Bioorganic & Medicinal Chemistry Letters 26 (2016) 2147–2151

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and evaluation of anticonvulsant properties of new *N*-Mannich bases derived from 3-(1-phenylethyl)- and 3-benzyl-pyrrolidine-2,5-dione

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ARTICLE INFO

Article history:
Received 29 January 2016
Revised 18 March 2016
Accepted 20 March 2016
Available online 21 March 2016

Keywords:
Pyrrolidine-2,5-dione
Anticonvulsant activity
In vivo studies

ABSTRACT

Two series of new derivatives of pyrrolidine-2,5-dione were synthesized and evaluated for their anticonvulsant properties. Initial screening for their anticonvulsant properties was performed in mice after intraperitoneal administration, using the maximal electroshock (MES), subcutaneous pentylenetetrazole (*sc*PTZ) and 6-Hz seizure tests. Quantitative pharmacological research revealed that the highest level of protection was demonstrated by compound *N*-[{4-methylpiperazin-1-yl}-methyl]-3-(1-phenylethyl)-pyrrolidine-2,5-dione monohydrochloride (**22**) which was effective both in the *sc*PTZ test (ED_{50} = 39 mg/kg) and in the 6-Hz test (ED_{50} = 36 mg/kg). This molecule showed higher potency than reference antiepileptic drugs such as ethosuximide, lacosamide and valproic acid. With the aim of explaining the possible mechanism of action of the selected molecule, its influence on sodium and calcium channels as well as NMDA and $GABA_A$ receptors binding properties were evaluated in vitro.

Epilepsy is a chronic disorder of the brain that affects people of all ages. Approximately 50 million people worldwide have epilepsy, which makes it one of the most common neurological diseases globally.[1] Epilepsy is defined as a discrete clinical event arising from transient, hypersynchronous, and abnormal neuronal behavior.[2,3] In spite of the fact that new generation antiseizure drugs have been produced, 30% of people with epilepsy are inadequately controlled by currently available medications.[4] Besides, anticonvulsant drugs presently on offer can cause serious side effects such as diminished attention span, impaired memory function, and information processing speed.[5] Consequently, there is the need for more efficient and less toxic antiepileptic drugs.

Incomplete information on the pathogenesis of human epilepsy and the complex mechanism of action of the majority of AEDs make it difficult to use rational methodologies in the search for new antiepileptic drugs. Therefore, the most important strategy to obtain new anticonvulsants is the ligand-based approach that utilizes existing biological data from old and new drugs, other historical compounds or different pharmacophore models established through the analysis of the structural characteristics of clinically effective AEDs as well as other anticonvulsant active compounds. This approach resulted in the successful discovery of several third-generation AEDs as well as compounds currently undergoing clinical trials.[6]

A lot of effort has been made in recent decades to identify the structural features essential for anticonvulsant activity. As a result, it has been established that a nitrogen heteroatomic system, usually cyclic imide, with at least one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system contributes significantly to anticonvulsant activity.[7,8] This aforementioned pattern is noticeable in the structures of first generation AEDs such as phenytoin and ethosuximide, and also in newer drugs like levetiracetam (Fig. 1). Additionally, our previous research enabled us to identify that pyrrolidine-2,5-dione differently substituted at position-1 and -3 is an interesting target for anticonvulsant activity. The structure–activity relationship (SAR) analysis has provided data indicating that the most promising were *N*-Mannich bases with aromatic substituents at position-3 and the phenylpiperazine moiety with an electron-withdrawing group at position-1 of the imide ring (Fig. 1).[9–13]

In the view of above-mentioned facts, and as a continuation of systematic SAR studies among variously substituted succinimides, the present study describes a library of 40 new *N*-Mannich which are the result of the following modifications. First of all, 1-phenylethyl or benzyl substituents were introduced at position-3 of the imide ring, while appropriately substituted 4-phenylpiperazines were attached to the imide core at position-1. Thereafter, the phe-

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http://dx.doi.org/10.1016/j.bmcl.2016.03.075
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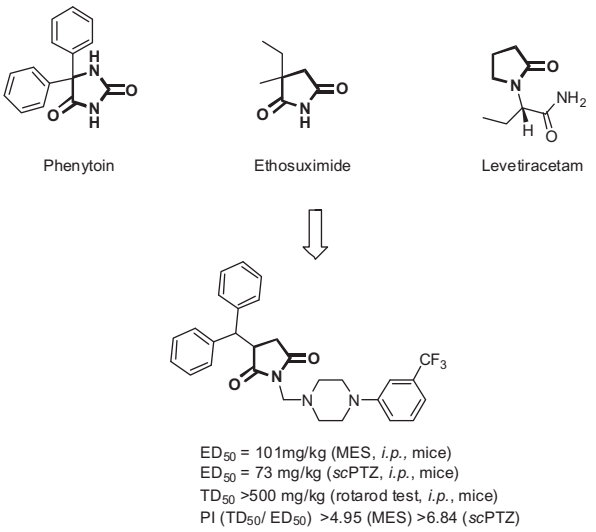

Figure 1. Structures of model AEDs based on five-membered heterocyclic rings and representative structure of active compound obtained in the previous studies.

nyl ring of 4-substituted piperazine was replaced with a benzyl, pyrimidinyl, hydroxyethyl, methyl or cyclohexyl group as well as the phenylpiperazine fragment was replaced with benzylpiperidine or morpholine (Fig. 2). The proposed modifications enabled the effect on anticonvulsant activity of the introduction of the above-mentioned substituents to be established both at position-1 and -3. Furthermore, the aim of these studies was the identification of derivatives with a broad-spectrum of anticonvulsant activity, which means these compounds should be effective in the maximal electroshock (MES) test-model of tonic–clonic seizures in humans, in the subcutaneous pentylenetetrazole (*sc*PTZ) test-model of absence epilepsy and/or in the 6-Hz test-model of pharmacoresistant limbic seizures.[14–17] Moreover, attempts to evaluate the possible mechanism of action were also undertaken.

Compounds **5**–**44** were synthesized according to Scheme 1. The starting 2-(1-phenylethyl)- (**1**) and 2-benzyl- (**2**) succinic acids were prepared according to the method described by Miller and Long.[18] Cyclocondensation reaction of **1** or **2** with the 25% ammonia at 180 °C for 1.5 h yielded 3-(1-phenylethyl)- (**3**) and 3-benzyl- (**4**) pyrrolidine-2,5-diones. The final compounds **5**–**44** were synthesized in an aminoalkylation (Mannich-type) reaction from the appropriately substituted 3-(1-phenylethyl)- (**3**) or 3-benzyl-pyrrolidine-2,5-dione (**4**), formaldehyde and corresponding 4-substituted piperazines, benzylpiperidine or morpholine. The reaction was carried out in 96% ethanol at room temperature for ca. 12 h.

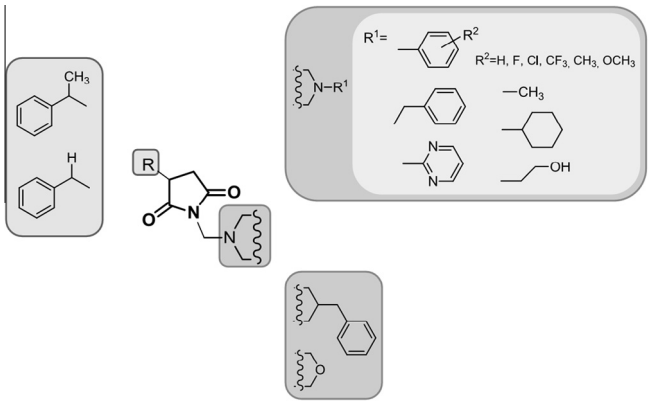

Figure 2. The proposed modifications.

The crude products were crystallized from 96% ethanol giving the final compounds in yields ranging from 40% to 90%. The final compounds were obtained as racemic mixtures. Except for compounds (**18**, **20**, **22**, **38**, **40** and **42**), which were isolated as hydrochloride salts, the other derivatives were obtained as free bases. The purities and homogeneity were assessed by thin-layer chromatography. The chemical structures were confirmed by both spectral (^{1}H NMR, ^{13}C NMR, ^{19}F NMR, LC/MS) and elemental (C, H, N) analyses (see Supplementary material).

Pre-clinical investigations into and development of new chemical agents with anticonvulsant properties are mainly based on the use of animal seizure models. It should be noticed that, in spite of significant advances that have been made in epilepsy research, the maximal electroshock test and subcutaneous pentylenetetrazole test are still recognized as the 'gold standards' in the early stages of the testing of new anticonvulsants.[14–17] Additionally, given the fact that pharmacoresistant epilepsy is a serious problem, the 6-Hz test was also used.

In the first step of pharmacological research the anticonvulsant activity of new compounds (**5**–**44**) was established in the MES and *sc*PTZ tests, after intraperitoneal (ip) injection into mice at doses of 30, 100 and 300 mg/kg. Observations were carried out at two different time intervals, namely 0.5 h and 4 h. Acute neurological toxicity was determined in the minimal motor impairment-rotarod screen (NT) in the same conditions. The in vivo results for **5**–**44** are shown in Table 1. Additionally, in order to identify molecules with a broad spectrum of anticonvulsant activity, for the selected compounds, which revealed activity in the MES and *sc*PTZ tests, the 6-Hz test was performed (Table 2). The experimental procedures used for in vivo studies are described in Supplementary material.

The obtained results of in vivo studies revealed diverse anticonvulsant activity for new derivatives with 1-phenylethyl- or benzyl-groups at position-3 of the imide ring. As shown in Table 1, twelve compounds, namely (**13**, **20**, **22**–**24**, **27**, **32**, **33**, **35**, **40**, **42** and **44**) exhibited anticonvulsant protection in the MES test at a dose of 300 mg/kg after 0.5 and/or 4 h. In terms of the *sc*PTZ test, sixteen compounds were effective. Molecules **16** and **22** revealed the highest protection, since they were active at a dose of 30 mg/kg after 0.5 h. Furthermore, compounds **21**, **28**, **32**, **35**, **36**, **38**, **39**, **42** and **44** revealed effectiveness at a dose of 100 mg/kg, whereas molecules **10**, **19**, **20**, **26** and **40** were efficient at a dose of 300 mg/kg. Compound **44** seems to be especially interesting, as it revealed activity at a dose of 100 mg/kg not only after 0.5 h, but also after 4 h.

In the rotarod test for acute neurological toxicity (NT) compounds **9**, **15**, **16**, **19**–**22**, **24**–**27**, **29**, **30**, **32**, **33**, **35**, **36**, **39**, **40** and **44** showed neurotoxicity at the maximum dose administered-300 mg/kg, while other derivatives did not cause motor impairment.

Subsequently, for selected compounds, namely **20**–**22**, **32**, **40**, **42** and **44** their activity in the 6-Hz test was evaluated. The most beneficial activity was revealed by molecules **20**–**22**, **40** and **44**, since they protected 100% of animals at a dose of 100 mg/kg after 0.5 h. Moreover, high activity at this time point was shown by compound **42** (active in 66% of animals), while compound **31** was inactive in this test (Table 2).

On the basis of the above preliminary screening data in mice, the most efficient molecules, namely **16**, **20**–**22**, **32**, **40**, **42** and **44**, were selected for quantification of their pharmacological parameters (ED_{50} and TD_{50}) in the *sc*PTZ and 6-Hz tests after ip administration in mice. The quantitative data were examined at a previously estimated time to peak effect (TPE). Results from these tests, along with the data for the standard drugs ethosuximide, lacosamide, valproic acid and levetiracetam, are shown in Table 3. It can be noticed that certain compounds (**16**, **21**, **22**, **32**, **42** and **44**)