_f = 0.80$ (S_1), $R_f = 0.74$ (S_4); UPLC: $t_R = 8.29$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.64 (t, 2H, piperazine, $J = 4.9$ Hz), 2.85 (t, 2H, piperazine, $J = 5.0$ Hz), 3.10 (d, 2H, CH_2, $J = 7.7$ Hz), 3.52 (t, 2H, piperazine, $J = 5.0$ Hz), 3.73 (t, 2H, piperazine, $J = 4.7$ Hz), 4.69 (t, 1H, CH, $J = 7.4$ Hz), 6.78–6.82 (dd, 1H, ArH, $J = 2.1$ Hz, $J = 5.4$ Hz), 7.11–7.33 (m, 12H, ArH); $C_{25}H_{24}N_2OCl_2$ (439.38); monoisotopic mass 438.13; $[M+H]^+ = 439.3$.

*1-[4-(3,4-Dichlorophenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3h**)*
White powdery crystals. Yield: 83%; mp 113–115°C; TLC: $R_f = 0.74$ (S_1), $R_f = 0.66$ (S_4); UPLC: $t_R = 8.76$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.78 (t, 2H, piperazine, $J = 5.0$ Hz), 2.99 (t, 2H, piperazine, $J = 5.1$ Hz), 3.09 (d, 2H, CH_2, $J = 7.4$ Hz), 3.45 (t, 2H, piperazine, $J = 5.1$ Hz), 3.68 (t, 2H, piperazine, $J = 5.1$ Hz), 4.67 (t, 1H, CH, $J = 7.6$ Hz), 6.64–6.68 (dd, 1H, ArH, $J = 2.8$ Hz, $J = 6.2$ Hz), 6.87 (d, 1H, ArH, $J = 2.8$ Hz), 7.15–7.31 (m, 11H, ArH); $C_{25}H_{24}N_2OCl_2$ (439.38); monoisotopic mass 438.13; $[M+H]^+ = 439.3$.

*Monohydrochloride 1-[4-(3-trifluoromethylphenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3i**)*
White powdery crystals. Yield: 73%; mp 175–176°C; TLC: $R_f = 0.16$ (S_1), $R_f = 0.12$ (S_4); UPLC: $t_R = 8.15$ min; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.08 (t, 4H, piperazine, $J = 4.9$ Hz), 3.16 (d, 2H, CH_2, $J = 7.6$ Hz), 3.52 (t, 2H, piperazine, $J = 4.7$ Hz), 3.64 (br s, 2H, piperazine), 4.49 (t, 1H, CH, $J = 7.6$ Hz), 7.09–7.18 (m, 3H, ArH), 7.21–7.33 (m, 10H, ArH), 7.42 (t, 1H, ArH, $J = 7.9$ Hz); $C_{26}H_{26}N_2OClF_3$ (474.94); amine: $C_{26}H_{25}N_2OF_3$ (438.50); monoisotopic mass 438.19; $[M+H]^+ = 438.2$.

*1-[4-(2-Methylphenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3j**)*
White powdery crystals. Yield: 78%; mp 84–86°C; TLC: $R_f = 0.83$ (S_1), $R_f = 0.77$ (S_4); UPLC: $t_R = 8.47$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.28 (s, 3H, CH_3), 2.57 (t, 2H, piperazine, $J = 4.9$ Hz), 2.73 (t, 2H, piperazine, $J = 4.9$ Hz), 3.11 (d, 2H, CH_2, $J = 7.4$ Hz), 3.48 (t, 2H, piperazine, $J = 4.9$ Hz), 3.69 (t, 2H, piperazine, $J = 4.7$ Hz), 4.70 (t, 1H, CH, $J = 7.4$ Hz), 6.88 (d, 1H, ArH, $J = 7.9$ Hz), 6.97–7.03 (m, 1H, ArH), 7.14–7.33 (m, 12H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 17.7, 38.7, 42.2, 46.3, 46.7, 51.6, 119.1, 123.7, 126.0, 127.3, 128.5, 131.1, 132.7, 144.1, 169.8; $C_{26}H_{28}N_2O$ (384.51); monoisotopic mass 384.22; $[M+H]^+ = 385.4$.

*1-[4-(3-Methylphenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3k**)*
White powdery crystals. Yield: 82%; mp 121–123°C; TLC: $R_f = 0.77$ (S_1), $R_f = 0.62$ (S_4); UPLC: $t_R = 8.07$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.31 (s, 3H, CH_3), 2.85 (t, 2H, piperazine, $J = 5.1$ Hz), 3.02 (t, 2H, piperazine, $J = 5.1$ Hz), 3.10 (d, 2H, CH_2, $J = 7.4$ Hz), 3.47 (t, 2H, piperazine, $J = 5.1$ Hz), 3.69 (t, 2H, piperazine, $J = 5.1$ Hz), 4.68 (t, 1H, CH, $J = 7.6$ Hz), 6.64–6.73 (m, 3H, ArH), 7.12–7.31 (m, 11H, ArH); $C_{26}H_{28}N_2O$ (384.51); monoisotopic mass 384.22; $[M+H]^+ = 385.4$.

*1-[4-(2-Methoxyphenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3l**)*
White powdery crystals. Yield: 59%; mp 116–118°C; TLC: $R_f = 0.67$ (S_1), $R_f = 0.52$ (S_4); UPLC: $t_R = 7.44$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.72 (t, 2H, piperazine, $J = 5.0$ Hz), 2.89 (t, 2H, piperazine, $J = 5.1$ Hz), 3.10 (d, 2H, CH_2, $J = 7.4$ Hz), 3.53 (t, 2H, piperazine, $J = 5.0$ Hz), 3.73 (t, 2H, piperazine, $J = 5.1$ Hz), 3.86 (s, 3H, OCH_3), 4.69 (t, 1H, CH, $J = 7.6$ Hz), 6.79–6.94 (m, 3H, ArH), 6.99–7.05 (m, 1H, ArH), 7.15–7.31 (m, 10H, ArH); $C_{26}H_{28}N_2O_2$ (400.51); monoisotopic mass 400.22; $[M+H]^+ = 401.4$.

*1-[4-(3-Methoxyphenyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3m**)*
White powdery crystals. Yield: 58%; mp 110–112°C; TLC: $R_f = 0.71$ (S_1), $R_f = 0.63$ (S_4); UPLC: $t_R = 7.63$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.84 (t, 2H, piperazine, $J = 5.1$ Hz), 3.03 (t, 2H, piperazine, $J = 5.1$ Hz), 3.09 (d, 2H, CH_2, $J = 7.4$ Hz), 3.46 (t, 2H, piperazine, $J = 5.1$ Hz), 3.69 (t, 2H, piperazine, $J = 5.1$ Hz), 3.78 (s, 3H, OCH_3), 4.68 (t, 1H, CH, $J = 7.6$ Hz), 6.38 (t, 1H, ArH, $J = 2.3$ Hz), 6.43–6.48 (m, 2H, ArH), 7.14–7.31 (m, 11H, ArH); $C_{26}H_{28}N_2O_2$ (400.51); monoisotopic mass 400.22; $[M+H]^+ = 401.4$.

*1-(4-Benzyl-piperazin-1-yl)-3,3-diphenylpropan-1-one (**3n**)*
White powdery crystals. Yield: 71%; mp 130–132°C; TLC: $R_f = 0.28$ (S_1), $R_f = 0.18$ (S_4); UPLC: $t_R = 5.34$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.14 (br s, 2H, piperazine), 2.30 (t, 2H, piperazine, $J = 5.0$ Hz), 3.03 (d, 2H, CH_2, $J = 7.4$ Hz), 3.34 (t, 2H, piperazine, $J = 4.9$ Hz), 3.43 (s, 2H, CH_2), 3.55 (br s, 2H, piperazine), 4.65 (t, 1H, CH, $J = 7.4$ Hz), 7.14–7.35 (m, 15H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.7, 41.6, 45.7, 47.3, 52.6, 52.8, 62.7, 126.4, 127.2, 127.8, 128.3, 128.5, 129.1, 144.1, 169.6; $C_{26}H_{28}N_2O$ (384.51); monoisotopic mass 384.22; $[M+H]^+ = 385.4$.

*3,3-Diphenyl-[4-(pyrimidin-2-yl)-piperazin-1-yl]-propan-1-one (**3o**)*
White powdery crystals. Yield: 63%; mp 193–194°C; TLC: $R_f = 0.42$ (S_1), $R_f = 0.53$ (S_4); UPLC: $t_R = 6.44$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.10 (d, 2H, CH_2, $J = 7.60$ Hz), 3.38 (dd, 2H, piperazine, $J = 6.5$ Hz, $J = 4.1$ Hz), 3.49–3.56 (m, 2H, piperazine), 3.59–3.65 (m, 2H, piperazine), 3.66–3.72 (m, 2H, piperazine), 4.70 (t, 1H, CH, $J = 7.7$ Hz), 6.51 (t, 1H, ArH, $J = 4.7$ Hz), 7.15–7.21 (m, 2H, ArH), 7.23–7.31 (m, 8H, ArH), 8.30 (d, 2H, ArH, $J = 4.7$ Hz); $C_{23}H_{24}N_4O$ (372.47); monoisotopic mass 372.20; $[M+H]^+ = 373.4$.

*1-(4-Cyclohexylpiperazin-1-yl)-3,3-diphenylpropan-1-one (**3p**)*
White powdery crystals. Yield: 72%; mp 100–101°C; TLC: $R_f = 0.18$ (S_1), $R_f = 0.06$ (S_4); UPLC: $t_R = 5.25$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.05–1.28 (m, 5H, cyclohexane), 1.62 (d, 1H, cyclohexane, $J = 11.3$ Hz), 1.77 (d, 2H, cyclohexane, $J = 6.4$ Hz), 2.16–2.25 (m, 3H, 1H cyclohexane, 2H piperazine), 2.39 (t, 2H, piperazine, $J = 5.1$ Hz), 3.04 (d, 2H, CH_2, $J = 7.4$ Hz), 3.32 (t, 2H, piperazine, $J = 5.1$ Hz), 3.53 (t, 2H, piperazine, $J = 5.0$ Hz), 4.66 (t, 1H, CH, $J = 7.4$ Hz), 7.13–7.30 (m, 10H, ArH); $C_{25}H_{32}N_2O$ (376.54); monoisotopic mass 376.25; $[M+H]^+ = 377.4$.

*Monohydrochloride 1-[4-(2-hydroxyethyl)-piperazin-1-yl]-3,3-diphenylpropan-1-one (**3q**)*
White powdery crystals. Yield: 60%; mp 215–216°C; TLC: $R_f = 0.14$ (S_1), $R_f = 0.11$ (S_4); UPLC: $t_R = 4.04$ min; ^{1}H NMR (300 MHz, DMSO-d_6): δ 3.11 (t, 4H, piperazine, $J = 4.9$ Hz), 3.17 (d, 2H, CH_2, $J = 7.6$ Hz), 3.34–3.46 (m, 5H, hydroxyethyl), 3.75 (t, 4H, piperazine, $J = 5.0$ Hz), 4.47 (t, 1H, CH, $J = 7.3$ Hz), 7.09–7.17 (m, 2H, ArH), 7.20–7.34 (m, 8H, ArH), 10.61–10.92 (m, 1H, HCl); $C_{21}H_{27}N_2O_2Cl$ (374.90); amine: $C_{21}H_{26}N_2O_2$ (338.45); monoisotopic mass 338.20; $[M+H]^+ = 339.4$.

*Monohydrohloride 1-(4-methyl-piperazin-1-yl)-3,3-diphenylpropan-1-one (**3r**)*
White powdery crystals. Yield: 65,8%; mp 264–266°C; TLC: $R_f = 0.16$ (S_1), $R_f = 0.12$ (S_4); UPLC: $t_R = 4.09$ min; ^{1}H NMR (300 MHz, DMSO-d_6): δ 2.68 (s, 3H, CH_3), 3.17 (d, 2H, CH_2), 3.33 (br s, 8H, piperazine), 4.46 (t, 1H, CH, $J = 7.3$ Hz), 7.09–7.17 (m, 2H, ArH), 7.20–7.34 (m, 8H, ArH), 11.02–11.62 (m, 1H, HCl); $C_{20}H_{25}N_2OCl$ (344.88); amine: $C_{20}H_{24}N_2O$ (308.43); monoisotopic mass 308.20; $[M+H]^+ = 309.4$.

*1-Morpholine-3,3-diphenylpropan-1-one (**3s**)*
White powdery crystals. Yield: 81%; mp 134–136°C; TLC: $R_f = 0.84$ (S_1), $R_f = 0.61$ (S_4); UPLC: $t_R = 5.97$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.04 (d, 2H, CH_2, $J = 7.7$ Hz), 3.31 (s, 4H, morpholine), 3.53 (s, 4H, morpholine), 4.66 (t, 1H, CH, $J = 7.6$ Hz), 7.16–7.31 (m, 10H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 38.5, 42.0, 46.2, 47.4, 66.3, 66.7, 126.5, 127.8, 128.3, 143.9, 169.9; $C_{19}H_{21}NO_2$ (295.38); monoisotopic mass 295.16; $[M+H]^+ = 296.3$.

*1-(4-Benzylpiperidin-1-yl)-3,3-diphenylpropan-1-one (**3t**)*
White powdery crystals. Yield: 52%; mp 95–97°C; TLC: $R_f = 0.87$ (S_1), $R_f = 0.70$ (S_4); UPLC: $t_R = 8.65$ min; ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.97–1.01 (m, 1H, piperidine), 1.47–1.69 (m, 4H, piperidine), 2.35–2.52 (m, 2H, CH_2, 1H, piperidine), 2.70–2.80 (m, 1H, piperidine), 2.95–3.12 (m, 2H, CH_2), 3.77 (d, 1H, piperidine, $J = 13.6$ Hz), 4.55 (d, 1H, piperidine, $J = 13.1$ Hz), 4.67 (t, 1H, CH, $J = 7.6$ Hz), 7.08–7.31 (m, 15H, ArH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 31.7, 32.2, 38.1, 38.7, 42.1, 42.9, 46.0, 47.4, 126.0, 126.3, 128.2, 129.0, 139.9, 144.2, 144.3, 169.4; $C_{27}H_{29}NO$ (383.53); monoisotopic mass 383.22; $[M+H]^+ = 384.4$.

Anticonvulsant activity

General

Male albino mice (CD-1 strain) weighing 16–26 g were used as experimental animals. The animals were housed in cages at room temperature of 22 ± 2°C, under a light/dark (12/12) cycle and had free access to food and water. 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 three to four animals (anticonvulsant screening) and each mouse was used only once. The compounds were suspended in 0.5% methylcellulose/water mixture. All the compounds were injected intraperitoneally into mice at the screening dose of 100 mg/kg with anticonvulsant activity and neurotoxicity assessment at 0.5 and 2 h after administration. Procedures involving animals and their care were conducted in accordance with current European Community (EU Directive 2010/63/EU) and Polish legislation on animal experimentation. The experimental protocols and procedures described in this manuscript were approved by the Local Ethics Committee at the Jagiellonian University Medical College in Cracow (Approval Nos. 100/2014 and 47/2015).

The maximal electroshock test (MES)
In the MES screen, electroconvulsions were produced by an electrical stimulus of 0.2 s in duration at a frequency of 50 Hz delivered via standard auricular electrodes by an electroshock generator (Hugo Sachs rodent shocker, Germany). The tonic hind-limb extension was taken as the end-point. Mice not displaying hind-limb tonic extension were considered protected from seizure [22].

Subcutaneous pentylenetetrazole seizure test (scPTZ)
*sc*PTZ-induced seizure was performed by subcutaneously injection of pentylenetetrazole (Sigma–Aldrich, Poland) at a dose of 85 mg/kg. This produced clonic convulsions lasting for at least five seconds in 97% of animal tested. The absence of clonic convulsions in the observed time period of 30 min was interpreted as the compound's ability to protect against PTZ-induced seizure [23].

The neurological toxicity (NT)
Neurological toxicity induced by compound was detected in mice using standardized rotarod test. Untreated control mice when placed on the rod, can maintain their equilibrium for a prolonged time period. The acute motor impairment can be demonstrated by the inability of animal to maintain equilibrium on the rod revolting at 10 rpm for at least 1 min [24].

6-Hz psychomotor seizures
The 6-Hz seizure model was performed according to the protocol originally described by Brown et al. [25] and more recently by Barton et al. [19]. Psychomotor seizures were induced via corneal stimulation (6 Hz, 32 mA, 0.2 ms rectangular pulse width, 3 s duration) using a constant-current device (ECT Unit 57800, Ugo Basile, Italy). A drop of 0.5% solution of lidocaine hydrochloride was applied to the mouse corneas before stimulation to provide local anesthesia and ensure optimal current conductivity. After the 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. The main seizure end-point was the duration of the immobility. Mice resuming normal behavior within 10 s from the stimulation were considered as protected [26].

Quantification studies
The ED_{50} is defined as the dose of a drug protecting 50% of animals against the MES, *sc*PTZ, and 6-Hz seizures. Groups of six animals received various doses of the compound until at least three points were established in the range of 10–90% seizure protection or minimal motor impairment. Both ED_{50} and TD_{50} values with 95% confidence limits were calculated by probit analysis [27]. The PI (protective index) value was calculated as the ratio of TD_{50} to ED_{50} ($TD_{50}/ED_{50} = PI$).

Radioligand binding assays
Sodium and calcium channels binding studies were performed commercially in Cerep Laboratories (Poitiers, France) using testing procedures described elsewhere (Na^+ channel – site 2 [28], L-type Ca^{2+} [29]).

We are pleased to acknowledge the generous financial support of this work by the grant of the Polish National Scientific Centre, Poland (Grant No. DEC-2013/11/B/NZ7/02081).

The authors have declared no conflict of interest.

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