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Anticonvulsant and antinociceptive activity of new amides derived from 3-phenyl-2,5-dioxo-pyrrolidine-1-yl-acetic acid in mice

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ABSTRACT

The aim of the present experiments was to examine the anticonvulsant and antinociceptive activity of five new amides derived from 3-phenyl-2,5-dioxo-pyrrolidine-1-yl-acetic acid in animal models of seizures and pain. The antiseizure activity was investigated in three acute models of seizures, namely, the maximal electroshock (MES), the subcutaneous pentylenetetrazole (*sc*PTZ), and 6 Hz psychomotor seizure tests in mice. The antinociceptive properties were estimated in the formalin model of tonic pain, and in the oxaliplatin-induced neuropathic pain model in mice. Considering drug safety evaluation, acute neurological toxicity was determined in the rotarod test. Three tested compounds (**3**, **4**, and **7**) displayed a broad spectrum of anticonvulsant activity and showed better protective indices than those obtained for MES/*sc*PTZ/6 Hz active reference drug – valproic acid. Furthermore, three compounds (**3**, **4**, and **6**) demonstrated a significant antinociceptive effect in the formalin test, as well as antiallodynic activity in the oxaliplatin-induced neuropathic pain model. Among the tested agents, compounds **3** and **4** displayed not only antiseizure properties, but also collateral prominent analgesic properties. The in vitro binding study indicated that the plausible mechanism of action of chosen compound (**4**) was the influence on neuronal voltage-sensitive sodium (site 2) and L-type calcium channels.

1. Introduction

Epilepsy is a chronic neurological disorder that is characterized by recurrent and unprovoked seizure episodes. Although numerous effective antiepileptic drugs are available in clinic, it is estimated that approximately 30% of epileptic patients are still improperly cured. Furthermore, adverse effects and poor tolerability of so-called first- and second-generation of anticonvulsant drugs necessitate the search for novel active molecules with more favourable anticonvulsant properties, improved efficacy and tolerability (Brodie et al., 2011; Löscher and Schmidt, 2011; Simonato et al., 2014).

Apart from epilepsy treatment anticonvulsant drugs are also extensively used as efficacious therapy of diverse non-epileptic conditions, including pain (neuropathic pain, migraine prophylaxis), neuromuscular disorders and psychiatric disorders (anxiety, bipolar affective disorder) (Mantegazza et al., 2010; Rogawski and Löscher, 2004). In particular, recent studies have documented beneficial effects of anticonvulsant drugs in ameliorating different types of neuropathic pain. Gabapentin and pregabalin have proven to be potent antiallodynic drugs in the treatment of diabetic neuropathic pain and post-herpetic neuralgia, whereas carbamazepine and oxcarbazepine are efficacious agents for the treatment of trigeminal neuralgia (Kukkar et al., 2013; Mendlik and Uritsky, 2015). However, evidence suggests that neuropathic pain is variable in its severity, as well as in response to cure. There have been reported the lack of satisfactory reduction of pain syndromes and/or severe adverse effects of currently available drugs, including analgesic adjuvants (i.e., certain antidepressant and antiepileptic medications, topical lidocaine, mexiletine, N-methyl-D-aspartate receptor antagonists, and topical capsaicin) (Dworkin et al., 2007). In view of the above mentioned reasons, there is a strong medical demand to search for novel, analgesically active chemical structures that present better analgesic efficacy and safety profile as compared to currently available drugs.

Therefore, as well as because of the increasing role of anticonvulsant drugs in the treatment of neuropathic pain, studies on the search for new anticonvulsant agents should also consider the evaluation of their usefulness in the treatment of this type of neurological disorder (Rogawski and Löscher, 2004).

Our previous research showed that some of the tested agents based on the core of pyrrolidino-2,5-dione revealed prominent

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anticonvulsant properties in 'classical' animal models of seizures, i.e., MES, *sc*PTZ, as well as in the 6 Hz model of pharmacoresistant limbic seizures and displayed distinctly better safety profile than clinically relevant anticonvulsant drugs (Kamiński et al., 2015a; Obniska et al., 2015a, 2015b; Rapacz et al., 2016; Rybka et al., 2016, 2015, 2014). Moreover, several compounds demonstrated analgesic activity in the formalin model of tonic pain in mice (Obniska et al., 2016, 2015a, 2015b; Rapacz et al., 2016).

Continuing our preclinical searching for new anticonvulsant drugs with collateral antinociceptive activity, in the present study, we have focused on a group of new amides derived from 3-phenyl-2,5-dioxo-pyrrolidine-1-yl-acetic acid. Herein, the evaluation of anticonvulsant and analgesic activity of five compounds, designated as **3**, **4**, **5**, **6**, and **7** is discussed. The antiseizure activity was assessed in three acute models of seizures, namely MES, *sc*PTZ, and 6 Hz tests. The antinociceptive properties were estimated in the formalin model of persistent pain, as well as in the oxaliplatin-induced neuropathic pain model in mice. Considering drug safety evaluation, which is important in the preclinical identification of new active substances, the acute neurological toxicity was determined in the rotarod test. To establish the plausible mechanism of anticonvulsant action for chosen compound, *in vitro* ion channel binding assays were also carried out.

2. Materials and methods

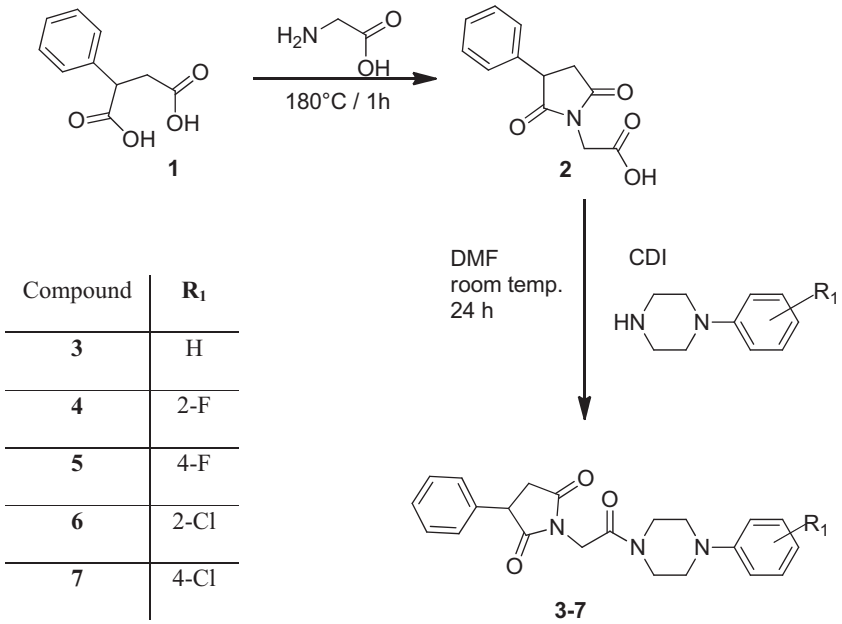
2.1. Animals

Male CD-1 mice weighing 18–24 g were used in the *in vivo* experiment (Obniska et al., 2015a, 2015b; Sałat et al., 2014). The animals were housed and fed in a laboratory kept at constant temperature of 20 ± 2 °C, under standard conditions (12:12 h light-dark cycle, standard pellet diet, tap water). The experimental groups consisted of four to ten animals (Laughlin et al., 2002; Obniska et al., 2015a, 2015b; Sałat et al., 2014), and all the animals were used only once. The experiments were performed between 8 AM and 3 PM. For the experiments the animals were selected in a random way and killed by cervical dislocation immediately after the assay. The experimental protocol was approved by the First Local Ethics Committee on Animal Testing at the Jagiellonian University in Kraków (No 100/2014), and was in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC).

2.2. Drugs and chemicals

The test compounds: **3** (1-[2-oxo-2-(4-phenylpiperazin-1-yl) ethyl]−3-phenylpyrrolidine-2,5-dione), **4** (1-{2-[4-(2-fluorophenyl)-piperazin-1-yl]−2-oxoethyl)−3-phenyl-pyrrolidine-2,5-dione), **5** (1-{2-[4-(4-fluorophenyl)-piperazin-1-yl]−2-oxoethyl}−3-phenyl-pyrrolidine-2,5-dione), **6** (1-{2-[4-(2-chlorophenyl)-piperazin-1-yl]−2-oxoethyl}−3-phenyl-pyrrolidine-2,5-dione) and **7** (1-{2-[4-(4-chlorophenyl)-piperazin-1-yl]−2-oxoethyl}−3-phenyl-pyrrolidine-2,5-dione) were synthesized at the Department of Medicinal Chemistry, Jagiellonian University, Medical College in Krakow (Scheme 1). For the in vivo studies the investigated compounds were suspended in a 0.5% aqueous solution of methylcellulose (Loba Chemie, Germany). Ethosuximide (Sigma-Aldrich, Germany) (Gören and Onat, 2007), lacosamide (Vimpat, UCB Pharma, Belgium) (Curia et al., 2009), levetiracetam (Sigma-Aldrich, Germany) (Dedeurwaerdere et al., 2006), oxaliplatin (Cayman Chemicals, USA) (Toyama et al., 2014), pentylenetetrazole (PTZ, Sigma-Aldrich, Germany) (Kupferberg, 2001), formaldehyde (POCh, Poland) (Porro and Cavazzuti, 1993), valproic acid (Sigma-Aldrich, Poland) (Nieoczym et al., 2013) were used. Ethosuximide, lacosamide, levetiracetam, PTZ, and valproic acid were dissolved in physiological saline solution. Formaldehyde was dissolved in distilled water. Oxaliplatin was prepared in a 5% aqueous solution of glucose. All drug solutions/suspensions were prepared freshly and administered intraperitoneally (i.p.) at a volume of 0.1 ml per 10 g body weight. Control animals were administered an equivalent volume of vehicle (methylcellulose) *via* the same route as the test compound. The anticonvulsant screening procedure was based upon the protocol used by the Anticonvulsant Screening Program (Stables and Kupferberg, 1997). In the initial anticonvulsant and neurotoxicity evaluations, the animals were

Synthetic protocol of the intermediate and target compounds **3-7**.



Compound	R_1
3	H
4	2-F
5	4-F
6	2-Cl
7	4-Cl

Scheme 1. Synthetic protocol of the intermediate and target compounds **3–7**.