Pharmacological Reports 68 (2016) 250–258

Contents lists available at ScienceDirect

Pharmacological Reports

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

Original research article

Pharmacological evaluation of novel 1-[4-(4-benzo[1,3]dioxol-5-ylmethyl-piperazin-1-yl)-phenyl]-3-phenyl-urea as potent anticonvulsant and antidepressant agent

Shikha Kumari [1], Chandra Bhushan Mishra [1], Manisha Tiwari *

Bio-Organic Chemistry Laboratory, Dr. B.R. Ambedkar Center for Biomedical Research, University of Delhi, New Delhi, India

ARTICLE INFO

Article history:
Received 4 June 2015
Received in revised form 10 August 2015
Accepted 21 August 2015
Available online 10 September 2015

Keywords:
Piperazine
Synthesis
Anticonvulsant
Antidepressant
Toxicity

ABSTRACT

Background: Earlier, we have identified a number of piperazine derivatives having good anticonvulsant activity *in vivo* and as a part of our ongoing search for potent anticonvulsant agent, we herein describes the synthesis of an aryl piperazine derivative "1-[4-(4-benzo[1,3]dioxol-5-ylmethyl-piperazin-1-yl)-phenyl]-3-phenyl-urea" (BPPU). The anticonvulsant and antidepressant activity of BPPU was checked in various *in vivo* models.

Methods: Anticonvulsant activity was assessed in maximal electroshock test (MES) and subcutaneous pentylenetetrazole (scPTZ) induced seizure tests. Moreover, plausible mechanistic studies were also performed by using several chemical induced seizure models. The antidepressant activity of BPPU was checked in forced swim test (FST) and tail suspension test (TST) in mice. Drug safety profile was studied in sub-acute toxicity rat model at a dose of 100 mg/kg, per oral for 14 days.

Results: BPPU exhibited excellent protection against seizures induced by MES and scPTZ in mice as well as rats. In pilocarpine induced model of status epilepticus (SE), BPPU demonstrated 50% protection at a dose of 100 mg/kg in rats. BPPU also successfully inhibited seizures induced by 3-mercaptopropionic acid (3-MPA) and thiosemicarbazide (TSC) in mice thus, suggested that BPPU might influence GABA-ergic neurotransmission in the brain. Moreover, BPPU showed good antidepressant activity and did not exhibit any significant toxicity.

Conclusion: BPPU displayed broad spectrum of anticonvulsant activity in several seizure models along with satisfactory antidepressant activity. Therefore, BPPU may be further developed as a potential therapeutic agent for therapy of epileptic disorders.

Introduction

Epilepsy is a complex brain disorder which is characterized by an enduring predisposition to generate epileptic seizures [1]. An epileptic seizure is defined as "a, transient occurrence of signs and/or symptoms due to abnormal excessive or synchronous neuronal activity in the brain" [1]. Status epilepticus (SE) is a life threatening condition where prolonged and recurrent seizures arise from brain [2]. At present, 50 million people worldwide are afflicted with epilepsy [3]. Even with the development of various antiepileptic drugs (AEDs) since 1993, the efficacy and tolerability of drug treatment of epilepsy has not substantially improved [4]. Moreover, 30–40% epileptic patients fail to experience sufficient control on their seizures [5]. In literature, there is a growing consensus that depression is predominant psycho-behavioral disorder associated with epilepsy [6]. Grzyb et al. suggested that the depressive signs are present in 40–60% patients with epilepsy [7]. Therefore, development of safer anticonvulsant agent with enhanced efficacy and which can also refurbish depressive symptoms may improve the management of epilepsy.

Piperazine scaffold has been recognized as a promising heterocycle for designing central nervous system (CNS) agents. Many studies suggested that piperazine derivatives possess good anticonvulsant and antidepressant activity in laboratory animals [8,9]. Previously, we have also reported several piperazine analogs as potent anticonvulsant agents [10]. Herein, we rationally designed and synthesized an novel aryl piperazine derivative 1-[4-(4-benzo[1,3]dioxol-5-ylmethyl-piperazin-1-yl)-phenyl]-3-phenyl-urea (BPPU) and evaluated it for anticonvulsant as well as antidepressant activity. Piperonylpiperazine moiety has been used to develop potent CNS active agents for

* Corresponding author.
E-mail address: mtiwari07@gmail.com (M. Tiwari).
[1] These authors have contributed equally.

http://dx.doi.org/10.1016/j.pharep.2015.08.013
1734-1140/

example Piribedil, a successful CNS drug exhibits wide range of CNS activities such as antiparkinsonian [11], anticonvulsant [12] and also antidepressant [13]. Therefore a piperonylpiperazine moiety was chosen to build up potent anticonvulsant agent. Moreover, other pharmacophoric features requirement for anticonvulsant activity such as hydrogen bond donor/acceptor unit were installed in piperonylpiperazine moiety led to development of piperonylpiperazine-phenyl urea hybrid compound BPPU (Fig. 1). The pharmacological profile of BPPU has been assessed in various *in vivo* models of epilepsy and depression. In addition, the sub-acute toxicity study of BPPU was also carried out using adult Wistar rats.

Materials and methods

In-silico *physicochemical and pharmacokinetic studies*

The physicochemical properties of BPPU were calculated by using Molinspiration online property explorer (Molinspiration Cheminformatics) [14]. The percentage absorption was calculated using the formula: Absorption (%ABS) = 109 − [0.345 × total polar surface area (TPSA)] [15]. The *in-silico* pharmacokinetic properties were calculated by using Discovery Studio 3.5 software (Accelrys, San Diego, CA, USA). The chemical structure of BPPU was drawn on Chemdraw version 10 to generate the possible conformations and the obtained optimized structure was saved in Mol 2 format which was utilized for pharmacokinetic estimation.

Chemicals and drugs

The detailed synthetic procedure and chemical characterization of tested compound (BPPU) has been given in supplementary data. The chemicals required for the synthesis of BPPU and biochemical used for pharmacological assays were purchased from Sigma-Aldrich (St. Louis, USA), Samarath Life Sciences (Mumbai, India) and Roche Ltd (Kolkata, India). For pharmacological tests BPPU was suspended in a 0.5–1.0% gum acacia solution and administered to animals either intraperitoneally (*ip*) or per oral (*po*). Vehicle represents 0.5% gum acacia solution in normal saline.

Animals and housing conditions

All the experimental protocols and procedures described here upon were prior approved by Institutional Animal Ethics Committee. The healthy Albino mice (Swiss, 25–30 g, 2–3 weeks) and Albino rats (Wistar, 100–150 g, 5–6 weeks) were obtained from animal house colony in the Dr. B.R. Ambedkar Center for Biomedical Research, Delhi University, India. The animals were kept under standard laboratory conditions (room temperature: 23 + 2° C; relative humidity: 60 + 5%; illumination: 12 h-light/dark cycle) and had freely access to food pellets and fresh water except for the short time duration when animals were removed for pharmacological testing. All experiments were performed between 9.00 AM and 2.00 PM.

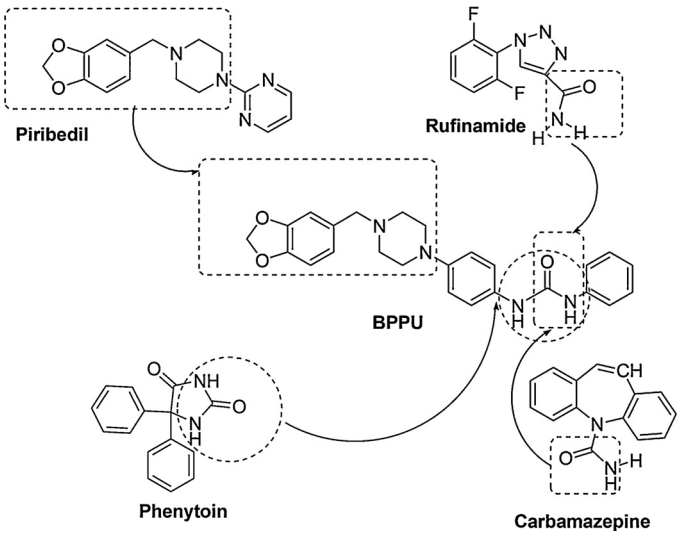

Fig. 1. Designing strategy of synthetic test compound BPPU.

Maximal electroshock (MES) seizure test

Seizures were induced by means of an electrical stimulus of 50 mA (in mice) and 150 mA (in rat) for 0.2 s, delivered *via* trans-auricular electrodes by a convulsiometer (Techno Electronics, Lucknow, India). In mice BPPU was injected *ip* at the doses of 30, 100 and 300 mg/kg and the anti-MES activity was assessed after 0.5 h and 4.0 h, respectively. In rats, oral dose of BPPU *i.e.* 30 and 100 mg/kg was used and anti-MES activity was recorded at five different (0.25, 0.5, 1.0, 2.0, 4.0 h) time point intervals. The complete abolition of the hind limb extension was defined as protection against MES-induced seizures [16].

Subcutaneous pentylenetetrazole (scPTZ) seizure test

The subcutaneous (*sc*) convulsive dose (CD_{97}) of 85 mg/kg (in mice) and 70 mg/kg (in rat) was utilized [16]. A threshold convulsion is defined as one episode of clonic spasms which persists for at least 5 s and the protection was defined as the absence of even a threshold convulsion. In mice, BPPU was administered *ip* at the doses of 30, 100 and 300 mg/kg and anti-PTZ activity was recorded after 0.5 and 4 h time intervals. To determine oral potential of BPPU in rats, oral doses of 30 and 100 mg/kg were given and anti-PTZ effect was assessed up to 2 h within time intervals of 0.25, 0.5, 1.0, and lastly 2.0 h.

Determination of the median effective dose (ED_{50}) in mice

The method of Litchfield and Wilcoxon [17] was used to calculate the ED_{50} and the respective 95% confidence intervals of BPPU in MES test. BPPU was administered *ip* to each group of animals at the varied doses until a minimum of three points was established between the dose level of 0% protection and 100% protection in MES seizure test. The ED_{50} and the 95% confidence intervals were calculated by the Graphpad prism 5.

Pilocarpine induced status prevention (PISP) model

BPPU was administered at the doses of 30 and 100 mg/kg (*ip*) to Wistar rats. Then, all animals received a challenge dose of pilocarpine (400 mg/kg, *ip*) and observed for the treatment effects of BPPU. The anticonvulsant activity was evaluated at the time zero, namely the time from first III seizures and at 30 min after a post-first stage III seizure. The seizure severity score was allotted according to Racine scale [18]. The results were taken as *N*/*F*, where *N* = number of animals protected and *F* = number of animals tested. In addition, the average weight change in 24 h post the first III seizures is reported for protected and non-protected rats, respectively.

3-Mercaptopropionic acid (3-MPA) induced seizures

The animals were randomly divided in to five groups (10 mice per group). Control group animals received vehicle only, group II received standard drug carbamazepine at the dose of 50 mg/kg (*ip*).