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New hybrid molecules with anticonvulsant and antinociceptive activity derived from 3-methyl- or 3,3-dimethyl-1-[1-oxo-1-(4-phenylpiperazin-1-yl)propan-2-yl]pyrrolidine-2,5-diones

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ABSTRACT

The purpose of this study was to synthetize the focused library of 34 new piperazinamides of 3-methyl- and 3,3-dimethyl-(2,5-dioxopyrrolidin-1-yl)propanoic or butanoic acids as potential new hybrid anticonvulsants. These hybrid molecules join the chemical fragments of well-known antiepileptic drugs (AEDs) such as ethosuximide, levetiracetam, and lacosamide. Compounds **5**–**38** were prepared in a coupling reaction of the 3-methyl- or 3,3-dimethyl-2-(2,5-dioxopyrrolidin-1-yl)propanoic (**1**, **2**) or butanoic acids (**3**, **4**) with the appropriately substituted secondary amines in the presence of the *N*,*N*-carbonyldiimidazole reagent. The initial anticonvulsant screening was performed in mice (ip) 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. The broad spectra of activity across the preclinical seizure models in mice ip displayed compounds **7**, **15**, and **36**. The most favorable anticonvulsant properties demonstrated **15** (ED_{50} MES = 74.8 mg/kg, ED_{50} *sc*PTZ = 51.6 mg/kg, ED_{50} 6 Hz = 16.8 mg/kg) which showed TD_{50} = 213.3 mg/kg in the chimney test that yielded satisfying protective indexes (PI MES = 2.85, PI *sc*PTZ = 4.13, PI 6 Hz = 12.70) at time point of 0.5 h. As a result, compound **15** displayed comparable or better safety profile than clinically relevant AEDs: ethosuximide, lacosamide or valproic acid. In the in vitro assays compound **15** was observed as relatively effective binder to the neuronal voltage-sensitive sodium and L-type calcium channels. Beyond the anticonvulsant properties, 6 compounds diminished the pain responses in the formalin model of tonic pain in mice.

1. Introduction

Epilepsy affects up to 1% of the world's population, making it the second most common neurological disorder after stroke. The latest proposal by the International League against Epilepsy (ILAE) defines epilepsy as the occurrence of at least one seizure with an enduring alteration in the brain structure or function.[1] Despite the therapeutic arsenal of old and new antiepileptic drugs (AEDs), approximately 30% of patients with epilepsy still suffer from seizures.[2] Data collected from eight of the biggest markets show that therapy-resistant epilepsy affects about 1.8 million people.[3] It should be emphasized that uncontrolled seizures are associated with increased morbidity and mortality, possessing a large economic burden on individuals and society. Moreover, the current data show that the prevalence increases with the age, and this fact becomes more and more serious due to demographic changes related with the aging of societies especially in industrialized countries. The patient compliance is also limited by adverse side effects most notably related to CNS exposure like diminished attention, executive function, intelligence, language skills, memory and processing speed.[4] Thus, there remains a substantial need for the development of more efficacious AEDs especially for patients with refractory seizures.[5]

The most successful for the design of new anticonvulsants is ligand-based pharmacophore approach.[6] It relies on the use of existing biological data for old and new drugs or other anticonvulsant active compounds. This method is applied mainly for

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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 maternal 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).

Taking into consideration the aforementioned facts, in the current studies, we aimed to obtain anticonvulsants with broad spectrum of activity in animal models of epilepsy, namely 'classical', such as maximal electroshock seizure (MES) and subcutaneous pentylenetetrazole (*sc*PTZ) tests as well as in the alternative 6 Hz model of pharmacoresistant limbic seizures.[7] Compounds with the mentioned pharmacological profile in the in vivo studies may be recognized as 'wide-spectrum' AEDs effective in therapy of different epilepsies including human generalized tonic–clonic, absence and therapy-resistant one. Another advantage of such molecules may be lower risk of drug–drug interactions compared to 'drug cocktails' that are in many cases required for satisfactory control of seizures.

The multifunctional ligands approach is one of the modern strategies in drug discovery especially in relation to diseases with complex pathomechanism, such as Alzheimer's disease,[8] cancer,[9] or epilepsy.[10] This strategy assumes that a single chemical entity able to modulate biological targets simultaneously, overcoming problems related to the use of 'multicomponent drugs' like different bioavailabilities or pharmacokinetics, as well as the poor compliance in case of 'drug cocktails'. The most useful for the design and development of new multiple ligand (ML) drugs is knowledge-based approach also known as framework combination. According to this method two different molecules, each selective for different target or with given biological properties, are combined in a single chemical entity to provide both activities. The two compounds can be connected via a linker (which sometimes can be cleaved in vivo), their frameworks can be attached together in the fused MLs or in case of the merged MLs, the two frameworks are integrated and overlapped in a common structure. The latter approach is the most suitable for medicinal chemistry because it allows to gain the multi target profile in one, small and simple molecule, hopefully with favorable physicochemical properties.[11,12]

Bearing in mind the assumptions of MLs strategy, and with the aim of obtaining substances active in the maximal electroshock (MES), subcutaneous pentylenetetrazole (*sc*PTZ), and six-Hertz (6 Hz) models, we proposed the structure of hybrid compounds derived from pyrrolidine-2,5-dione core (Fig. 1). These compounds join on the one chemical template the structural fragments of well-known ADEs such as ethosuximide (pyrrolidine-2,5-dione derivative, active in the *sc*PTZ seizures), levetiracetam (pyrrolidine-2-one derivative with the butanamide moiety, effective in the 6 Hz test), and lacosmide (classified as functionalized amino acid, active in the MES and 6 Hz seizures). It should be stressed that each of the mentioned drugs possesses different indications and various mechanisms of pharmacodynamic activity.[13–18] Furthermore, apart from the structural fragments of aforementioned AEDs, we have introduced the different phenylpiperazines which presence in the structure was crucial for the activity in the MES seizures among structurally diversified pyrrolidin-2,5-diones described previously.[19,20]

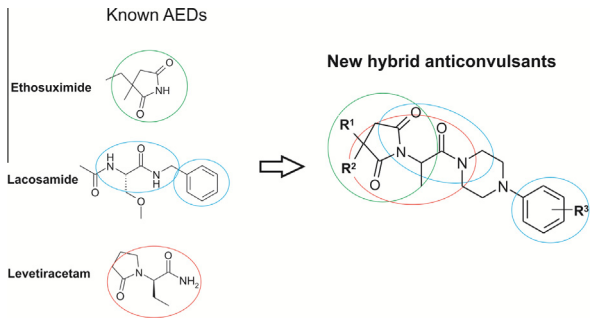

Figure 1. The general structure of target compounds.

Antiepileptic drugs are increasingly used for the treatment of neurological and psychiatric disorders. In neurology, they are used primarily for the treatment of epilepsy, but they also play an important role in the therapy of migraine and exclusively neuropathic pain. Moreover, it is believed that AEDs are likely to be effective in the treatment of withdrawal syndromes, schizophrenia and schizoaffective illness, personality disorders, anxiety disorders, eating disorders, or post-traumatic stress.[21] Considering the important role of AEDs in the treatment of neuropathic pain, studies on the search for new AEDs should also consider the evaluation of their usefulness in the treatment of this type of neurological disorder. Thus, for chosen anticonvulsant active compounds their antinociceptive activity has been studied in the formalin test in mice persistent pain model.

2. Results and discussion

2.1. Chemistry

Compounds **5**–**38** were synthesized in a two-step reaction according to Scheme 1. First, the condensation reaction of commercially purchased 3-methyl- or 3,3-dimethylsuccinic acids, with DL-α-alanine or DL-2-aminobutyric acid, yielded corresponding intermediates **1**–**4**. In the next step, **1**–**4** was converted to **5**–**38** by coupling with the appropriate phenylpiperazine derivative or 4-methylpiperazine in the presence of *N*,*N*-carbonyldiimidazole (CDI), which is a commonly used reagent for the synthesis of amides from carboxylic acids and amines through the acyl imidazole intermediate.[22] 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 **5**–**38** were obtained with yields ranging between 67% and 83%. All compounds were prepared as racemic mixtures. The final substances were fully characterized by elemental analyses (C, H, N) and ^{1}H NMR, ^{13}C NMR, ^{19}F NMR, and LC/MS spectra (details are shown in Section 4; see also Supplementary materials).

2.2. Anticonvulsant activity

Empirical screening has successfully led to the identification of many clinically relevant AEDs. Thus, the anticonvulsant activity of final molecules **5**–**38** was determined using the MES test, a mechanism-independent animal seizure model which enables the 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.[23] 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.[24] Compounds **5**–**38** were administered to mice via the intraperitoneal (ip) route at the fixed dose of 300 mg/kg. An observation was carried out at four pretreatment time points, namely, 0.25, 0.5, 1, and 2 h. The method applied herein allowed to determine the percentage of animals (in a group consisting of four mice), which were protected against electrically induced seizures and to estimate the time-course of anticonvulsant