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Article

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Design, Synthesis and Anticonvulsant Activity of New Hybrid Compounds Derived from 2-(2,5-Dioxopyrrolidin-1-yl)propanamides and 2-(2,5-Dioxopyrrolidin-1-yl)butanamides

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ABSTRACT

The library of 27 new 1-(4-phenylpiperazin-1-yl)- or 1-(morpholin-4-yl)-(2,5-dioxopyrrolidin-1-yl)propanamides and (2,5-dioxopyrrolidin-1-yl)butanamides as potential new hybrid anticonvulsant agents was synthesized. These hybrid molecules join the chemical fragments of well-known antiepileptic drugs (AEDs) such as ethosuximide, levetiracetam and lacosamide. Compounds **5**, **10**, **11**, and **24** displayed the broad spectra of activity across the preclinical seizure models, namely the maximal electroshock (MES) test, the subcutaneous pentylenetetrazole (*sc*PTZ) test, and the six-hertz (6 Hz) model of pharmacoresistant limbic seizures. The highest protection demonstrated **11** (ED_{50} MES = 88.4 mg/kg, ED_{50} *sc*PTZ = 59.9 mg/kg, ED_{50} 6 Hz = 21.0 mg/kg). This molecule did not impair the motor coordination of animals in the chimney test even at high doses (TD_{50} >1500 mg/kg) that yielded superb protective indexes (PI MES >16.97, PI PTZ >25.04, PI 6 Hz >71.43). As a result, **11** displayed distinctly better safety profile than clinically relevant AEDs ethosuximide, lacosamide or valproic acid.

INTRODUCTION

Epilepsy affects approximately 50 million people worldwide, making it the second most common neurological disorder after stroke.[1,2] For many years epilepsy has been recognized as a disease of young people, as the first symptoms occur usually before the age of 10. However, current data show that the prevalence increases with the age, being 0.7% in range of 55–64 years and 1.2% in population over age of 85.[3,4] This fact becomes more and more serious owing to the demographic changes related to the aging of societies in industrialized countries. Despite the significant advances in epilepsy research, convulsions in 30% of epileptics are still inadequately controlled by standard drug therapy.[5,6] Furthermore, compliance is often limited by adverse side effects most notably related to the central nervous

system (CNS) exposure like diminished attention, executive function, intelligence, language skills, memory, and processing speed.[7] Data collected from eight of the biggest markets show that therapy-resistant epilepsy affects about 1.8 million people.[8] In recent times, several new drugs such as levetiracetam, felbamate, lamotrigine, gabapentin, and topiramate have been implemented for the treatment of epilepsy. Although these drugs have been shown to be effective in epileptic syndromes in a number of patients, their efficacy does not appear to be superior to that of the established AEDs. Therefore, the ideal AED should prevent different types of seizures without producing side effects that adversely affect patients' quality of life. Taking into consideration the aforementioned facts, the continued search for safer and more effective antiepileptic drugs is both urgent and necessary.

The incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of majority AEDs make it difficult to use the rational drug design technique that is based on the three-dimensional structure of the biological target. Conceptually, there are two different methods of obtaining new AEDs, namely, ligand-based approach and screening approach.[9,10] The ligand-based approach relies on the use of existing biological data for old and new drugs or other anticonvulsant active compounds. This method is applied mainly for structural modifications of the currently available AEDs, with the aim of obtaining more efficacious substances that will suppress different types of seizures and/or drugs with minimal or no adverse effects compared to original AEDs. It was successfully used in the discovery of several third-generation AEDs (e.g., eslicarbazepine, fluorofelbamate, pregabalin) as well as compounds that are currently in Phase 3 of clinical trials (e.g. brivaracetam or seletracetam). The screening approach involves a comprehensive screening process of either diverse or focused compound libraries and utilizes rodent models of human epilepsies.[11,12]

Previous research from our laboratory identified pyrrolidine-2,5-diones differently substituted at position-1 and -3 as candidates for new AEDs.[13–17] Many of these compounds were effective in the maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests. Studies on the structure–activity relationship (SAR) demonstrated the potent and wide spectrum of anticonvulsant activity for the 2-(2,5-dioxopyrrolidin-1-yl)acetamides containing phenylpiperazines with highly electronegative chlorine, fluorine, or trifluoromethyl substituents at the amine function.[18]

Considering the aforementioned facts, the aim of the study was to obtain anticonvulsants with a broad spectrum of activity in "classic" animal models of epilepsy, MES and *sc*PTZ, as well as in the 6 Hz model of pharmacoresistant limbic seizures. Thus, the hypothesis assumes that the combination of chemical fragments of AEDs active in the MES test (lacosamide), PTZ test (ethosuximide), and 6 Hz seizures (levetiracetam, lacosamide) in one hybrid molecule may yield substances effective in all aforementioned preclinical animal seizure models.

In view of the above hypothesis in the current studies, the library of new 1-(4-phenylpiperazin-1-yl)- or 1-(morpholin-4-yl)-(2,5-dioxopyrrolidin-1-yl)propanamides and (2,5-dioxopyrrolidin-1-yl)butanamides was synthesized. The alkylamide moiety, namely, propanamide or butanamide, connected with the five-membered pyrrolidine-2,5-dione ring approximated the structures of new hybrid molecules to first generation AED - ethosuximide and levetiracetam which is one of the newest AEDs (Figure 1 A).

In the next step, the synthesis of the 3-methoxypropanamide analog of the most effective compound from the series of 2-(2,5-dioxopyrrolidin-1-yl)alkylamides was carried out. The introduction of the methoxy functional group at the alkylamide linker allowed the imitation of the mode of substitution to that observed for lacosamide. This AED, chemically classified as functionalized amino acid (FAA),[19–23] has been recently approved for the

treatment of partial-onset seizures with or without secondary generalization as monotherapy or adjunctive therapy in humans.[24,25] The proposed structural modification is shown in Figure 1 B.

The final compounds were tested *in vivo* for anticonvulsant activity using the maximal electroshock-induced seizure test (MES), which is the primary animal model of epilepsy used for the discovery and development of new AEDs.[26] In addition to the MES screening, the most potent substances were tested in the subcutaneous pentylenetetrazole (*sc*PTZ) and the six-hertz (6 Hz) seizure models. To determine the plausible mechanism of action for the most effective compounds *in vitro* binding assays were carried out. Considering drug safety evaluation, which is important in the preclinical identification of new drug candidates, the antiproliferative potential of the most promising molecule, and its influence on function of recombinant human CYP3A4 cytochrome isoenzyme were studied *in vitro.* Furthermore, the metabolic stability was examined using the *in silico* and *in vitro* methods.

RESULTS AND DISCUSSION

Chemistry. Compounds **3**–**28** were synthesized in a two-step reaction according to Scheme 1. First, the condensation reaction of commercially purchased succinic anhydride, with DL-α-alanine or DL-2-aminobutyric acid, yielded corresponding intermediates **1** and **2**. In the next step, **1** or **2** was converted to **3**–**28** by coupling with the appropriate phenylpiperazine derivative or morpholin in the presence of carbonyldiimidazole (CDI), which is a commonly used reagent for the synthesis of amides from carboxylic acids and amines through the acyl imidazole intermediate.[27,28] The reaction was carried out in dry tetrahydrofuran (THF) at room temperature. The progress of the reaction was monitored using HPLC chromatography (completion at approx. 24 h). Compounds **3**–**28** were obtained with yields ranging between 73% and 86%. The synthesis of 3-methoxypropanamide derivative **30**

was accomplished as shown in Scheme 2. First, the coupling reaction of 2-chloro-3-methoxypropanoic acid and 1-[3-(trifluoromethyl)phenyl]piperazine in the presence of CDI yielded intermediate **29**, which was then used for alkylation of pyrrolidine-2,5-dione to form the desired compound, **30**. Alkylation in a biphasic system was carried out in dry acetone at 60 °C for approx. 24 h in the presence of anhydrous potassium carbonate and potassium iodide. Due to the high reactivity of the chlorine atom at the α-position of 3-methoxypropanoic amide **29**, the target compound **30** was obtained in high yield–84%. All compounds were isolated as racemic mixtures. The final substances were fully characterized by elemental analyses (C, H, N), ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and LC/MS spectra (details are shown in the Supporting Information).

Pharmacology. The anticonvulsant activity profile of final molecules **3**–**28** and **30** was determined using the MES test, a mechanism-independent animal seizure model which enables identification of compounds preventing seizure spread. This test is thought to be an experimental model of tonic–clonic epilepsy and of partial convulsions with or without secondary generalization in humans.[12,29] It should be noted that despite significant advances in epilepsy research in the past several years, the MES model still persists as the most useful tool for the identification of new anticonvulsants.[26] Compounds **3**–**28** and **30** were administered in mice intraperitoneally (*i.p.*) at the fixed dose of 300 mg/kg, and the anticonvulsant protection was observed at four pretreatment times–0.25, 0.5, 1 and 2 h. The method applied here allowed the determination of the number of animals (in a group consisting of four mice) protected against electrically induced seizures as well as the estimation of the time course of anticonvulsant activity together with time of peak effect (TPE). The protection profiles for 2-(2,5-dioxopyrrolidin-1-yl)propanamides (**3**–**15**, **30**) and 2-(2,5-dioxopyrrolidin-1-yl)butanamides (**16**–**28**) are shown in Table 1.

The preliminary pharmacological screening revealed that only two compounds (**15**, **28**), representing morpholine derivatives, were completely devoid of anticonvulsant activity. In the whole series, 10 molecules **5**, **7**–**11**, **16**, **18**, **24**, and **30** revealed rapid onset and long-lasting anticonvulsant protection showing activity up to 2 h. Except for weak active molecules **4**, **12**, **19**, **22**, **23**, **25**, and **27**, all other compounds showed 50–100% protection lasting from 0.25 h up to 2 h. In the whole series, the most potent was **11** with three points of maximal protection at 0.5, 1, and 2 h. Slightly weaker efficacy was observed for its, 3-chlorine (**5**), 3-fluorine (**8**), 2-trifloromethyl (**10**), and butanamide (**24**) analogs. With the exclusion of highly active compound **26**, the exchange of electronegative trifluoromethyl group or chlorine atom in the *meta*-position of the phenylpiperazine moiety with electron-donating methyl or methoxy substituents yielded less active molecules **13**, **14**, and **27**. The analysis of preliminary pharmacological data revealed that the presence of highly electronegative atoms or groups in position 3 seems preferential for anticonvulsant properties. Their removal or replacement into *ortho*- or *para*-position caused a decrease in anticonvulsant activity. It is worth noting that higher protection was observed in general for compounds representing 2-(2,5-dioxopyrrolidin-1-yl)propanamides. Finally, slight weakening of activity was observed in case of replacement of propanamide moiety of compound **11** into 3-methoxypropanamide of **30**. It should be stressed, however, that anticonvulsant protection of the latter substance increased over time from 25% at 0.25 h to 100% at 1 h and 2 h. This observation may suggest its delayed and prolonged activity.

On the basis of the above preliminary data, 17 active compounds were screened in mice (*i.p.*) at the fixed dose of 100 mg/kg, at four pretreatment times 0.25, 0.5, 1, 2 h (Table 2).

The results obtained at the dose of 100 mg/kg revealed that 12 molecules **3**, **5**–**11**, **18**, **24**, **26**, and **30** showed satisfactory activity (at least of 50% protection in MES seizures).

Among these molecules, **5**, **10**, **11**, and **30** protected mice starting from 0.25 h till 1 h with different TPEs, i.e., 0.5 h for **5** and **11** (75% protection) or 0.25 h for **10** and **30** (100% protection). In general, other compounds showed short duration of anticonvulsant activity with peak protection at time point of 0.25 h.

In the aim of finding new hybrid anticonvulsants with wide spectrum of activity in the preclinical studies, 5 the most potent compounds in the MES test **5**, **10**, **11**, **24**, and **30** (see Table 2) were studied in the subcutaneous pentylenetetrazole (*sc*PTZ) test and psychomotor 6 Hz seizure model.

The *sc*PTZ test employs chemically induced myoclonic seizures and is proposed to identify the agents raising the seizure threshold. This test is related to human generalized absence seizures.[29] It should be stressed that ethosuximide being the chemical prototype for compounds proposed herein is an anti-absence medication effective in the PTZ seizures, however inactive in the MES test.[30] The results after *i.p.* injection in mice (doses of 300 and 100 mg/kg) are summarized in Tables 3 and 4.

As is shown in Table 3, all molecules displayed potent activity at dose of 300 mg/kg, protecting at least 75% of animals in all time points. It should be emphasized that compound **11** showed maximal protection starting from 0.25 till 2 h. This molecule displayed also the most potent activity at the dose of 100 mg/kg, with one peak of 100% protection at 0.5 h, and points of 75% protection at 1 and 2 h (Table 4). Other compounds screened at the dose of 100 mg/kg revealed at least of 50% protection in different time intervals. Notably, **11** and **30** displayed prolonged and satisfactory activity in all the time points.

Traditionally, most screening programs assess efficacy of AED candidates against either electrically (e.g. MES) or chemically (e.g. 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 PTZ seizures even at high doses, whereas showed high efficacy in the 6 Hz model of pharmacoresistant limbic seizures.[31] Bearing in mind the aforementioned facts and the structural similarities of new compounds with levetiracetam (see Figure 1), 5 molecules **5**, **10**, **11**, **24**, and **30** tested before in the MES and PTZ seizures, were screened in the 6 Hz model. The results after *i.p.* administration are summarized in Table 5.

As is shown in Table 5, all compounds tested displayed satisfactory activity in the 6 Hz seizures. The most beneficial anticonvulsant properties was observed for **11**, which displayed three points of 100% protection – 0.5, 1, and 2 h. Furthermore, high efficacy with two points of the maximal activity revealed also **5**, **24**, and **30**, whereas **10** protected 100% of animals only at 0.25 h after *i.p.* administration.

Based on the preliminary results, in the next step of the pharmacological studies, the median effective doses (ED_{50}) were determined for compounds which showed minimum 50% protection at the dose of 100 mg/kg (MES, PTZ or 6 Hz tests). Moreover, for compounds with the best ED_{50} values (<120 mg/kg), the median neurotoxic doses (TD_{50},) were estimated in the chimney test. This data was used to calculate the protective indexes (PIs), which are the measure of the benefit-to-risk ratio of the therapeutic agent. In parallel, the same studies were performed for model AEDs active in the MES (lacosamide), PTZ (ethosuximide), 6 Hz (levetiracetam, lacosamide) tests as well as effective in all seizure models (valproic acid) (Table 6).

The quantitative *i.p.* data in mice confirmed the wide spectrum and potent anticonvulsant activity of **5**, **10**, **11**, and **24** containing the 3-chloro- (**5**), 2-(trifluoromethyl)- (**10**) or 3-(trifluoromethyl)phenylpiperazines (**11**, **24**) as amine function. Among these

compounds, the most promising was **11** which did not impair motor coordination of animals even at high doses (TD_{50} >1500 mg/kg) that yielded superb protective indexes (>16.97, MES; >25.04, PTZ; >71.43, 6 Hz) at time point of 0.5 h. As a result, **11** displayed distinctly better safety profile than all model AEDs. Despite compound **11** showing lower protection in the MES and 6 Hz seizures compared to the lacosamide, it showed better safety profile in the chimney test that resulted in 4.7-fold (MES) and 13.6-fold (6 Hz) more beneficial PI values. Notably, this molecule revealed comparable efficacy to levetiracetam (6 Hz test), and distinctly higher activity in comparison with PTZ-active ethosuximide (2.5-fold), as well as MES/PTZ/6 Hz-active valproic acid as follows: 2.4-fold (MES), 4.6-fold (PTZ), and 6.2-fold (6 Hz). Additionally, compound **11** displayed potent activity and markedly low neurotoxicity 1 h after administration. High activity and wide spectrum of anticonvulsant protection was observed also for other trifluoromethyl analogs **10**, **24**, and *meta*-chloro derivative **5**; however, these molecules were more neurotoxic in the chimney test, resulting in worsening of PIs compared to model AEDs (ethosuximide, lacosamide, and levetiracetam). It should be stressed that all aforementioned substances were more potent compared to valproic acid (MES, PTZ, 6 Hz seizures), as well as provided better protection in the *sc*PTZ test than model PTZ-active drug – ethosuximide. Notably, the *meta*-chloro derivative **5** displayed ED_{50} of 15.6 mg/kg in the 6 Hz test 0.25 h after *i.p.* injection (data not indicated in Table 6). The quantitative data revealed that in case of the most effective substance **11**, the introduction of the methoxy group into the propanamide fragment (compound **30**) increased activity in the MES test, however decreased the protection in the 6 Hz seizures. Furthermore, it was not possible to determine ED_{50} value in the *sc*PTZ test up to dose of 120 mg/kg. This observation may result from the presence of 3-methoxypropanamide fragment that is common for both **30** and lacosamide, which is known to be inactive in the PTZ seizures. Despite the lower PI of **30** compared to parent compound **11**, the aforementioned structural modification may lead to

highly effective substances and will be examined precisely in the next studies. In conclusion, the results of the current studies as well as our previous research in a group of *N*-benzyl-2-(2,5-dioxopyrrolidin-1-yl)propanamide and 2-(2,5-dioxopyrrolidin-1-yl)butanamide derivatives,[32] highly support the proposed hypothesis of hybrid anticonvulsants based on the pyrrolidine-2,5-dione core fragment.

In vitro radioligand binding studies. Intensive research into the physiological and biochemical events which occur during epileptic seizures has provided insight into the molecular mechanisms by which these might be controlled. Thus, AEDs act on diverse molecular targets to selectively modify the excitability of neurons so that seizure-related firing is blocked without disturbing non-epileptic activity. This occurs largely through effects on voltage-gated sodium and calcium channels, or by promoting inhibition mediated by $GABA_A$ (γ-aminobutyric acid, type A) receptors. The fundamental role in establishing and regulating the excitability of CNS neurons as well as suppression of seizures is ascribed to voltage-gated sodium channels (VGSCs), and voltage-dependent calcium channels (VDCC).[30] Thus, the brain voltage-gated sodium and calcium channels are the molecular targets of numerous chemically diverse AEDs.[33–36] Using radioligand binding techniques, Willow and associates have found an allosteric interaction between classical and clinically relevant AEDs - phenytoin and carbamazepine, and the batrachotoxinin (BTX) binding site of sodium channels from rat brain.[37,38] Electrophysiological studies of neuroblastoma cells demonstrate a frequency- and voltage-dependent blockade of sodium currents by aforementioned anticonvulsants.[39,40] These findings suggest that blockade of sodium channel activity by these agents underlies their anticonvulsant actions. Such mode of action is characteristic of substances active in the MES test in animals. Due to the activity of the compounds reported in this paper in the MES seizures, for 3 the most active molecules (**11**, **24**, **30**), and carbamazepine (as a reference AED), the binding assays for Na^+ channel (site 2) were

performed using the [^{3}H]BTX as radioligand.[41] Compound binding was expressed as a percentage of inhibition of the binding of a radioactively labeled ligand. The inhibition values for **11**, **24**, and **30** were determined at four concentrations, 1 μM, 10 μM, 100 μM, and 500 μM (Table 7).

Compounds **11**, **24**, and **30** were observed as relatively effective binders to the neuronal voltage-sensitive Na^+ channel at the highest concentration–500 μM. The moderate activity was observed also for **11**, and **30** at a concentration of 100 μM. Notably, carbamazepine, which is known to be the anticonvulsant that acts as Na^+ channel blocker, showed a moderate effect only at 500 μM, and was weaker binder compared to **11**, **24**, and **30**. The above results may suggest that the mechanism of anticonvulsant protection of compounds tested is probably related to their influence on voltage-gated sodium channel. Furthermore, this observation was strengthened by the *in vivo* quantitative data (Table 6), namely the more potent compound in the electrically evoked seizures (MES test) the higher Na^+ channel affinity. Compound **11** has a broad spectrum of anticonvulsant activity (MES, *sc*PTZ, 6 Hz models), most likely reflecting its multiple sites of action. Thus, with the aim of investigating the more precise mechanisms by which **11** is believed to exert its anti-seizure effects, the binding assays for voltage-gated N-type Ca^{2+} channel and L-type Ca^{2+} channel were carried out. In parallel, the same tests were performed for **30** which was inactive in the *sc*PTZ seizures (ED_{50} > 120 mg/g), and provided weaker protection in the 6 Hz model. As shown in Table 8, **11** revealed distinctly stronger affinity to L-type Ca^{2+} channel than **30**, and both compounds did not bind to N-type Ca^{2+} channel. This data indicate that interaction of **11** with voltage-gated L-type Ca^{2+} channel may play an important role for its activity in the PTZ seizures especially, however also in the 6 Hz test. It should be emphasized that a modulation of neuronal L-type Ca^{2+} channel activity is an essential mechanism of action for topiramate (TPM), which is the novel AED with broad therapeutic spectrum.[42,43] Notably, compound **11**

proved to be a more effective binder to the voltage-gated L-type Ca^{2+} channel compared to the mentioned AED at a concentration of 100 μM.

In vitro and in silico assays. In the modern approach to the drug development process, drug-like properties of the new compounds should be evaluated in parallel with the study on their efficacy at early discovery phases.[44,45] In the present study, compound **11**, as the most promising in the pharmacological studies, was chosen for preliminary *in vitro* assays of selected ADME–Tox parameters, such as antiproliferative activity and metabolic stability. Additionally, the bioluminescence assay was used for the prediction of the influence of compound **11** on the activity of recombinant cytochrome P450 3A4, considering the potential drug–drug interactions (DDIs).

Antiproliferative assay. The incubation of human embryonic kidney cell line (HEK–293) in the presence of the most promising molecule **11** for 48 h showed the decreasing viability of examined cells above the 10 μM concentration (Figure 2). The calculated IC_{50} value of **11** against HEK-293 was 50.73 μM. However, in comparison with the IC_{50} value of 0.46 μM for the reference antiproliferative drug–doxorubicin (DX), compound **11** possesses over 100-fold lower antiproliferative activity. Thus, the results obtained qualify compound **11** as safe in relation to the antiproliferative effect.

Metabolic stability. The metabolic stability of compound **11** was examined first *in silico* by using MetaSite software.[46] The plot of MetaSite predictions for sites of metabolism for **11** using the liver computational model is shown in Figure 3. The darker red color of atoms marked with circles indicate their higher probability to be involved in the metabolism pathway. The blue circle marks the site of compound **11** involved in metabolism with the highest probability (100% score). According to *in silico* data, the highest probability of metabolism will occur at the position-4 of phenyl moiety. Moreover, compound **11** will be likely metabolized also at the alkylamide linker and at pyrrolidine-2,5-dione ring. The

metabolic stability of **11** was also evaluated *in vitro* using human liver microsomes (HLMs). A full scan chromatogram of compound **11** after 2 h of incubation with HLMs showed the presence of four metabolites–**M1**, **M2**, **M3**, and **M4** (Figure 4 A). The LC/MS analysis provided the molecular masses of obtained metabolites: **M1** $[M+H]^+$ = 382.27 *m/z*, **M2** $[M+H]^+$ = 416.31 *m/z*, **M3** $[M+H]^+$ = 402.28 *m/z*, and **M4** $[M+H]^+$ = 400.29 *m/z* (Figure 4 B). To confirm the structures of HLM metabolites of compound **11**, the ion fragment analysis was performed. The metabolite structures generated on the basis of *in silico* studies and the ion fragment analysis considered the following metabolic pathways of **11**: (i) the dehydrogenation of piperazine moiety (**M1**); (ii) double-hydroxylation of the alkylamide linker and pyrrolidine-2,5-dione moiety (**M2**); (iii) hydroxylation of the alkylamide linker with the reduction of one carbonyl group of pyrrolidine-2,5-dione ring (**M3**); and (iv) the introduction of the hydroxyl group next to the trifluoromethyl substituent in the phenylpiperazine moiety (**M4**) (Figure 5).

Influence on recombinant human CYP3A4 P450 cytochrome. Many drug–drug interactions are metabolism based and are mediated primarily *via* the microsomal cytochrome P450 (CYP) family of enzymes. Ten CYP isoforms are expressed in a typical human liver and six of them (CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A4) appear to be employed in the biotransformation of most drugs. Notably, among these isoforms, the CYP3A4 is responsible for the metabolism of more than 50% of medicines and the associated drug–drug interactions. The inhibition of these enzymes may decrease the metabolic clearance of a co-administered drug, resulting in elevated blood concentration, which may cause adverse drug effects or toxicity. As detailed in the draft guidance document for drug–drug interactions,[47] the FDA has placed emphasis on evaluating the inhibition potential of a new chemical entity (NCE) at an earlier stage in drug development to avoid the development of compounds with the potential to yield adverse drug interactions.

To evaluate the potential inhibition or induction of cytochrome CYP3A4 by compound **11**, we used the luminescence CYP3A4 P450-Glo™ assay based on the conversion of the luciferin-PPXE (the beetle D-luciferin derivative) into D-luciferin by recombinant human CYP3A4 isoenzyme. After the addition of firefly luciferase, the measured amount of light produced in the reaction was proportional to the concentration of D-luciferin.[48] As it is shown in the following (Figure 6), compound **11** inhibited slightly the cytochrome activity at 25 μM (68.4% of control activity). However, comparing with the effect of ketoconazole which completely inhibited the CYP3A4 activity at 10 μM (calculated IC_{50}=0.14 μM), compound **11** may be considered as a very weak CYP3A4 inhibitor. Thus, the data suggest that compound **11** is unlikely to show inhibitory interactions with other CYP3A4 metabolized drugs *in vivo*.

CONCLUSION

The purpose of this study was to design and synthetize the focused library of 27 new 1-(4-phenylpiperazin-1-yl)- or 1-(morpholin-4-yl)-(2,5-dioxopyrrolidin-1-yl)propanamides and (2,5-dioxopyrrolidin-1-yl)butanamides as potential new hybrid anticonvulsant agents. These hybrid molecules join the chemical fragments of well-known AEDs such as ethosuximide, levetiracetam, and lacosamide. The initial anticonvulsant screening was performed in mice (*i.p.*) using the 'classical' maximal electroshock (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests as well as in the six-hertz (6 Hz) model of pharmacoresistant limbic seizures. The acute neurological toxicity was determined applying the chimney test. Four compounds **5**, **10**, **11**, and **24** showed the broad spectra of activity across all the preclinical seizure models. The quantitative pharmacological studies in mice *i.p.* demonstrated the highest protection and a substantial safety profile in the chimney for **11**. This compound emerged as the most promising molecule with superb protective indexes (PI

MES >16.97, PI PTZ >25.04, PI 6 Hz >71.43). The *in vitro* binding studies proved that the most plausible mechanism of action for **11** was the influence on the neuronal voltage-sensitive sodium and L-type calcium channels. The results obtained in the current studies support the proposed hypothesis of hybrid anticonvulsants based on the pyrrolidine-2,5-dione core fragment.

EXPERIMENTAL SECTION

Chemistry. *General*. All chemicals and solvents were purchased from Sigma-Aldrich (St. Louis, USA) and were used without further purification. Melting points (mp) were determined in open capillaries on a Büchi 353 melting point apparatus (Büchi Labortechnik, Flawil, Switzerland) and are uncorrected. The purity and homogeneity of the compounds were assessed by TLC and gradient HPLC chromatography. The thin–layer chromatography (TLC) was performed on Merck silica gel 60 F_{254} pre-coated aluminium sheets (Merck; Darmstadt, Germany), using developing system consisting of: S_1 – dichloromethane : methanol (9 : 1; *v/v*), S_2 – dichloromethane : methanol (9 : 0.3; *v/v*). Spots were detected by their absorption under UV light (λ = 254 nm). HPLC analyses were run on the HPLC Waters 2695 Separation Module (Waters, Milford, USA) equipped with photodiode array detector Waters 2998 (Waters, Milford, USA). The Chromolith RP–18 SpeedROD column (4.6 × 50 mm) was used. Conditions applied were as follow: eluent A (water/0.1% TFA), eluent B (acetonitrile/0.1% TFA); flow rate of 5 mL/min, gradient of 0–100% B over 3 min were used, injection volume was 10 μL. Standard solutions (1 mg/mL) of each compound were prepared in analytical grade acetonitrile and analyzed at wave lengths 214 and 254 nm. Retention times (t_R) are given in minutes. The purity of all final compounds determined by use of chromatographic HPLC method was >95%. Elemental analysis for C, H, and N were carried out by a micro method using the elemental Vario EI III Elemental analyzer (Hanau, Germany). The results of

elemental analyses were within ±0.4 % of the theoretical values. ^{1}H NMR,^{13}C NMR and ^{19}F NMR spectra were obtained in a Varian Mercury spectrometer (Varian Inc., Palo Alto, CA, USA), in $CDCl_3$ operating at 300 MHz (^{1}H NMR), 75 MHz (^{13}C NMR), and 282 MHz (^{19}F NMR). Chemical shifts are reported in δ values (ppm) relative to TMS δ = 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), ddd (double double doublet), t (triplet), td (triplet of doublets), q (quartet), m (multiplet). The mass spectra (LC-MS) were obtained on Waters ACQUITY™ TQD system with the TQ Detector (Waters, Milford, USA). The ACQUITY UPLC BEH C18, 1.7 μm, 2.1 × 50 mm column was used (Waters, Milford, USA). Preparative column chromatography was performed using silica gel 60 (particle size 0,063–0,200 mm; 70–230 Mesh ASTM) purchased from Merck (Darmstadt, Germany).

General procedure for the preparation of the 2-(2,5-dioxopyrrolidin-1-yl)propanoic acid (1) and 2-(2,5-dioxopyrrolidin-1-yl)butanoic acid (2). Succinic anhydride (5.0 g, 0.05 mol) was suspended in 20 mL of water and DL-α-alanine (4.4 g, 0.05 mol) or DL-2-aminobutyric acid (5.2 g, 0.05 mol) was gradually added. The mixture was heated in a term–regulated 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 column chromatography (dichloromethane : methanol, 9 : 1, *v/v*) to afford intermediates **1** and **2** as yellow oils.

2-(2,5-Dioxopyrrolidin-1-yl)propanoic acid (1). Yellow oil. Yield 75%; TLC: R_f = 0.61 (S_1); HPLC (purity 100%): t_R = 0.278 min.; ESI–MS: 172.1 ($C_7H_9NO_4$ $[M+H]^+$). Anal calcd for $C_7H_9NO_4$ (171.15): C, 49.12; H, 5.30; N, 8.18. Found: C, 49.20; H, 5.18; N, 8.25.

2-(2,5-Dioxopyrrolidin-1-yl)butanoic acid (2). Yellow oil. Yield 72%; TLC: R_f = 0.71 (S_1); HPLC (purity 99.2%): t_R = 0.524 min.; ESI–MS: 185.1 ($C_8H_{11}NO_4$ $[M+H]^+$). Anal calcd for $C_8H_{11}NO_4$ (185.15): C, 51.89; H, 5.99; N, 7.56. Found: C, 51.95; H, 5.90; N, 7.66.

General method for the preparation of 2-(2,5-dioxopyrrolidin-1-yl)propanamides (3-15) and 2-(2,5-dioxopyrrolidin-1-yl)butanamides (16–28). Carbonyldiimidazole (0.97 g, 0.006 mol) in 5 mL of dry THF was added to a solution of intermediate **1** (1.02g, 0.006 mol) or **2** (1.11g, 0.006 mol) dissolved in 10 mL of anhydrous THF while stirring. After the end of gaseous (carbon dioxide) evolution (approx. 0.5 h), the secondary amine (0.006 mol) dissolved in 5 mL of anhydrous THF was added dropwise. The mixture was stirred at room temperature (approx. 24 h) and evaporated to dryness. The crude product was purified by column chromatography (dichloromethane : methanol, 9 : 0.3, *v/v*). The final amides were obtained as solid substances after concentration of organic solvents under reduced pressure.

1-[1-Oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl]pyrrolidine-2,5-dione (3). White solid. Yield: 77%; mp 129–130 ºC; TLC: R_f = 0.45 (S_2); HPLC (purity 100%): t_R = 0.975 min.; ESI–MS: 316.1 ($C_{17}H_{21}N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{17}H_{21}N_3O_3$ (315.37): C: 64.74, H: 6.71, N:13.32; Found C: 64.88, H: 6.62, N: 13.50.

1-{1-[4-(2-Chlorophenyl)piperazin-1-yl]-1-oxopropan-2-yl}pyrrolidine-2,5-dione (4). White solid. Yield: 73%; mp 118–119 ºC; TLC: R_f = 0.52 (S_2); HPLC (purity 100%): t_R = 1.247 min.; ESI–MS: 350.2 ($C_{17}H_{20}ClN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{17}H_{20}ClN_3O_3$ (349.81): C: 58.37, H: 5.76, N: 12.01; Found C: 58.55, H: 5.85, N: 11.88.

1-{1-[4-(3-Chlorophenyl)piperazin-1-yl]-1-oxopropan-2-yl}pyrrolidine-2,5-dione (5). White solid. Yield: 78%; mp 148–149 ºC; TLC: R_f = 0.56 (S_2); HPLC (purity 100%): t_R = 1.380 min.; ESI–MS: 350.2 ($C_{17}H_{20}ClN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{17}H_{20}ClN_3O_3$ (349.81): C: 58.37, H: 5.76, N: 12.01; Found C: 58.46, H: 5.80, N: 11.94.

1-{1-[4-(4-Chlorophenyl)piperazin-1-yl]-1-oxopropan-2-yl}pyrrolidine-2,5-dione (6). White solid. Yield: 80%; mp 137–138 ºC; TLC: R_f = 0.57 (S_2); HPLC (purity 100%): t_R = 1.413 min.; ESI–MS: 350.2 ($C_{17}H_{20}ClN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{17}H_{20}ClN_3O_3$ (349.81): C: 58.37, H: 5.76, N: 12.01; Found C: 58.22, H: 5.83, N: 12.08.

1-{1-[4-(2-Fluorophenyl)piperazin-1-yl]-1-oxopropan-2-yl}pyrrolidine-2,5-dione (7). White solid. Yield: 77%; mp 122–123 ºC; TLC: R_f = 0.49 (S_2); HPLC (purity 100%): t_R = 1.241 min.; ESI–MS: 334.1 ($C_{17}H_{20}FN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{17}H_{20}FN_3O_3$ (333.36): C: 61.25, H: 6.05, N: 12.61; Found C: 61.19, H: 5.98, N: 12.68.

1-{1-[4-(3-Fluorophenyl)piperazin-1-yl]-1-oxopropan-2-yl}pyrrolidine-2,5-dione (8). White solid. Yield: 79%; mp 121–122 ºC; TLC: R_f = 0.52 (S_2); HPLC (purity 100%): t_R = 1.235 min.; ESI–MS: 334.1 ($C_{17}H_{20}FN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{17}H_{20}FN_3O_3$ (333.36): C: 61.25, H: 6.05, N: 12.61; Found C: 61.20, H: 5.92, N: 12.65.

1-{1-[4-(4-Fluorophenyl)piperazin-1-yl]-1-oxopropan-2-yl}pyrrolidine-2,5-dione (9). White solid. Yield: 81%; mp 83–84 ºC; TLC: R_f = 0.50 (S_2); HPLC (purity 100%): t_R = 1.104 min.; ESI–MS: 334.1 ($C_{17}H_{20}FN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{17}H_{20}FN_3O_3$ (333.36): C: 61.25, H: 6.05, N: 12.61; Found C: 61.18, H: 5.99, N: 12.59.

1-(1-Oxo-1-{4-[2-(trifluoromethyl)phenyl]piperazin-1-yl}propan-2-yl)pyrrolidine-2,5-dione (10). White solid. Yield: 82%; mp 118–119 ºC; TLC: R_f = 0.62 (S_2); HPLC (purity 100%): t_R = 1.518 min.; ESI–MS: 384.2 ($C_{18}H_{20}F_3N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{20}F_3N_3O_3$ (383.36): C: 56.39, H: 5.26, N: 10.96; Found C: 56.44, H: 5.29, N: 10.79.

1-(1-Oxo-1-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}propan-2-yl)pyrrolidine-2,5-dione (11). White solid. Yield: 80%; mp 135–136 ºC; TLC: R_f = 0.68 (S_2); HPLC (purity 100%): t_R = 1.590 min.; ESI–MS: 384.2 ($C_{18}H_{20}F_3N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{20}F_3N_3O_3$ (383.36): C: 56.39, H: 5.26, N: 10.96; Found C: 56.42, H: 5.33, N: 10.84.

1-(1-Oxo-1-{4-[4-(trifluoromethyl)phenyl]piperazin-1-yl}propan-2-yl)pyrrolidine-2,5-dione (12). White solid. Yield: 80%; mp 147–148 ºC; TLC: R_f = 0.67 (S_2); HPLC (purity 100%): t_R = 1.465 min.; ESI–MS: 384.2 ($C_{18}H_{20}F_3N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{20}F_3N_3O_3$ (383.36): C: 56.39, H: 5.26, N: 10.96; Found C: 56.45, H: 5.27, N: 10.90.

1-{1-[4-(3-Methylphenyl)piperazin-1-yl]-1-oxopropan-2-yl}pyrrolidine-2,5-dione (13). White solid. Yield: 86%; mp 105–106 ºC; TLC: R_f = 0.55 (S_2); HPLC (purity 100%): t_R = 0.994 min.; ESI–MS: 330.20 ($C_{18}H_{23}N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{23}N_3O_3$ (329.39): C: 65.63, H: 7.04, N: 12.76; Found C: 65.69, H: 7.15, N: 12.88.

1-{1-[4-(3-Methoxyphenyl)piperazin-1-yl]-1-oxopropan-2-yl}pyrrolidine-2,5-dione (14). White solid. Yield: 80%; mp 131–132 ºC; TLC: R_f = 0.54 (S_2); HPLC (purity 100%): t_R = 1.004 min.; ESI–MS: 346.1 ($C_{18}H_{23}N_3O_4$ $[M+H]^+$). Anal. calcd for $C_{18}H_{23}N_3O_4$ (345.39): C: 62.59, H: 6.71, N: 12.17; Found C: 62.68, H: 6.80, N: 12.18.

1-[1-(Morpholin-4-yl)-1-oxopropan-2-yl]pyrrolidine-2,5-dione (15). White solid. Yield: 75%; mp 124–125 ºC; TLC: R_f = 0.44 (S_1); HPLC (purity 100%): t_R = 0.811 min.; ESI–MS: 241.1 ($C_{11}H_{16}N_2O_4$ $[M+H]^+$). Anal. calcd for $C_{11}H_{16}N_2O_4$ (240.26): C: 54.99, H: 6.71, N: 11.66; Found C: 54.88, H: 6.82, N: 11.70.

1-[1-Oxo-1-(4-phenylpiperazin-1-yl)butan-2-yl]pyrrolidine-2,5-dione (16). White solid. Yield: 77%; mp 116–117 ºC; TLC: R_f = 0.53 (S_2); HPLC (purity 100%): t_R = 1.039 min.; ESI–MS: 330.1 ($C_{18}H_{23}N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{23}N_3O_3$ (329.39): C: 65.63, H: 7.04, N:12.76; Found C: 65.73, H: 7.08, N: 12.88.

1-{1-[4-(2-Chlorophenyl)piperazin-1-yl]-1-oxobutan-2-yl}pyrrolidine-2,5-dione (17). White solid. Yield: 73%; mp 129–130 ºC; TLC: R_f = 0.62 (S_2); HPLC (purity 100%): t_R = 1.351 min.; ESI–MS: 364.3 ($C_{18}H_{22}ClN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{22}ClN_3O_3$ (363.84): C: 59.42, H: 6.09, N: 11.55; Found C: 59.55.88, H: 6.20, N: 11.60.

1-{1-[4-(3-Chlorophenyl)piperazin-1-yl]-1-oxobutan-2-yl}pyrrolidine-2,5-dione (18). White solid. Yield: 78%; mp 108–109 ºC; TLC: R_f = 0.65 (S_2); HPLC (purity 100%): t_R = 1.545 min.; ESI–MS: 364.3 ($C_{18}H_{22}ClN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{22}ClN_3O_3$ (363.84): C: 59.42, H: 6.09, N: 11.55; Found C: 59.46, H: 6.34, N: 11.77.

1-{1-[4-(4-Chlorophenyl)piperazin-1-yl]-1-oxobutan-2-yl}pyrrolidine-2,5-dione (19). White solid. Yield: 80%; mp 166–167 ºC; TLC: R_f = 0.63 (S_2); HPLC (purity 99.8%): t_R = 1.415 min.; ESI–MS: 364.4 ($C_{18}H_{22}ClN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{22}ClN_3O_3$ (363.84): C: 59.42, H: 6.09, N: 11.55; Found C: 59.50, H: 6.29, N: 11.60.

1-{1-[4-(2-Fluorophenyl)piperazin-1-yl]-1-oxobutan-2-yl}pyrrolidine-2,5-dione (20). White solid. Yield: 77%; mp 128-129 ºC; TLC: R_f = 0.58 (S_2); HPLC (purity 100%): t_R = 1.311 min.; ESI–MS: 348.1 ($C_{18}H_{22}FN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{22}FN_3O_3$ (347.38): C: 62.23, H: 6.38, N: 12.10; Found C: 62.19, H: 6.50, N: 12.11.

1-{1-[4-(3-Fluorophenyl)piperazin-1-yl]-1-oxobutan-2-yl}pyrrolidine-2,5-dione (21). White solid. Yield: 79%; mp 147–148 ºC; TLC: R_f = 0.57 (S_2); HPLC (purity 100%): t_R = 1.277 min.; ESI–MS: 348.1 ($C_{18}H_{22}FN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{22}FN_3O_3$ (347.38): C: 62.23, H: 6.38, N: 12.10; Found C: 62.28, H: 6.54, N: 12.16.

1-{1-[4-(4-Fluorophenyl)piperazin-1-yl]-1-oxobutan-2-yl}pyrrolidine-2,5-dione (22). White solid. Yield: 81%; mp 154–155 ºC; TLC: R_f = 0.59 (S_2); HPLC (purity 99.7%): t_R = 1.209 min.; ESI–MS: 348.1 ($C_{18}H_{22}FN_3O_3$ $[M+H]^+$). Anal. calcd for $C_{18}H_{22}FN_3O_3$ (347.38): C: 62.23, H: 6.38, N: 12.10; Found C: 62.11, H: 6.48, N: 12.04.

1-(1-Oxo-1-{4-[2-(trifluoromethyl)phenyl]piperazin-1-yl}butan-2-yl)pyrrolidine-2,5-dione (23). White solid. Yield: 82%; mp 148–149 ºC; TLC: R_f = 0.72 (S_2); HPLC (purity 100%): t_R = 1.589 min.; ESI–MS: 398.1 ($C_{19}H_{22}F_3N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{19}H_{22}F_3N_3O$ (397.39): C: 57.43, H: 5.58, N: 10.57; Found C: 57.30, H: 5.59, N: 10.79.

1-(1-Oxo-1-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}butan-2-yl)pyrrolidine-2,5-dione (24). White solid. Yield: 80%; mp 128–129 ºC; TLC: R_f = 0.76 (S_2); HPLC (purity 100%): t_R = 1.646 min.; ESI–MS: 398.1 ($C_{19}H_{22}F_3N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{19}H_{22}F_3N_3O$ (397.39): C: 57.43, H: 5.58, N: 10.57; Found C: 57.45, H: 5.68, N: 10.65.

1-(1-Oxo-1-{4-[4-(trifluoromethyl)phenyl]piperazin-1-yl}butan-2-yl)pyrrolidine-2,5-dione (25). White solid. Yield: 85%; mp 148–149 ºC; TLC: R_f = 0.77 (S_2); HPLC (purity 100%): t_R = 1.537 min.; ESI–MS: 398.1 ($C_{19}H_{22}F_3N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{19}H_{22}F_3N_3O$ (397.39): C: 57.43, H: 5.58, N: 10.57; Found C: 57.50, H: 5.70, N: 10.60.

1-{1-[4-(3-Methylphenyl)piperazin-1-yl]-1-oxobutan-2-yl}pyrrolidine-2,5-dione (26). White solid. Yield: 75%; mp 95–96 ºC; TLC: R_f = 0.60 (S_2); HPLC (purity 99.8%): t_R = 1.148 min.; ESI–MS: 344.3 ($C_{19}H_{25}N_3O_3$ $[M+H]^+$). Anal. calcd for $C_{19}H_{25}N_3O_3$ (343.42): C: 66.45, H: 7.34, N: 12.24; Found C: 66.35, H: 7.50, N: 12.30.

1-{1-[4-(3-Methoxyphenyl)piperazin-1-yl]-1-oxobutan-2-yl}pyrrolidine-2,5-dione (27). White solid. Yield: 80%; mp 144–145 ºC; TLC: R_f = 0.58 (S_2); HPLC (purity 100%): t_R = 1.109 min.; ESI–MS: 360.3 ($C_{19}H_{25}N_3O_4$ $[M+H]^+$). Anal. calcd for $C_{19}H_{25}N_3O_4$ (359.42): C: 63.49, H: 7.01, N: 11.69; Found C: 63.52, H: 7.20, N: 11.75.

1-[1-(Morpholin-4-yl)-1-oxobutan-2-yl]pyrrolidine-2,5-dione (28). White solid. Yield: 75%; mp 117–118 ºC; TLC: R_f = 0.56 (S_1); HPLC (purity 100%): t_R = 0.923 min.; ESI–MS: 255.1 ($C_{12}H_{18}N_2O_4$ $[M+H]^+$). Anal. calcd for $C_{12}H_{18}N_2O_4$ (254.28): C: 56.68, H: 7.13, N: 11.02; Found C: 56.72, H: 7.25, N: 11.07.

Synthesis of 2-chloro-3-methoxy-1-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}propan-1-one (29). Carbonyldiimidazole (0.97 g, 0.006 mol) in 5 mL of dry THF was added to a solution of 2-chloro-3-methoxypropanoic acid (0.83 g, 006 mol) dissolved in 10 mL of anhydrous THF while stirring. After the end of gaseous (carbon dioxide) evolution (approx. 0.5 h), 1–[3 (trifluoromethyl)phenyl]piperazine (1.38 g, 0.006 mol) dissolved in 5

mL of anhydrous THF was added dropwise. The mixture was stirred at room temperature (approx. 24 h), and evaporated to dryness. The crude product was purified by column chromatography (dichloromethane : methanol, 9 : 0.5, *v/v*). After concentration of organic solvents under reduced pressure compound **29** was obtained as light oil. Yield: 66%; TLC: R_f = 0.77 (S_2); HPLC(purity 98.5%): t_R = 1.688 min.; ESI–MS: 351.3 ($C_{15}H_{18}ClF_3N_2O_2$ $[M+H]^+$). Anal. calcd for $C_{15}H_{18}ClF_3N_2O_2$ (350.76): C: 51.36, H: 5.17, N: 7.99; Found C: 51.50, H: 5.20, N: 8.05.

1-{1-[4-(3-Trifluoromethylphenyl)piperazin-1-yl]-3-methoxy-1-oxopropan-2-yl}pyrrolidine-2,5-dione (30). A mixture of pyrrolidine-2,5-dione (0.0075 mol), 2-chloro-3-methoxy-1-{4-[3-(trifluoromethyl)phenyl]piperazin-1-yl}propan-1-one (**29**), anhydrous potassium carbonate (0.0375 mol) and potassium iodide (0.2 g) in 15 mL of acetone was stirred at 60 °C for approx. 24 h. Then, the inorganic solid was filtered off and acetone was evaporated to dryness. The oily residue obtained was purified by column chromatography using a dichloromethane : methanol, 9 : 0.7 (*v/v*) mixture as a solvent system. Compound **30** was isolated as white solid after concentration of organic solvents under reduced pressure. Yield: 84%; mp 164–166 ºC; TLC: R_f = 0.80 (S_1); HPLC (purity 100%): t_R = 1.576 min.; ESI–MS: 414.3 ($C_{19}H_{22}F_3N_3O_4$ $[M+H]^+$). Anal. calcd for $C_{19}H_{22}F_3N_3O_4$ (413.39): C: 55.20, H: 5.36, N: 10.16; Found C: 55.25, H: 5.20, N: 10.30.

ASSOCIATED CONTENT

Supporting Information Available: Molecular Formula Strings Spreadsheet (CSV), ^{1}H NMR, ^{13}C NMR, and ^{19}F NMR spectra description, animal procedures, *in vitro* and *in silico* studies reports are available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS USED

ADME-Tox, absorption, distribution, metabolism, excretion, toxicity; CDI, carbonyldiimidazole; DX, doxorubicin; FDA, Food and Drug Administration; HLM, human liver microsomes; MES, maximal electroshock seizure test; PI, protective index (TD_{50}/ED_{50}); *sc*PTZ, subcutaneous pentylenetetrazole seizure test; 6 Hz, six-hertz test; TPE, time of peak effect.

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Table 1. Anticonvulsant activity–MES test in mice *i.p.* (dose of 300 mg/kg)

compd	R_1	R_2	Pretreatment times[a]			
			0.25 h	0.5 h	1 h	2 h
3	CH_3	H	**4/4**	**2/4**	**2/4**	0/4
4	CH_3	2-Cl	0/4	0/4	**1/4**	0/4
5	CH_3	3-Cl	**3/4**	**4/4**	**3/4**	**2/4**
6	CH_3	4-Cl	**4/4**	**4/4**	0/4	0/4
7	CH_3	2-F	**4/4**	**2/4**	**2/4**	**1/4**
8	CH_3	3-F	**4/4**	**2/4**	**3/4**	**4/4**
9	CH_3	4-F	**4/4**	**2/4**	**1/4**	**2/4**
10	CH_3	2-CF_3	**4/4**	**4/4**	**3/4**	**3/4**
11	CH_3	3-CF_3	**3/4**	**4/4**	**4/4**	**4/4**
12	CH_3	4-CF_3	**1/4**	**1/4**	0/4	0/4
13	CH_3	3-CH_3	**4/4**	**2/4**	0/4	0/4
14	CH_3	3-OCH_3	**3/4**	**2/4**	0/4	0/4
15	CH_3	morpholine	0/4	0/4	0/4	0/4
16	C_2H_5	H	**3/4**	**3/4**	**3/4**	**1/4**
17	C_2H_5	2-Cl	**2/4**	**1/4**	**2/4**	0/4
18	C_2H_5	3-Cl	**3/4**	**2/4**	**4/4**	**1/4**
19	C_2H_5	4-Cl	0/4	0/4	**1/4**	**1/4**
20	C_2H_5	2-F	**4/4**	**4/4**	**2/4**	0/4
21	C_2H_5	3-F	**3/4**	**4/4**	**3/4**	0/4
22	C_2H_5	4-F	0/4	**1/4**	**1/4**	0/4
23	C_2H_5	2-CF_3	**1/4**	**1/4**	0/4	0/4
24	C_2H_5	3-CF_3	**3/4**	**2/4**	**4/4**	**2/4**
25	C_2H_5	4-CF_3	**1/4**	0/4	0/4	0/4
26	C_2H_5	3-CH_3	**4/4**	**4/4**	**3/4**	0/4
27	C_2H_5	3-OCH_3	**1/4**	0/4	0/4	0/4
28	C_2H_5	morpholine	0/4	0/4	0/4	0/4
30	CH_3	3-CF_3	**1/4**	**2/4**	**4/4**	**4/4**

Ratios where at least one animal was protected have been highlighted in bold for easier data interpretation.
[a] Data indicate: number of mice protected / number of mice tested. The animals were examined at four pretreatment times–0.25, 0.5, 1 and 2 h.

Table 2. Anticonvulsant activity–MES test in mice *i.p.* (dose of 100 mg/kg)

compd	R_1	R_2	Pretreatment times[a]			
			0.25 h	0.5 h	1 h	2 h
3	CH_3	H	**2/4**	0/4	0/4	0/4
5	CH_3	3-Cl	**2/4**	**3/4**	**2/4**	0/4
6	CH_3	4-Cl	**2/4**	**1/4**	0/4	0/4
7	CH_3	2-F	**3/4**	0/4	0/4	0/4
8	CH_3	3-F	**3/4**	0/4	0/4	0/4
9	CH_3	4-F	**3/4**	0/4	0/4	0/4
10	CH_3	2-CF_3	**4/4**	**1/4**	**1/4**	0/4
11	CH_3	3-CF_3	**2/4**	**3/4**	**2/4**	0/4
13	CH_3	3-CH_3	0/4	0/4	0/4	0/4
14	CH_3	3-OCH_3	**1/4**	0/4	0/4	0/4
16	C_2H_5	H	**1/4**	**1/4**	0/4	0/4
18	C_2H_5	3-Cl	**2/4**	**2/4**	0/4	0/4
20	C_2H_5	2-F	0/4	0/4	0/4	0/4
21	C_2H_5	3-F	0/4	0/4	0/4	0/4
24	C_2H_5	3-CF_3	**3/4**	**2/4**	0/4	0/4
26	C_2H_5	3-CH_3	**2/4**	0/4	0/4	0/4
30	CH_3	3-CF_3	**4/4**	**3/4**	**2/4**	0/4

Ratios where at least one animal was protected have been highlighted in bold for easier data interpretation.
[a] Data indicate: number of mice protected / number of mice tested. The animals were examined at four pretreatment times–0.25, 0.5, 1, and 2 h.

Table 3. Anticonvulsant activity–PTZ test in mice *i.p.* (dose of 300 mg/kg)

compd	R_1	R_2	Pretreatment times[a]			
			0.25 h	0.5 h	1 h	2 h
5	CH_3	3-Cl	**3/4**	**3/4**	**3/4**	**3/4**
10	CH_3	2-CF_3	**4/4**	**4/4**	**3/4**	**2/4**
11	CH_3	3-CF_3	**4/4**	**4/4**	**4/4**	**4/4**
24	C_2H_5	3-CF_3	**4/4**	**3/4**	**3/4**	**3/4**
30	CH_3	3-CF_3	**4/4**	**4/4**	**3/4**	**3/4**

Ratios where at least one animal was protected have been highlighted in bold for easier data interpretation.
[a] Data indicate: number of mice protected / number of mice tested. The animals were examined at four pretreatment times–0.25, 0.5, 1, and 2 h.

Table 4. Anticonvulsant activity–PTZ test in mice *i.p.* (dose of 100 mg/kg)

compd	R_1	R_2	Pretreatment times[a]			
			0.25 h	0.5 h	1 h	2 h
5	CH_3	3-Cl	**2/4**	**3/4**	**2/4**	**1/4**
10	CH_3	2-CF_3	**4/4**	**3/4**	**1/4**	0/4
11	CH_3	3-CF_3	**2/4**	**4/4**	**3/4**	**3/4**
24	C_2H_5	3-CF_3	**2/4**	**3/4**	**1/4**	**1/4**
30	CH_3	3-CF_3	**2/4**	**2/4**	**2/4**	**2/4**

Ratios where at least one animal was protected have been highlighted in bold for easier data interpretation.
[a] Data indicate: number of mice protected / number of mice tested. The animals were examined at four pretreatment times–0.25, 0.5, 1, and 2 h.

Table 5. Anticonvulsant activity–6 Hz test in mice *i.p.* (dose of 100 mg/kg)

compd	R_1	R_2	Pretreatment times[a]			
			0.25 h	0.5 h	1 h	2 h
5	CH_3	3-Cl	**4/4**	**4/4**	**3/4**	**1/4**
10	CH_3	2-CF_3	**4/4**	**1/4**	0/4	0/4
11	CH_3	3-CF_3	**3/4**	**4/4**	**4/4**	**4/4**
24	C_2H_5	3-CF_3	**4/4**	**4/4**	**3/4**	**2/4**
30	CH_3	3-CF_3	**4/4**	**4/4**	**3/4**	**1/4**

Ratios where at least one animal was protected have been highlighted in bold for easier data interpretation.
[a] Data indicate: number of mice protected / number of mice tested. The animals were examined at four pretreatment times–0.25, 0.5, 1, and 2 h.

Table 6. The quantitative pharmacological parameters ED_{50}, TD_{50} and PI values in mice *i.p.*

compd	TPE (h)[a]	ED_{50} MES (mg/kg)[b]	ED_{50} PTZ (mg/kg)[c]	ED_{50} 6 Hz (mg/kg)[d]	TD_{50} (mg/kg)[e]	PI (TD_{50}/ED_{50})[f]
3	0.25	164.5 (140.9–192.1)	-	-	-	-
5	0.5	119.2 (100.9–140.9)	69.6 (54.9–88.2)	50.4 (33.8–72.9)	201.5 (189.7–212.5)	1.69 (MES) 2.90 (PTZ) 4.00 (6 Hz)
7	0.25	151.2 (114.0–200.4)	-	-	-	-
8	0.25	137.2 (119.2–172.4)	-	-	-	-
9	0.25	129.8 (100.1–168.2)	-	-	-	-
10	0.25	75.5 (65.3–87.02)	87.1 (79.9–95.0)	74.2 (55.6–98.9)	149.0 (127.6–173.9)	1.97 (MES) 1.71 (PTZ) 2.01 (6 Hz)
11	0.5	88.4 (73.2–106.8)	59.9 (52.5–68.3)	21.0 (12.4–35.7)	>1500	>16.97 (MES) >25.04 (PTZ) >71.43 (6 Hz)
11	1.0	85.1 (75.0–96.5)	88.7 (80.1–93.5)	35.0 (21.7–56.5)	823.6 (630.1–1076.5)	9.68 (MES) 9.28 (PTZ) 23.53 (6 Hz)
16	0.25	148.8 (125.0–177.2)	-	-	-	-
18	0.5	137.7 (107.4–176.7)	-	-	-	-
24	0.25	58.9 (51.8–67.0)	83.2 (75.0–89.6)	19.9 (11.7–33.9)	199.2 (169.7–233.7)	3.38 (MES) 2.39 (PTZ) 10.00 (6 Hz)
24	0.5	91.7 (75.0–112.1)	78.6 (64.3–96.0)	42.3 (36.5-48.9)	172.7 (153.1–195.0)	1.88 (MES) 2.19 (PTZ) 4.08 (6 Hz)
30	0.25	58.1 (52.7–63.9)	>120	57.6 (50.8–65.4)	202.7 (187.8–218.7)	3.49 (MES) 3.52 (6 Hz)
30	0.5	85.1 (75.0–96.5)	>120	74.7 (57.3–97.3)	197.3 (167.4–232.6)	2.32 (MES) 2.64 (6 Hz)
ETX[g]	0.25	>500	147.8 (130.7–167.3)	>200	722.1 (647.0–805.8)	4.89 (PTZ)
LCS[g]	0.5	9.4 (8.1–10.7)	>500	6.4 (3.5–11.5)	33.7 (28.8–38.7)	3.60 (MES) 5.26 (6 Hz)
LEV[g]	1.0	>500	>500	14.8 (11.2–18.4)	>500	>33.78 (6 Hz)
VPA[g]	0.5	216.9 (207.5–226.3)	239.4 (209.2–274.1)	130.1 (116.3–143.9)	372.9 (356.0–389.8)	1.72 (MES) 1.56 (PTZ) 2.87 (6 Hz)

Values in parentheses are 95% confidence intervals determined by probit analysis.[49]
[a] Time to peak effect.
[b] ED_{50} (MES-maximal electroshock seizure test).
[c] ED_{50} (*sc*PTZ-pentylenetetrazole seizure test).
[d] ED_{50} (6 Hz-psychomotor seizure test).
[e] TD_{50} (NT-acute neurological toxicity determined in the chimney test).
[f] Protective index (TD_{50}/ED_{50}).
[g] Reference AEDs: Ethosuximide (ETX), Lacosamide (LCS), Levetiracetam (LEV), and Valproic acid (VPA) tested in the same conditions. TPEs for model AEDs taken from literature.[50]
A dash indicates-not tested.

Table 7. *In vitro* Na^+ channel (site 2) binding assays

compd	Concentration [μM]	% Inhibition of control specific binding[a]
11	1	3.5
	10	12.2
	100	37.4
	500	62.3
24	1	8.4
	10	9.5
	100	8.8
	500	69.5
30	1	5.7
	10	14.6
	100	38.7
	500	77.6
CBZ[b]	1	2.7
	10	4.6
	100	17.4
	500	33.6

[a] Compounds were each evaluated in synaptoneurosomal preparations from rat cerebral cortex as inhibitors of the specific binding of [^{3}H]BTX to the voltage-sensitive sodium channel. 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] Carbamazepine (CBZ), reference AED tested in the same conditions.

Table 8. *In vitro* Ca^{2+} channels binding assays

compd	Concentration [μM]	% Inhibition of control specific binding[a]	
		N-type Ca^{2+} (antagonist radioligand)	L-type Ca^{2+} (dihydropyridine site, antagonist radioligand)
11	100	13.9	64.2
30*	200	3.2	36.2
TPM[b]	100	3.1	7.9

[a] Compounds were each evaluated in synaptoneurosomal preparations from rat cerebral cortex as inhibitors of the specific binding of [^{125}I]ω-conotoxin GVIA for N-type Ca^{2+} channel or [^{3}H]nitrendipine for L-type Ca^{2+} channel. 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] Topiramate (TPM), reference AED tested in the same conditions.

*Inactive at concentration of 100 μM.

Figure and Scheme captions:

Figure 1. The main modifications and the general structure of compounds designed

Figure 2. Activity of DX (standard) and compound **11** against HEK-293 cell line

Figure 3. The plot of MetaSite predictions for sites of metabolism of compound **11**

Figure 4. **A**–The UPLC spectrum after 2 h reaction of **11** with HLMs, **B**–MS spectra of compound **11** and its metabolites in the total ion chromatogram

Figure 5. MS/MS spectra and ion fragments analysis of compound's **11** metabolites in the total ion chromatogram

Figure 6. The effect of compound **11** and ketoconazole on CYP3A4 activity

Scheme 1. Synthesis of intermediates **1**, **2** and target compounds **3**–**28**

Scheme 2. Synthesis of intermediate **29** and target compound **30**

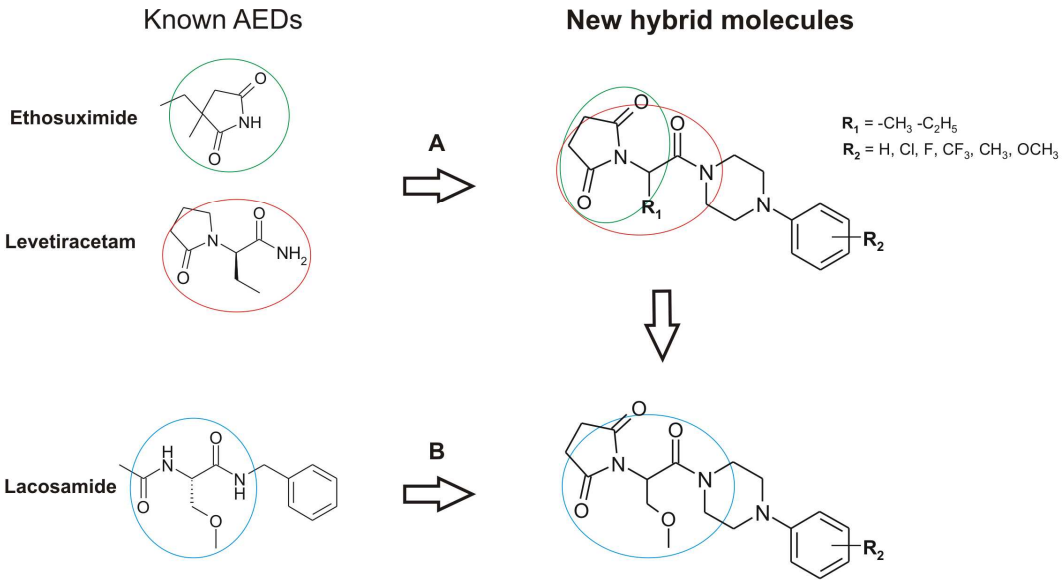

Known AEDs
New hybrid molecules
Ethosuximide
Levetiracetam
Lacosamide
A
B
R1 = -CH3 -C2H5
R2 = H, Cl, F, CF3, CH3, OCH3

Figure 1.

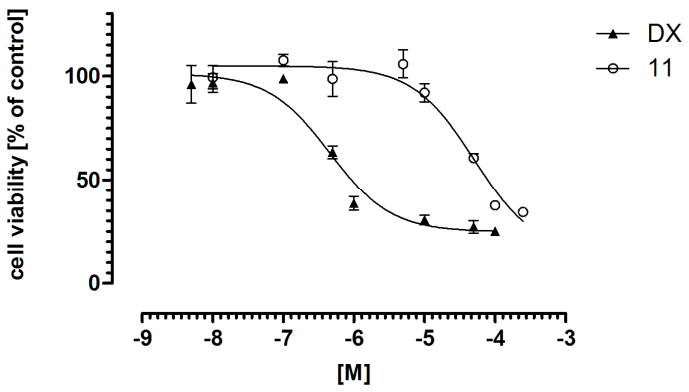


Figure 2.

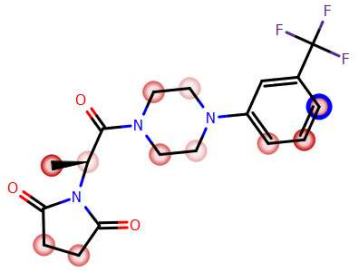


Figure 3.

A

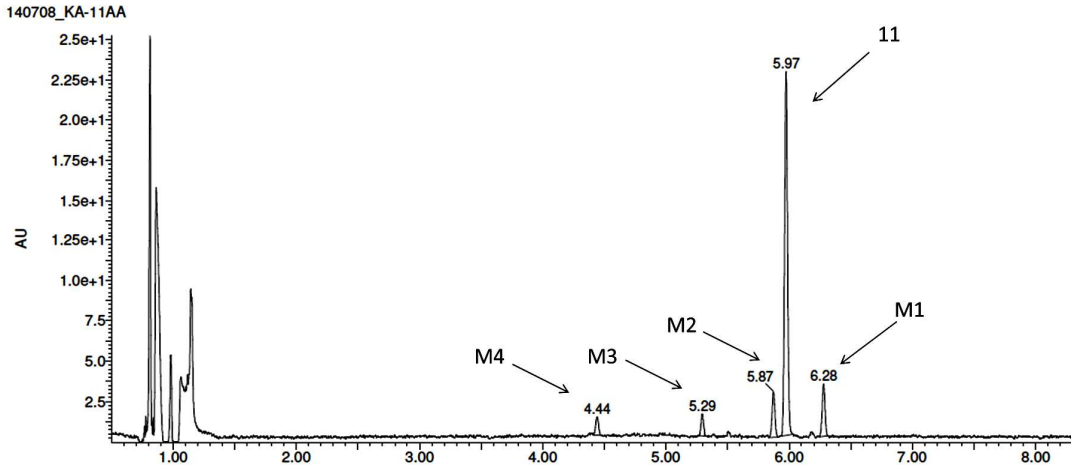

140708_KA-11AA
AU
2.5e+1
2.25e+1
2.0e+1
1.75e+1
1.5e+1
1.25e+1
1.0e+1
7.5
5.0
2.5
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
11
5.97
M1
6.28
M2
5.87
M3
5.29
M4
4.44

Figure 4 A.

B

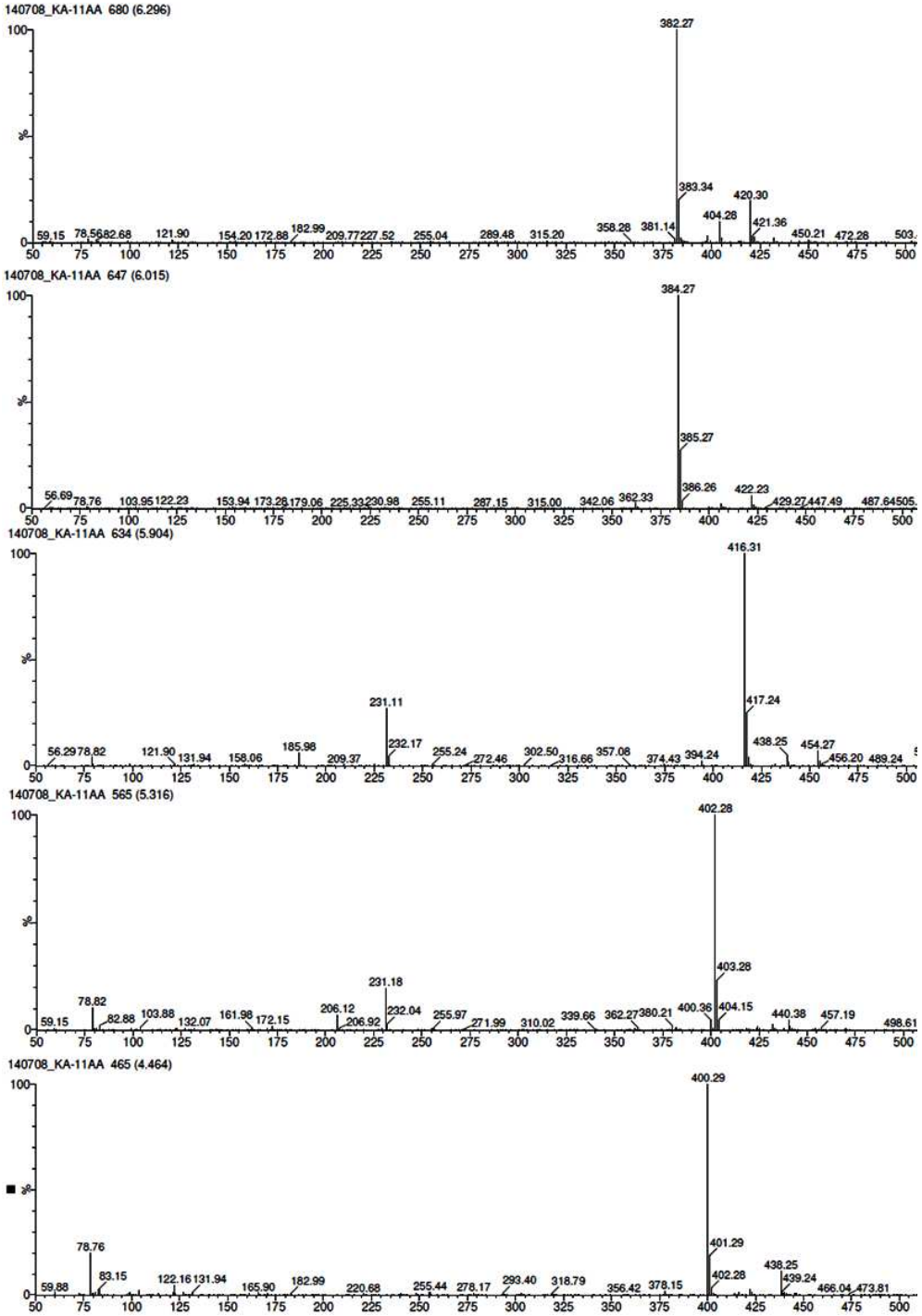

140708_KA-11AA 680 (6.296)
382.27
383.34
420.30
404.26
421.36
140708_KA-11AA 647 (6.015)
384.27
385.27
386.26
140708_KA-11AA 634 (5.904)
416.31
417.24
231.11
232.17
140708_KA-11AA 565 (5.316)
402.28
403.28
231.18
78.82
140708_KA-11AA 465 (4.464)
400.29
401.29
78.76
438.25

Figure 4 B.

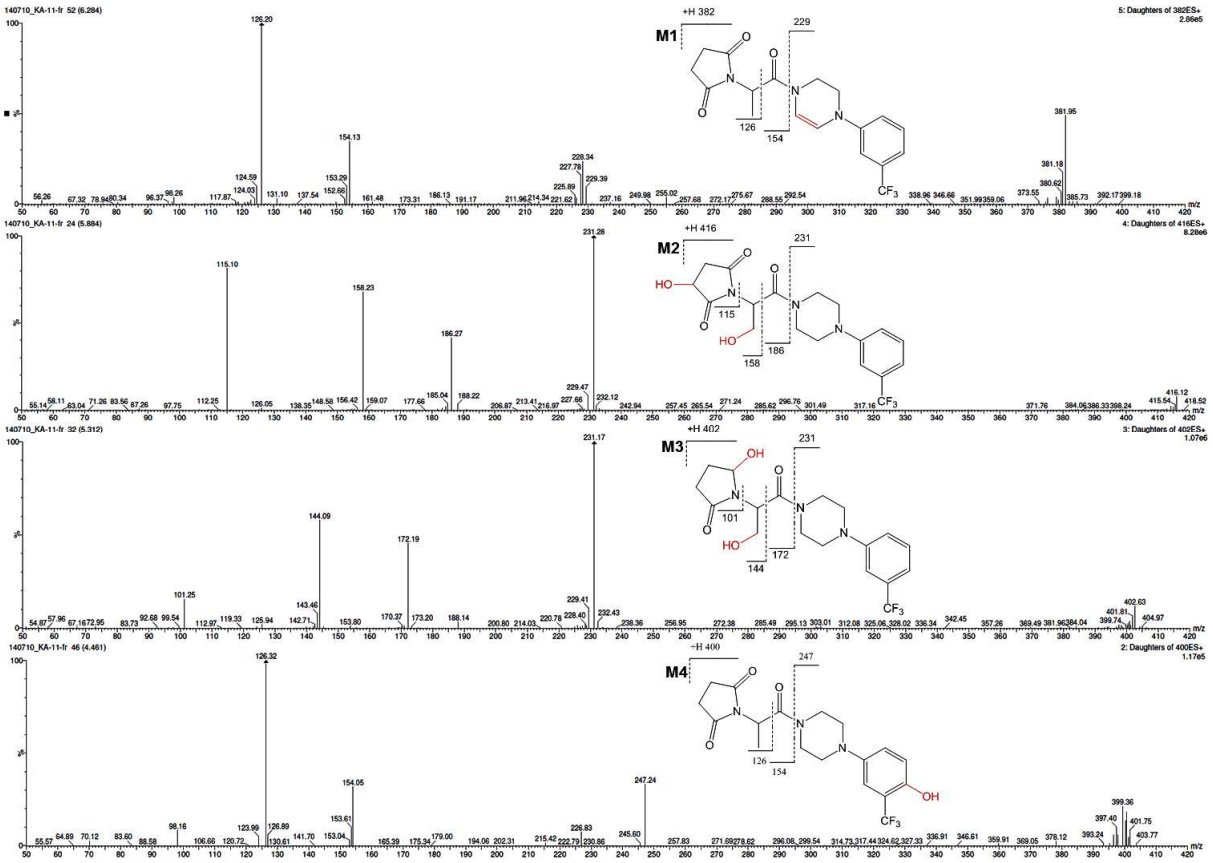


Figure 5.

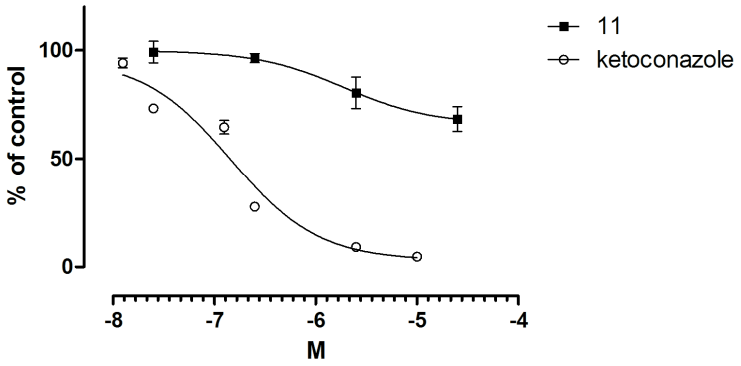
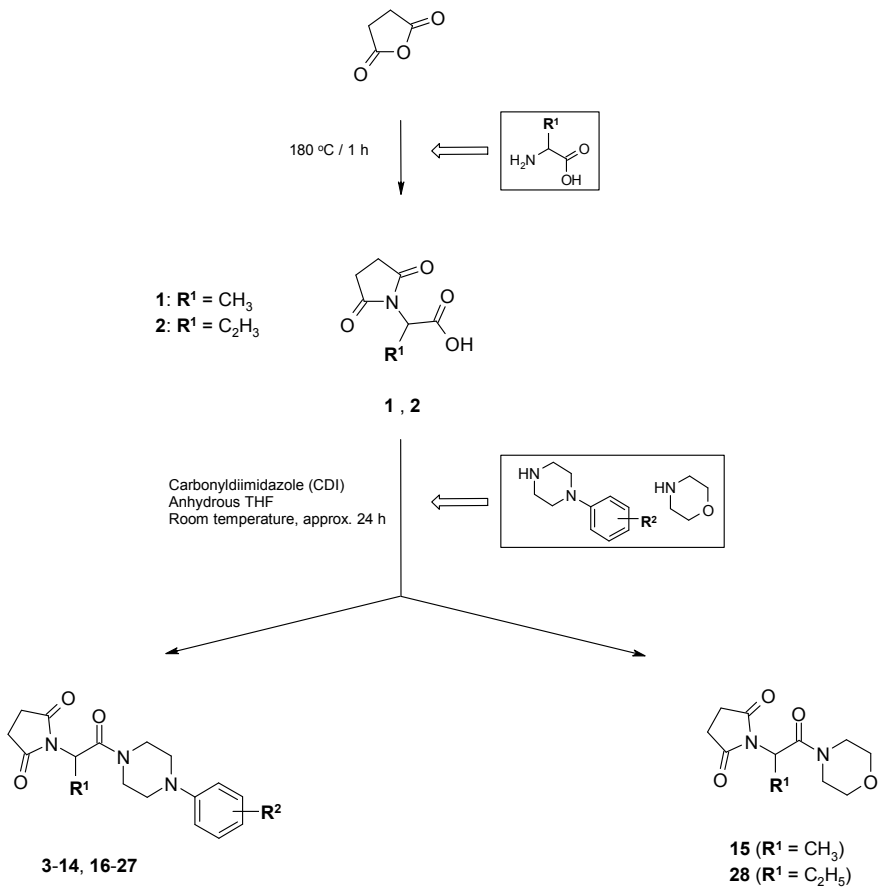


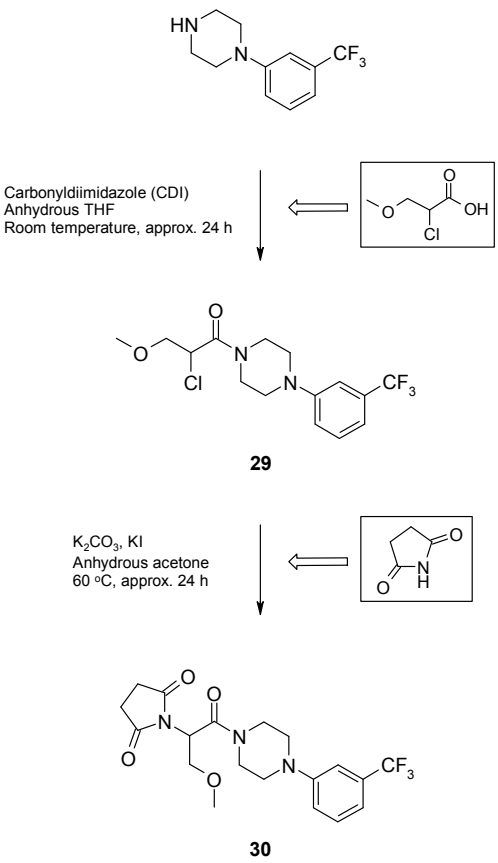
Figure 6.



3: **R¹** = CH_3, **R²** = H
4: **R¹** = CH_3, **R²** = 2-Cl
5: **R¹** = CH_3, **R²** = 3-Cl
6: **R¹** = CH_3, **R²** = 4-Cl
7: **R¹** = CH_3, **R²** = 2-F
8: **R¹** = CH_3, **R²** = 3-F
9: **R¹** = CH_3, **R²** = 4-F
10: **R¹** = CH_3, **R²** = 2-CF_3
11: **R¹** = CH_3, **R²** = 3-CF_3
12: **R¹** = CH_3, **R²** = 4-CF_3
13: **R¹** = CH_3, **R²** = 3-CH_3
14: **R¹** = CH_3, **R²** = 3-OCH_3

16: **R¹** = C_2H_5, **R²** = H
17: **R¹** = C_2H_5, **R²** = 2-Cl
18: **R¹** = C_2H_5, **R²** = 3-Cl
19: **R¹** = C_2H_5, **R²** = 4-Cl
20: **R¹** = C_2H_5, **R²** = 2-F
21: **R¹** = C_2H_5, **R²** = 3-F
22: **R¹** = C_2H_5, **R²** = 4-F
23: **R¹** = C_2H_5, **R²** = 2-CF_3
24: **R¹** = C_2H_5, **R²** = 3-CF_3
25: **R¹** = C_2H_5, **R²** = 4-CF_3
26: **R¹** = C_2H_5, **R²** = 3-CH_3
27: **R¹** = C_2H_5, **R²** = 3-OCH_3

Scheme 1.


HN
N
CF3
Carbonyldiimidazole (CDI)
Anhydrous THF
Room temperature, approx. 24 h
O
OH
Cl
O
29
K2CO3, KI
Anhydrous acetone
60 °C, approx. 24 h
O
N
H
O
30

Scheme 2.

Table of Contents graphic