Med Chem Res (2012) 21:2300–2306
DOI 10.1007/s00044-011-9760-8

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of substituted 5-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones against pentylenetetrazole-induced convulsant in mice

Karan Deep Singh Puri · Shailja Sood · Arunachalam Muthuraman

Received: 4 October 2010 / Accepted: 19 July 2011 / Published online: 29 July 2011

Abstract This study was designed to synthesis of substituted 5-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones followed by evaluation against pentylenetetrazole-(PTZ) induced convulsant in mice. The titled compounds were confirmed by IR and ^{1}H-NMR spectral techniques. Pre-treatment of compound **4c** showed significant anticonvulsant activity at 40 mg/kg which was comparable to that of PTZ and sodium valproate pre-treated groups. The results show the importance of barbituric acid derivative (i.e., compound **4c** (R = *p*-OH, *m*-OCH_3) for this anti-convulsant activity. It may be due to its anti-oxidative and neuroprotective potential. Therefore, compound **4c** emerged as the most active molecules in the management of convulsive disorder.

Keywords Anti-convulsant · Barbituric acid · Mus musculas · Pentylenetetrazole

Introduction

Epilepsy is one of the most common neurodegenerative disorders and is affecting at least 50 million people worldwide. Epilepsy is major neurological problem in children and young people among the developed and developing countries. Moreover, prevalence rates are 3.6 to 4.2 per 1000 children (Blom *et al*., 1978; Skinner *et al*., 2010). The incidence of epilepsy is higher in men than in women and appears to be higher in African-Americans than in Caucasians (Haerer *et al*., 1986). In fact, epilepsy is most frequent neurological disorder after cerebrovascular disease and dementia in elder people (Kramer, 2001). Cardinal feature of epilepsy syndromes are the predisposition of recurrent unprovoked seizures and convulsions. Seizures, in turn, are sudden brief attacks of altered consciousness; motor, sensory, cognitive, psychic, or autonomic disturbances; or inappropriate behavior caused by abnormal excessive or synchronous neuronal activity in the brain, whereas, convulsions are violent and involuntary contractions of voluntary muscles (Tuchman *et al*., 2010; Kossoff and Andermann, 2010). Epilepsy is associated with high prevalence of reproductive disorders and leads to infertility (Kim *et al*., 2010). Electrical discharges alter the secretion of pituitary hormones and results in the reproductive dysfunction (Kim *et al*., 2010). Therefore, emerging therapies are needed for these disorders.

Barbiturate therapy for acute brain insults achieves a better clinical outcome than the standard treatment and number of studies reporting disappointing results for both traumatic brain injury (DeDeyne, 2010; Molina *et al*., 2009) and acute cerebral ischemia (Chang *et al*., 2008). Moreover, a high incidence of adverse effects have been reported in association with barbiturate therapy including cardio-respiratory depression, impaired white cell function, hypokalemia, stroke, severe cutaneous reactions, hepatic, and renal injury (Shaik and Mehvar, 2010; Mamishi *et al*., 2009; English and Davis, 2010; Hashizume *et al*., 2002).

Barbiturates were considered as drug of choice in only intensive care unit (Thuong *et al*., 2008). On the other

K. D. S. Puri · S. Sood · A. Muthuraman
Rayat Institute of Pharmacy, Nawanshahr District, Near Ropar, Railmajra, Punjab 144533, India

A. Muthuraman (✉)
Rayat Institute of Pharmacy, District Shaheed Bhagat Singh Nagar, Railmajra, Punjab 144533, India
e-mail: arunachalammu@gmail.com

Present Address:
A. Muthuraman
Department of Pharmaceutical Sciences & Drug Research, Punjabi University, Patiala, Punjab 147002, India

hand, long-time continuous administration of barbiturates is associated with an increase of the incidence of side effects including immunosuppression (Stover and Stocker, 1998). However, barbiturates exert pleiotropic effects in vivo. The neuroprotective effect of barbiturates was initially attributed to their ability to reduce cerebral metabolism (Wakamatsu *et al*., 2009). Paradoxically, barbiturate therapy reduces CBF, CMRO2 and increases cerebral vascular resistance in patients with severe head injury (Neil and Dale, 2009). Acute and chronic intracranial hypertension is also significantly reduced with a concomitant elevation in cerebral perfusion pressure (Cordato *et al*., 2003). Several studies, since 1970 suggested that barbiturate therapy has a major impact on the prognosis of patients with intractable intracranial hypertension and other neurological disorders (An *et al*., 2010; Ghahremanzadeh *et al*., 2010). Further, the currently available, other anticonvulsants drugs (AEDs) are also effective in reducing the severity and number of seizures in less than 70% of patients. Moreover, their usage is associated with undesirable side effects ranging from cosmetic (gingival hyperplasia) to life threatening diseases such as hepatotoxicity and megaloblastic anemia (Greenwood, 2000; Llompart-Pou *et al*., 2007). Therefore, new molecules with safer and more effective anti-epileptic drugs are needed for the management of epileptic disorder. This study focused on the synthesis and evaluation of pharmacologically active substituted 5-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones in the management of epileptic disease.

Materials and methods

Drugs and chemicals

Chemicals such as barbituric acid, acetic acid, hydrazine hydrate, benzaldehyde, anisaldehyde, vanillin, chloroacetyl chloride, and triethylamine were obtained from SD Fine Chemicals, Loba Chemicals, Nice Chemicals and Merck Chemicals. Standard reference drugs sodium valproate (VPA) and pentylenetetrazole (PTZ) were provided as gift sample from Ranbaxy laboratory, Mumbai. All the reagents used in this study were of analytical grade.

Animals

The mice weighing 15–25 g of either sex were obtained from National Institute of Pharmaceutical Education and Research (NIPER), Mohali. The animals were housed under standard laboratory conditions maintained under a natural light and dark cycle, and had free access to food and water. A 12 h light–dark cycle was maintained throughout the experimental protocol. Each animal was used only once. The experimental protocol was duly approved by Institutional Animal Ethics Committee (IAEC) and care for the animals was taken as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India (Reg No: 874/ac/05/CPCSEA).

Synthesis of substituted 5-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones

Synthesis of 5-bromopyrimidine-2,4,6(1H,3H,5H)-trione ***(1)***

A suspension of barbituric acid (12.8 g, 0.1 mol) in excess of glacial acetic acid was prepared and to this bromine (10.29 ml, 0.2 mol) was added dropwise. After complete addition of bromine, the reaction mixture was stirred for 10 h and poured into ice-cold water and then left overnight at room temperature and the completion of reaction was monitored by TLC. The resultant precipitates were filtered, thoroughly washed with distilled water, dried, and crystallized from methanol to yield of compound **1** (47.65%), mp 196°C, IR (KBr): 648 (C–Br), 1338 (C–N), 1715 (C=0), 3300 cm^{-1} (N–H). ^{1}H-NMR (DMSO-d_6): 11.27 (s, 2H, 2N*H*CO), 5.5 (s, 1H, C*H*–Br).

Synthesis of 5-hydrazinylpyrimidine-2,4,6(1H,3H,5H)-trione ***(2)***

The mixture of compounds **1** (20.6 g, 0.1 mol) and hydrazine hydrate (9.8 ml, 0.2 mol) in methanol was refluxed for 8 h and the completion of reaction was monitored by TLC. The solvent was removed under reduced pressure and crushed ice was added to it. The solid obtained was filtered, washed with distilled water, and crystallized from methanol to give compound **2** (58.25%), mp 210°C, IR (KBr): 1292 (N–N), 1349 (C–N), 1720 (C=0), 3221 (NH–NH_2), 3286 cm^{-1} (N–H), ^{1}H-NMR (DMSO-d_6): 11.23 (s, 2H, 2NHCO), 5.3 (d, 1H, CH–NH–NH_2), 4.7 (s, 3H, NH–NH_2).

Synthesis of 5-(2-benzylidenehydrazinyl)pyrimidine-2,4,6(1H,3H,5H)-trione ***(3a)***

To a solution of (**2**) (15.8 g, 0.1 mol) in methanol, anisaldehyde and few drops of acetic acid were added. The resultant solution was refluxed for 8 h and poured into ice-cold water. The completion of reaction was monitored by TLC. The precipitates thus obtained were filtered, washed with distilled water, dried, and crystallized from methanol to yield compound **3a**.

3a. (51.23%), mp 230°C, IR (KBr): 1270 (N–N), 1340 (C–N), 1610 (C–C), 1670 (C–N), 1713 (C=0), 3100 (C–H), 3290 (N–H) cm^{-1}, ^{1}H-NMR (DMSO-d_6): 11.22 (s, 2H, 2NHCO), 8.60 (s, 1H, =CH–Ar), 8.25–7.85 (m, 5H, Ar–H), 5.2 (d, 1H, CH–NH), 4.5 (s, 1H, CH–NH).

3b. (58.82%), mp 185°C, IR (KBr): 1050 (C–O–C), 1290 (N–N), 1335 (C–N), 1600 (C–C), 1670 (C–N), 1713 (C=0), 3100 (C–H), 3280 (N–H) cm^{-1}, ^{1}H-NMR (DMSO-d_6): 11.24 (s, 2H, 2NHCO), 8.59 (s, 1H, =CH–Ar), 8.20–7.80 (m, 4H, Ar–H), 5.4 (d, 1H, CH–NH), 4.4 (s, 1H, CH–NH), 3.40 (s, 3H, OCH_3).

3c. (63.25%), mp 246°C, IR (KBr): 1060 (C–O–C), 1290 (N–N), 1300 (C–N), 1600 (C–C), 1700 (C=0), 3150 (C–H), 3240 (N–H), 3420 (O–H) cm^{-1}, ^{1}H-NMR (DMSO-d_6): 12.22 (s, 1H, OH), 11.25 (s, 2H, 2NHCO), 8.58 (s, 1H, =CH–Ar), 8.20–7.80 (m, 3H, Ar–H), 5.5 (d, 1H, CH–NH), 4.2 (s, 1H, CH–NH), 3.45 (s, 3H, OCH_3).

Synthesis of 5-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)pyrimidine-2,4,6(1H,3H,5H)-trione ***(4a)***

The mixture of compounds **3a** (2.46 g, 0.01 mol) in dimethylformamide (DMF) and chloroacetyl chloride (1.12 ml, 0.01 mol) with catalytic amount of triethylamine was placed in round bottom flask and was refluxed at water bath for 10 h. The completion of reaction was confirmed by TLC. The resulting reaction mixture was then poured into ice-cold water and filtered. The product was crystallized from methanol to give compound **4a** (52.84%), mp 260°C, *m/z* (322) IR (KBr): 780 (C–Cl), 1280 (N–N), 1342 (C–N), 1600 (C–C), 1715 (C=O), 3105 (C–H), 3275 (N–H) cm^{-1}. ^{1}H-NMR (DMSO-d_6): 11.20 (s, 2H, 2N*H*CO), 8.30–7.80 (m, 5H, Ar–*H*), 5.90 (s, 1H, C*H*–Ar), 5.3 (d, 1H, C*H*–NH), 4.5(s, 1H, CH–N*H*), 4.1 (d, 1H, C*H*–Cl). $C_{13}H_{11}ClN_4O_4$ (Combound 4a): Calcd.: C, 48.38; H, 3.44; Cl, 10.99; N, 17.36; O, 19.83; Found: C, 48.71; H, 3.64; Cl, 10.65; N, 17.58; O, 19.63. **4b** (54.34%), mp 247°C, *m/z* (352)IR (KBr): 782 (C–Cl), 1340 (N–N), 1610 (C–C), 1715 (C=O), 3110 (C–H), 3280 (N–H) cm^{-1}, ^{1}H-NMR (DMSO-d_6): 11.20 (s, 2H, 2N*H*CO), 8.25–7.80 (m, 4H, Ar–*H*), 5.8 (s, 1H, C*H*–Ar), 5.4 (d, 1H, C*H*–NH), 4.5(s, 1H, CH–N*H*), 4.2 (d, 1H, C*H*–Cl), 3.42 (s, 3H, OC*H*$_3$). $C_{14}H_{13}ClN_4O_5$ (Compound 4b): Calcd.: C, 47.67; H, 3.71; Cl, 10.05; N, 15.88; O, 22.68; Found: C, 47.83; H, 3.66; Cl, 10.41; N, 15.61; O, 22.93. **4c** (62.06%), mp 291°C, *m/z* (368) IR (KBr): 780 (C–Cl), 1305 (N–N), 1600 (C–C), 1715 (C=O), 3150 (C–H), 3245 (N–H) cm^{-1}, 3420 (O–H) cm^{-1}. ^{1}H-NMR (DMSO-d_6): 12.25 (s, 1H, O*H*), 11.22 (s, 2H, 2N*H*CO), 8.15–7.65 (m, 3H, Ar–*H*), 5.8 (s, 1H, C*H*–Ar), 5.3 (d, 1H, C*H*–NH), 4.5(s, 1H, CH–N*H*), 4.2 (d, 1H, C*H*–Cl), 3.39 (s, 3H, OC*H*$_3$). $C_{14}H_{13}ClN_4O_6$ (Compound 4c): Calcd.: C, 45.60; H, 3.55; Cl, 9.61; N, 15.19; O, 26.03; Found: C, 45.72; H, 3.39; Cl, 9.49; N, 15.41; O, 26.31.

Acute toxicity studies

Acute toxicity studies were performed based on Organization of Economical Cooperation and Development (OECD) guidelines for the synthesized compound. Synthesized compounds (**4a**, **4b**, and **4c**) were administrated (100, 200, and 1000 mg/kg, *i.p.*) in three different groups, each groups comprised of three mice. Acute toxicity signs and mortality were observed. The LD50 value was determined by calculating the geometric means of the lowest dose that caused death and the highest dose for which the animals survived as described method of Lorke (1983).

Induction of kindled seizure by administration of PTZ

PTZ-induced seizure test was performed according to the method of Olivera *et al.* (2004). Mice (15–25 g) were subjected for the administration of PTZ (60 mg/kg, *s.c.*) for the development of seizures. Animals were observed for a period of 30 min post-PTZ administration. The parameters noted were mean onset of time of convulsions, duration of convulsions, and % protection. Sodium valproate (80 mg/kg, *i.p.*) was used as a standard drug and was injected 30 min before PTZ administration. Further, all the newly synthesized barbituric acid derivatives (**4a**, **4b**, **and 4c**) were tested for their anticonvulsant activity.

Experimental protocol for anticonvulsant studies in mice

Nine groups, each comprising of six mice weighing about 15–25 g, were employed in this study.

Group I (*Normal control group*)*:* Mice were subjected to administration of 1 ml of saline (0.9% w/v, *i.p.*) on the day of experiment.

Group II (*Negative control group*)*:* Mice were subjected to administration of pentylenetetrazole (PTZ, 60 mg/kg, *s.c.*) for the development of seizures on the day of experiment.

Group III (*Positive control group*)*:* Mice were subjected to administration of sodium valproate (80 mg/kg, *i.p.*) before 30 min of PTZ administration on the day of experiment.

Group IV and V (*Compound 4a*)*:* Mice were subjected to pretreatment with compound **4a** (10 and 40 mg/kg, *i.p.*) before 30 min of PTZ administration on the day of experiment.

Group VI and VII (*Compound 4b*)*:* Mice were subjected to pretreatment with compound **4b** (10 and 40 mg/kg, *i.p.*) before 30 min of PTZ administration on the day of experiment.

Group VIII and IX (*Compound* 4c)*:* Mice were subjected to pretreatment with compound **4c** (10 and 40 mg/kg, *i.p.*) before 30 min of PTZ administration on the day of experiment.

Biochemical evaluation

Following the behavioral testing, the animals were decapitated under ether anesthesia, and the brains were quickly removed, cleaned with ice-cold saline, and stored at −80°C. Brain tissue samples were thawed and homogenized with 10 times (w/v) ice-cold 0.1 M phosphate buffer (pH 7.4) using a glass homogenizer. Aliquots of homogenates from rat brain were used to determine the lipid peroxidation, reduced glutathione, nitric oxide, and carbonyl protein.

Estimation of thiobarbituric reactive substances (TBARS)

Estimation of TBARS level as an index of lipid peroxidation product (MDA; malondialdehyde) was determined by thiobarbituric acid (TBA) reaction as described method of Ohkawa *et al.* (1979). The absorbance was determined spectrophotometrically at 543 nm.

Estimation of tissue reduced glutathione

Estimation of reduced glutathione (GSH) level as an index of oxidative stress marker was determined in the tissue homogenate as described method of Ellman (1959). The absorbance was determined spectrophotometrically at 412 nm.

Estimation of nitrite level

Estimation of nitrite (NO^{2-}) and nitrate (NO^{3-}) were estimated by Griess reaction as an index of NO production as described method of Cortas and Wakid (1990). The absorbance was determined spectrophotometrically at 543 nm.

Estimation of carbonyl protein content

Estimation of carbonyl protein content was determined by the method described of Yan *et al.* (1995). The absorbance was determined spectrophotometrically at 370 nm.

Estimation of total protein content

Estimation of total protein concentration was estimated according to the method of Lowry *et al.* (1951). The absorbance was determined spectrophotometrically at 750 nm.

Statistical analysis

All the results were expressed as standard error of means (±SEM). The data were statistically analyzed by one-way analysis of variance (ANOVA) followed by Tukey's multiple range tests by using Sigma stat Version-2.0 Software. The *P-value* <0.05 was considered to be statistically significant.

Results and discussions

Synthesized compound

Melting points of synthesized compounds were measured using Buchi melting point apparatus and are uncorrected. The ^{1}H-NMR spectra were recorded on a Bruker AC-300F, 300 MHz instrument using DMSO-d_6 as solvent, and tetramethyl silane (TMS) as internal reference standard. Thin layer chromatography was performed on silica gel thin layer chromatography plates using ethyl acetate–hexane (7:3 v/v) as the mobile phase.

5-hydrazinylpyrimidine-2,4,6(1H,3H,5H)-trione ***(2)***

5-bromopyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione **(1)** was prepared by carrying out bromination of barbituric acid in the presence of acetic acid which was followed by reaction with hydrazine hydrate to obtain compound 5-hydrazinylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione **(2)** (Fig. 1). ^{1}H-NMR spectrum exhibited two singlet at δ 11.27 (s, 2H, N*H*CO) and δ 5.5 ppm (s, 1H, C*H*–Br) for the compound **1**. IR band for C–Br stretching was observed at 648 cm^{-1}. The compound **2** exhibited singlet at δ 4.7 (s, 3H, CH–N*H*–NH_2). IR band at 1292 (N–N) cm^{-1} further supported the structure.

Substituted 5-(2-benzylidenehydrazinyl)pyrimidine-2,4,6(1H,3H,5H)-triones ***(3a–c)***

Reaction of compound **2** with different aromatic aldehydes in the presence of methanol afforded compounds **3a**, **3b**, and **3c** (Fig. 1). The structure of these compounds was elucidated by spectral analysis. ^{1}H-NMR spectrum exhibited singlet at δ 8.60 (1H, =C*H*–Ar) and multiplet at δ 8.25–7.85 (5H, Ar–*H*) for the compound **3a**. A peak at 1670 (C–N) cm^{-1} appeared in IR spectrum. Similarly, anisaldehyde derivative **3b** spectrum showed singlet at δ 3.40 (3H, OCH_3), and also a peak at 1050 (C–O–C) cm^{-1} in IR spectrum. For compound **3c**, spectrum exhibited two singlets at δ 12.22 (1H, O*H*) at δ 3.45 (3H, OCH_3). IR spectrum band was also observed at 3420 (O–H) cm^{-1} (Fig. 1).

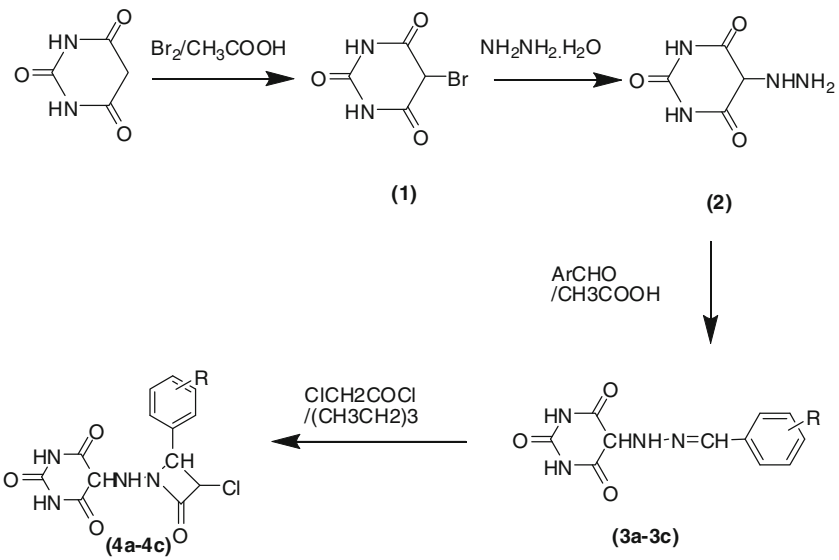

Fig. 1 The synthesis of 5-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones from 5-bromopyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione from barbituric acid by bromination, hydrazination, attachment of aromatic ring, and cyclization reaction. Compound **(1)** 5-bromopyrimidine-2,4,6(*1H,3H,5H*)-*trione*; **(2)** 5-hydrazinylpyrimidine-2,4,6(*1H,3H,5H*)-1trione; **(3a)** R = H; **(3b)** R = *p*-OCH_3; **(3c)** R = *p*-OH, *m*-OCH_3; **(4a)** R = H; **(4b)** R = *p*-OCH_3; **(4c)** R = *p*-OH, *m*-OCH_3

*Substituted 5-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)pyrimidine-2,4,6(1H,3H,5H)-triones **(4a–c)***

Compound **3a**, **3b**, and **3c** undergoes cycloaddition with chloroacetylchloride in the presence of triethyl amine to yield compound **4a**, **4b**, and **4c** (Fig. 1). These compounds have been characterized by IR and ^{1}H NMR studies. The formation of compound **4a** was evidenced by appearance of signal at δ 4.1 due to (d, 1H, C*H*–Cl) in azetidinone ring and IR band at 780 (C–Cl) cm^{-1}. The ^{1}H NMR spectra of **4b** showed the azetidinone ring proton δ 4.2 (d, 1H, C*H*–Cl). The later compound showed IR bands at 1775 (C=O) cm^{-1}. Similarly, azetidinone derivative **4c** exhibited doublet at doublet δ 4.2 (d, 1H, C*H*–Cl). A peak at (C=O) cm^{-1} appeared in IR spectrum.

Acute toxicity studies

Acute toxicity studies were performed on the basis of OECD guidelines, for the synthesized compound (**4a**, **4b** and **4c**) as described method of Lorke (1983). No toxicity or death was observed for synthesized compounds on administration of a dose of 100, 200, and 1000 mg/kg. At 1000 mg/kg dose, there were slight behavioral changes and 70% mortality was found. Based on this observation, a dose of 20 and 40 mg/kg of synthesized compounds were selected for the evaluation of their anti-convulsant activities.

*Effect of compound **4a**, **4b**, and **4c** in PTZ-induced convulsant*

PTZ is a well-known $GABA_A$ antagonist. PTZ-induced convulsions can be prevented or attenuated by antioxidants or reactive species scavengers in a dose-dependent manner. The results obtained from sodium valproate showed significant protection against PTZ-induced convulsions as depicted in Table 1. Pretreatment of compound **4a**, **4b**, and **4c** attenuated the convulsions induced by PTZ in a dose-dependent manner. Further, it was noted that all compounds show significant anticonvulsant activity at high dose level which is comparable with standard reference drug, i.e., sodium valproate. Among all the newly synthesized barbituric acid derivatives, compound **4c** showed 83% protection at higher dose level whereas, at lower dose showed 66% protection which was comparable to that of standard drug sodium valproate. It was found that the presence of hydroxyl and methoxy group in the molecular framework has shown significant anticonvulsant activity. Some studies reported that PTZ-induced convulsions are attenuated by phenyl-butyl-nitrone, tocopherol, glutathione, melatonin, high doses of ascorbate, and sodium valproate (Oliveira *et al*., 2004; Bashkatova *et al*., 2004).

*Effect of compound **4a**, **4b**, and **4c** in PTZ-induced biochemical changes*

Literature survey revealed that PTZ-induced convulsion and brain bio & neurochemical changes can be prevented or attenuated by various antioxidants or reactive species scavenging molecule (Aldarmaa *et al*., 2010). Table 2 shows the changes of PTZ-induced brain biochemical changes (i.e., TBARS, GSH, nitrite level, and carbonyl protein content) in mice. PTZ is potentially induced biochemical abnormality in mice brain. Pretreatment of compound **4a**, **4b**, and **4c** attenuated the alteration biomarker changes in a dose-dependent manner. Further, it was noted that compound **4c** shows significant biochemical

Table 1 Effect of compounds **4a**, **4b**, and **4c** on PTZ-induced convulsion in mice

Groups	Dose	Mean onset of time (s)	Duration of seizure (s)	% Protection
Naive mice	(0.9% w/v, NaCl)	258.61 ± 6.42	4.38 ± 2.1	0
PTZ	60 mg/kg	261.34 ± 6.42	4.56 ± 3.1	0
PTZ + Sodium valproate	80 mg/kg	300.23 ± 2.61[a]	1.1 ± 0.4[a]	100
PTZ + Compound **4a**	20 mg/kg	263.13 ± 4.34[b]	4.21 ± 2.6[b]	16
PTZ + Compound **4a**	40 mg/kg	267.48 ± 6.24[a]	2.73 ± 2.5[a]	16
PTZ + Compound **4b**	20 mg/kg	270.48 ± 1.29[b]	3.75 ± 1.7[b]	33
PTZ + Compound **4b**	40 mg/kg	278.44 ± 2.16[a]	2.31 ± 2.6[a]	66
PTZ + Compound **4c**	20 mg/kg	282.91 ± 3.98[b]	1.98 ± 1.7[b]	66
PTZ + Compound **4c**	40 mg/kg	290.58 ± 4.34[acd]	1.60 ± 0.6[acd]	83

Data were expressed as standard error of mean (SEM, $n = 6$), [a] $P < 0.05$ versus PTZ control group, [b] $P < 0.05$ versus sodium valproate pretreated group, [c,d] $P < 0.05$ versus compound **4a** and **4b** (20 mg/kg) pretreated group, respectively

Table 2 Effect of compounds **4a**, **4b**, and **4c** on PTZ-induced biomarker changes in mice

Groups	Dose	TBARS (nmol/mg of protein)	GSH (μmol/mg of protein)	Nitrite level (μmol/mg of protein)	Carbonyl protein (nmol/mg of protein)
Naive mice	0.9% w/v, NaCl	26.34 ± 0.34	0.093 ± 0.004	0.55 ± 0.03	18.14 ± 0.24
PTZ	60 mg/kg	52.26 ± 0.11	0.011 ± 0.002	0.46 ± 0.01	31.56 ± 0.58
PTZ + Sodium valproate	80 mg/kg	28.13 ± 0.54[a]	0.089 ± 0.003[a]	0.54 ± 0.04[a]	19.06 ± 0.39[a]
PTZ + Compound **4a**	20 mg/kg	48.36 ± 0.28[b]	0.031 ± 0.006[b]	0.46 ± 0.02[b]	30.74 ± 0.61[b]
PTZ + Compound **4a**	40 mg/kg	44.04 ± 0.16[b]	0.036 ± 0.002[b]	0.48 ± 0.01[b]	29.61 ± 0.42[b]
PTZ + Compound **4b**	20 mg/kg	47.11 ± 0.09[b]	0.043 ± 0.007[b]	0.47 ± 0.09[b]	27.53 ± 0.71[b]
PTZ + Compound **4b**	40 mg/kg	42.91 ± 0.16[b]	0.047 ± 0.001[b]	0.49 ± 0.02[b]	25.94 ± 0.64
PTZ + Compound **4c**	20 mg/kg	35.19 ± 0.13[ac]	0.081 ± 0.002[ac]	0.53 ± 0.02[ac]	21.68 ± 0.32[ac]
PTZ + Compound **4c**	40 mg/kg	30.68 ± 0.26[acd]	0.084 ± 0.005[acd]	0.54 ± 0.01[acd]	20.48 ± 0.53[acd]

Data were expressed as standard error of mean (SEM, $n = 6$), [a] $P < 0.05$ versus PTZ control group, [b] $P < 0.05$ versus sodium valproate pretreated group, [c,d] $P < 0.05$ versus compound **4a** and **4b** (20 mg/kg) pretreated group, respectively

alteration at high dose level which is comparable with standard reference drug, i.e., sodium valproate. Recent report evidenced that accumulation of PTZ-induced mitochondria derived reactive oxygen and nitrogen species has reported in the deleterious effects on the brain tissue leads to convulsant action (Aldarmaa *et al*., 2010). We examined the anticonvulsant effects of barbiturate derivatives on PTZ-induced seizures in mice and the possible mechanisms of protection against oxidative damage of brain tissue. Our study indicated that PTZ causes the rise in oxidative stress marker such as TBARS, nitrite, and carbonyl protein levels and decreases the reduced glutathione level in brain homogenates. Similar results were observed in other laboratories (Aldarmaa *et al*., 2010; Uma Devi *et al*., 2006).

The variety barbiturate derivatives exhibit a different of biological activities, such as central nervous depression, sedative-hypnosis, anti-depression, muscle relaxant, local anesthesia, anti-bacterial, anti-fungal, and anti-convulsant activities (Uciechowska *et al*., 2008; Yan *et al*., 2009; Kidwai *et al*., 2005). In our research, 5-(3-chloro-2-(4-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-ylamino)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione, i.e., compound **4c** as the lead compound showed a positive anticonvulsant activity with an effective dose of 40 mg/kg in the PTZ-induced convulsant model.

Conclusion

To conclude, we can state that the substituted 5-(3-chloro-2-oxo-4-phenylazetidin-1-ylamino)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-triones may be served as the potential candidate for the management of epileptic disorders due to its potential of antioxidant property. Moreover, the elaborative studies are necessary to explore their molecular mechanism of its anticonvulsant activity.

Acknowledgment Thanks to Dr. A.C. Rana and all faculty members of Rayat Institute of Pharmacy for their encouragement and support. We are also grateful to Rayat & Bahra Educational and Research Trust for their unconditional help to carry out this project.

Conflict of interest There was no conflict of interest in this study.

References

Aldarmaa J, Liu Z, Long J, Mo X, Ma J, Liu J (2010) Anti-convulsant effect and mechanism of *Astragalus mongholicus* extract in vitro and in vivo: protection against oxidative damage and mitochondrial dysfunction. J Neurochem Res 35:33–41

An HS, Cho BM, Kang JH, Kim MK, Oh SM, Park SH (2010) Efficacy of low dose barbiturate coma therapy for the patients with intractable intracranial hypertension using the bispectral index monitoring. J Korean Neurosurg Soc 47:252–257

Bashkatova V, Narkevich V, Vitskova G, Vanin A (2004) The influence of anticonvulsant and antioxidant drugs on nitric oxide level and lipid peroxidation in the rat brain during penthylenetetrazole-induced epileptiform model seizures. Prog Neuropsychopharmacol Biol Psychiatry 27:487–492

Blom S, Heijbel J, Bergfors PG (1978) Incidence of epilepsy in children: a follow-up study three years after the first seizure. Epilepsia 19:343–350

Chang YJ, Ryu SJ, Chen JR, Hu HH, Yip PK, Chiu TF (2008) Consensus group of Taiwan stroke society. Guidelines for the general management of patients with acute ischemic stroke. Acta Neurol Taiwan 17:275–294

Cordato DJ, Herkes GK, Mather LE, Morgan MK (2003) Barbiturates for acute neurological and neurosurgical emergencies—do they still have a role? J Clin Neurosci 10:283–288

Cortas NK, Wakid NW (1990) Determination of inorganic nitrate in serum and urine by a kinetic cadmium-reduction method. Clin Chem 36:1440–1443

DeDeyne CS (2010) Therapeutic hypothermia and traumatic brain injury. Curr Opin Anaesthesiol 23:258–262

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82:70–77

English J, Davis B (2010) Case report: death associated with stroke following intracarotid amobarbital testing. Epilepsy Behav 17:283–284

Ghahremanzadeh R, Fereshtehnejad F, Bazgir A (2010) Chromeno[2,3-d]pyrimidine-triones synthesis by a three-component coupling reaction. Chem Pharm Bull 58:516–520

Greenwood RS (2000) Adverse effects of antiepileptic drugs. Epilepsia 41:S42–S52

Haerer AF, Anderson DW, Schoenberg BS (1986) Prevalence and clinical features of epilepsy in a biracial United States population. Epilepsia 27:66–75

Hashizume H, Takigawa M, Tokura Y (2002) Characterization of drug-specific T cells in phenobarbital-induced eruption. J Immunol 168:5359–5368

Kidwai M, Thakur R, Mohan R (2005) Eco friendly synthesis of novel antifungal (thio) barbituric acid derivatives. Acta Chim Slov 52:88–92

Kim GH, Lee HW, Park H, Lee SK, Lee SA, Kim YI, Song HK, Shin DJ, Hong SB (2010) Seizure exacerbation and hormonal cycles in women with epilepsy. Epilepsy Res 90:214–220

Kossoff EH, Andermann F (2010) Migraine and epilepsy. Semin Pediatr Neurol 17:117–122

Kramer G (2001) Epilepsy in the elderly: some clinical and pharmacotherapeutic aspects. Epilepsia 42:55–59

Llompart-Pou JA, Perez-Bárcena J, Raurich JM, Burguera B, Ayestaran JI, Abadal JM, Homar J, Ibanez J (2007) Effect of barbiturate coma on adrenal response in patients with traumatic brain injury. J Endocrinol Invest 30:393–398

Lorke D (1983) A new approach to acute toxicity testing. Arch Toxicol 54:275–287

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with folin phenol reagent. J Biol Chem 193:265–275

Mamishi S, Fattahi F, Pourpak Z, Aghaee FM, Moinfar Z, Mohammadi M, Ashrafi M, Moin M (2009) Severe cutaneous reactions caused by barbiturates in seven Iranian children. Int J Dermatol 48:1254–1261

Molina DK, McCutcheon JR, Rulon JJ (2009) Head injuries, pentobarbital and the determination of death. Am J Forensic Med Pathol 30:75–77

Neil MJ, Dale MC (2009) Hypokalaemia with severe rebound hyperkalaemia after therapeutic barbiturate coma. Anesth Analg 108:1867–1868

Ohkawa H, Ohishi N, Yagi K (1979) Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 95:351–358

Oliveira MS, Furian AF, Royes LFF, Fighera MR, Myskiw JC, Fiorenza NG, Mello CF (2004) Ascorbate modulates pentylenetetrazol-induced convulsions biphasically. Neuroscience 128:721–728

Shaik IH, Mehvar R (2010) Cytochrome P450 induction by phenobarbital exacerbates warm hepatic ischemia-reperfusion injury in rat livers. Free Radic Res 44:441–453

Skinner HI, Dubon-Murcia SA, Thompson AR, Medina MT, Edwards JC, Nicholas JS, Holden KR (2010) Adult convulsive status epilepticus in the developing country of Honduras. Seizure 19:363–367

Stover JF, Stocker R (1998) Barbiturate coma may promote reversible bone marrow suppression in patients with severe isolated traumatic brain injury. Eur J Clin Pharmacol 54:529–534

Thuong M, Matta B, Menon D (2008) Sedation and analgesia assessment tools in ICU patients. Ann Fr Anesth Reanim 27:581–595

Tuchman R, Cuccaro M, Alessandri M (2010) Autism and epilepsy: historical perspective. Brain Dev 32:709–718

Uciechowska U, Schemies J, Neugebauer RC, Huda EM, Schmitt ML, Meier R, Verdin E, Jung M, Sippl W (2008) Thiobarbiturates as sirtuin inhibitors: virtual screening, free-energy calculations and biological testing. Chem Med Chem 3:1965–1976

Uma Devi P, Pillai KK, Vohora D (2006) Modulation of pentylenetetrazole-induced seizures and oxidative stress parameters by sodium valproate in the absence and presence of *N*-acetylcysteine. Fundam Clin Pharmacol 20:247–253

Wakamatsu T, Tanaka T, Oda S, Nishi K, Harada H, Daijo H, Takabuchi S, Kai S, Fukuda K, Hirota K (2009) The intravenous anesthetics barbiturates inhibit hypoxia-inducible factor 1 activation. Eur J Pharmacol 617:17–22

Yan LJ, Traber MG, Packer L (1995) Spectrophotometric method for determination of carbonyls in oxidatively modified apolipoprotein B of human low-density lipoproteins. Anal Biochem 228:349–351

Yan Q, Cao R, Yi W, Chen Z, Wen H, Ma L, Song H (2009) Inhibitory effects of 5-benzylidene barbiturate derivatives on mushroom tyrosinase and their antibacterial activities. Eur J Med Chem 44:4235–4243