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Design, synthesis and pharmacological evaluation of novel *N*-(2-(1, 1-dimethyl-5, 7-dioxo-4, 6-diazaspiro[2.4]heptan-6-yl)ethyl) sulfonamide derivatives as potential anticonvulsant agents

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ABSTRACT

A series of new *N*-(2-(1,1-dimethyl-5,7-dioxo-4,6-diazaspiro[2.4]heptan-6-yl)ethyl) sulfonamide derivatives (**8a**–**i**) and ethyl 2,2-dimethyl-1-(3-(2-(sulfonamido)ethyl)ureido) cyclopropanecarbox-ylate derivatives (**9a**–**i**) were designed, synthesized and evaluated for their anticonvulsant activities using maximal electroshock shock (MES) and subcutaneous pentylenetetrazole (*scPTZ*) seizure models in mice. *N*-(2-(1,1-dimethyl-5,7-dioxo-4,6-diazaspiro[2.4]heptan-6-yl)ethyl)-4-fluoroben- zenesulfonamide (**8f**) and *N*-(2-(1,1-dimethyl-5,7-dioxo-4,6-diazaspiro[2.4]heptan-6-yl)ethyl)-4- methylbenzenesulfonamide (**8e**) have shown promising anticonvulsant activities in MES model. The most active compound **8f** has shown the MES-induced seizures with ED_{50} value of 28.05 mg/kg and TD_{50} value of 561 mg/kg after intraperitoneal injection to mice, which provided compound **8f** with a protective index (TD_{50}/ED_{50}) of 20 in the MES test. Further, rotarod toxicity method was used to study the acute neurotoxicity profile of selected compounds.

1. Introduction

Epilepsy is a chronic brain disorder characterized by excessive temporary neuronal discharge and affects about 1% of the world's population [1]. A number of anti-epileptic drugs (AEDs) such as topiramate [2], lamotrigine [3], tiagabine [4], felbamate [5], vigabatrin [6], and zonisamide [7] have been introduced to treat epilepsy diseases. However, 20–30% of patients are failed to control seizures by current medications. Substantial increases in drug resistance and undesirable side effects such as neurotoxicity of current AEDs make it necessary to develop more efficacious anti-epileptic and anti-epileptogenic drug along with less side effects. Over last decade, anticonvulsant drug development has become one of the most active research areas in medicinal chemistry.

Hydantoins show various types of biological activities [8,9]. Phenytoin, a hydantion derivative has been known for anticonvulsant activity since 1938. It is still prescribed as one of most common drugs for treating grand mal. 5, 5-Cyclopropanespirohydantoins have also been synthesized and demonstrated to have good anticonvulsant activities with less toxicity than that of phenytoin [10–12]. Those interesting biological activities have drawn us more attention on the development of the novel 5, 5-cyclopropanespirohydantoin as the core structure of potential anticonvulsant drugs (Fig. 1). Acetazolamide (Fig. 1), an old AED, containing a sulfonamide group, has displayed excellent anticonvulsant activity [13]. It was found that *N*- (2, 2, 3, 3-tetramethylcyclopropanecarboxamide)-*p*-phenylsulfonamide (compound **I**) exhibits high potency and a wide protective index ($PI = TD_{50}/ED_{50}$) during the rat-MES seizure test [14]. Our research mainly focuses on the synthesis and biological studies of compound **II** containing a sulfone moiety [11]. As an extension, we set out to introduce a sulfonamide group to 5, 5- cyclopropanespirohydantoin, in order to enhance biological activity. Therefore, a series of compounds (type **I**–**IV**) are designed and synthesized as listed in Fig. 1. Unfortunately, type I and type II compounds are not stable enough (The synthetic route of type I and type II compounds was shown in the supporting information). Herein, type III and type IV compounds are synthesized and their pharmacological activities as potential anticonvulsant agents are

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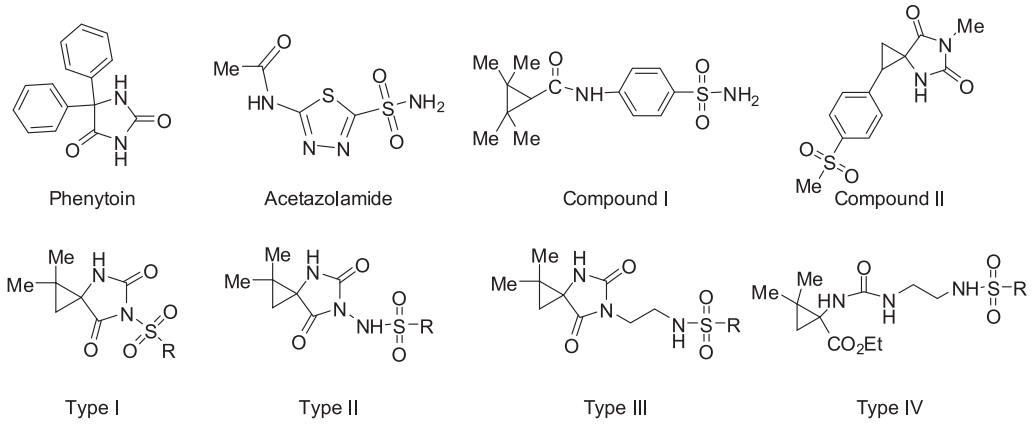

Fig. 1. Structure of designed compounds and model AEDs.

evaluated in this paper.

2. Results and discussion

2.1. Chemistry

The synthetic route of compounds **8a**–**i** and **9a**–**i** was shown in Scheme 1. Compound **2** was prepared by condensation of diethyl malonate with isobutyraldehyde in the presence of piperidine and acetic acid. The reaction of compound **2** with NBS and AIBN in chloroform afforded the desired bromo compound **3**. The resulting compound **3** underwent cyclopropane formation by Michael initiated ring closure (MIRC) reaction to afford compound **4**. Then monoester **5** was obtained after monohydrolysation in a 1 N NaOH/ethanol-water solution at room temperature for 12 h. It was then converted to corresponding acyl azide by using ethyl chloroformate with triethylamine followed by reaction with sodium azide in a one-pot synthesis. Compound **6** was successfully made by a Curtius reaction. Then it was treated with hydrazine in THF to provide compound **7**. The subsequent sulfonylation of compound **7** with a variety of different sulfonylating reagents gave the target compounds **8a**–**i**. Reactions between compound **6** and a variety of sulfonyl ethylenediamines resulted in the formation of target compounds **9a**–**i**.

The chemical structures of the titled compounds were elucidated on the basis of ^{1}H NMR, ^{13}C NMR and mass spectra. The detailed physical and analytical data are listed in Section 4. All of these compounds were prepared as racemic mixtures and no attempt was made to resolve the enantiomers.

2.2. Anticonvulsant activity

It is highly unlikely that any animal model will ever successfully predict the full therapeutic potential of an investigational AED due to its highly heterogenous nature of seizure disorders in humans and the complexity of the seizure phenotypes [15]. A number of animal models have been used in the search for more efficacious AEDs. For example, maximal electroshock (MES) and subcutaneous pentylenetetrazole (*scPTZ*) tests are reported to detect the effects on generalized tonic-clonic seizures and generalized absence seizures, respectively [16,17].

In the present study, maximal electroshock seizure (MES), subcutaneous pentylenetetrazole (*scPTZ*) were chosen in the early stages of testing because they are recognized as a gold standard [18]. Neurotoxicity is also determined in the minimal motor impairment-rotarod screening. All the tested compounds were administrated intraperitoneally (ip.) into the Kunming mice (18–22 g) using dose of 300, 100 and 30 mg/kg and the observations were taken at 0.5 h and 4.0 h after injection and the results are summarized in Table 1.

As shown in Table 1, compounds **8e** and **8f**, which showed protection at a dose of 30 mg/kg after 0.5 h, were found to be the most active in the mice ip. MES screen. It's worth noting that compound **8f** protected the tested mice from seizures at a dose of 30 mg/kg after 4 h while compound **8e** was active at the same time but at a higher dose of 100 mg/kg. The data suggest that compound **8f** has a long duration of anticonvulsant activity. Compounds **8b**, **8d**, **8h**, **8i**, **9b**, **9e**, **9f** and **9i** were active at 100 mg/kg after 0.5 h in MES test. Among those compounds, **8b** and **9f** kept active at the same dose after 4 h. In the *scPTZ* test, all of the compounds were ineffective to protect the tested mice except **8e** and **8f** which were active at a dose of 300 mg/kg after 0.5 h. Given $R_1 = R_2$, it is self-evident that *N*-(2-(1, 1-dimethyl-5, 7 -dioxo-4, 6-diazaspiro [2.4] heptan- 6-yl) ethyl) sulfonamide derivatives (**8a**–**i**) were more active than that of their ring-open derivatives (**9a**–**i**). For example, compound **8f** showed protection at a dose of 30 mg/kg following 0.5 h while compound **9f** exhibited activity at a higher dose of 100 mg/kg. Furthermore, introduction of methyl or fluorine group at *para* position of the phenyl ring gave the best anticonvulsant activity. However, the activity was decreased when nitro substituent was on phenyl ring. In the neurotoxicity test, compounds **8e, 8h** and **9d** showed neurotoxicity at the maximum dose. Compound **8d** was neurotoxic at a dose of 100 mg/kg after 0.5 h and it was also found to reveal neurotoxicity after 4 h at a dose of 300 mg/kg. The rest compounds did not cause any motor impairment at all.

Crossing the blood–brain barrier (BBB) is an important factor influencing anticonvulsant activity. It is believed that log P values between 1 and 2 is sufficient value for crossing BBB. The optimum compound show favorable anticonvulsant activity [21]. The Clog P of the tested compounds is summarized in Table 1. Compounds **8a** and **8c** with low ClogP values (−1.015 and −1.159 respectively) showed no anticonvulsant activity at the max dose (300 mg/kg). The most active compounds **8e** and **8f** showed optimal ClogP values of 1.273 and 1.2194 respectively.

In view of the performance of those tested compounds in initial evaluation, compounds **8e** and **8f** with the highest anticonvulsant potencies are selected for the quantification of TD_{50} and ED_{50} parameters. The estimated time of peak effect (TPE) is evaluated after administrated intraperitoneally (ip.) into mice. The results are listed in Table 2. It is seen that compounds **8e** and **8f** showed a higher protective index (PI = TD_{50}/ED_{50}) than that of standard drug phenytoin. All freshly synthesized cyclopropanespirohydantoins