Med Chem Res
DOI 10.1007/s00044-014-1315-3

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Anticonvulsant activity of 1,2,4-triazine derivatives with pyridyl side chain: synthesis, biological, and computational study

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Received: 11 August 2014 / Accepted: 9 December 2014

Abstract A series of 5,6-bisaryl-1,2,4-triazine-3-thiol-substituted derivatives were synthesized by condensation of 1,2-diketones and thiosemicarbazide under microwave irradiations and subsequent alkylation of thiol group by chloromethylpyridinium chloride. Evaluation of anticonvulsant activity of compounds was performed by maximal electroshock and pentylenetetrazole-induced seizures tests. In order to evaluate their neuroprotective potential, the ability of compounds to inhibit soybean 15-lipoxygenase was also assessed. Further molecular modeling and docking study on Na+ channel and $GABA_A$ receptor was performed to elucidate their mechanisms of action and necessary interactions in the active site. Compounds **2c** and **2d** with bis(4-bromophenyl) and pyridyl substituents showed highest protection up to 70 and 80 % in PTZ and MES-induced seizures, respectively, compared to the control group. Molecular docking study revealed their possible antiseizure mechanism of action through $GABA_A$ receptor, and in silico assessment of their BBB permeability indicated them as CNS active agents.

Keywords Synthesis · 1,2,4-Triazine · Anticonvulsant · 15-LOX · Molecular docking

Introduction

Epilepsy affects approximately 1 % (50 million people) of the world's population, and one in 10 people will suffer a seizure in their lifetime. Epilepsy is as common as breast cancer in America and takes many lives. Although administration of available antiepileptic drugs (AEDs) in patients successfully controls seizures in 50 % of patients developing partial seizures and 60–70 % of those developing generalized seizures, remaining are not responsive to

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Published online: 18 December 2014

the conventionally available AEDs. Moreover, the efficacy of many AEDs is limited by the dose-dependent toxicities and adverse drug reactions such as minimal brain impairment, megaloblastic anemia to death from aplastic anemia, or hepatic failure. Thus, there is a substantial need for further development of safer agents and novel mechanistic approaches in the treatment of epilepsy (Landmark, 2007); (Siddiqui and Ahsan, 2010).

However, oxidative injury may play a role in the initiation and progression of epilepsy, and therapies targeted at reducing oxidative stress may ameliorate tissue damage and favorably alter the clinical course of the disease (Devi *et al.*, 2008).

Several in vivo evidences of oxidative stress were found in animal models of epilepsy. Accordingly, antioxidant therapies with the aim of reducing oxidative injury have received remarkable attention in the treatment of epilepsy (Acharya *et al.*, 2008).

According to this fact, anticonvulsant agents with neuroprotective properties have received considerable attention in the treatment of epilepsy. Several studies reported that neurodegeneration is the major neurobiological abnormality in epileptic brain (Naegele, 2007). Since epileptic seizure and neurodegeneration are very similar in some aspects of their basic pathophysiology therefore, some anticonvulsive agents are equally effective neuroprotectives (Stanislaw *et al.*, 2007).

Previous studies on oxidative stress model in neuronal cells have revealed a role for the lipoxygenases (LOX), lipid oxidizing enzymes, to produce neuronal damage. 15-LOX is one of the key inflammatory mediators in neurodegenerative disease because it is stimulated by reactive oxygen species (ROS). Activation of 15-LOX tends to generate lipid hydroperoxides (15-HPETE) that help to amplify oxidative stress (Loscalzo, 2008).

Increased level of 15-LOX has been found in early stages of Alzheimer disease in human and in experimental model of stroke in mice (Van Leyen *et al.*, 2006; Pratico *et al.*, 2004). Therefore, inhibition of 15-LOX may protect a variety of neuronal cell types, and 15-LOX inhibitors could be potential neuroprotective agents.

Several heterocycle-containing compounds have been reported to have considerable antiepileptic activities. 1,2,4-triazine is among the usually occurred heterocyclic nuclei possessing the wide range of biological applications such as anticonvulsant (Irannejad *et al.*, 2014), antiparkinsonism (Congreve *et al.*, 2012), and potent neuroprotective (Irannejad *et al.*, 2010) activities. Among these biological activities, 1,2,4-triazine derivatives have been a promising scaffold which is mainly used in the novel anticonvulsant drugs such as lamotrigine (Fig. 1). Lamotrigine shares similar mode of action on neuronal sodium channel as phenytoin.

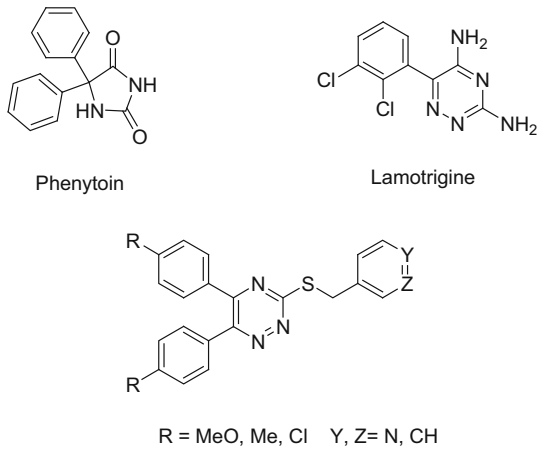


Fig. 1 Schematic representation of anticonvulsant drugs phenytoin and lamotrigine, and the previously synthesized compounds as anticonvulsant agents

Recently, we synthesized and evaluated anticonvulsant activity of some 1,2,4-triazine-substituted derivatives (Fig. 1). Among the compounds tested, we found out the prominent anticonvulsant activity of 3-((pyridin-4-ylmethyl)thio)-5,6-di-*p*-tolyl-1,2,4-triazine as a lead compound in both maximal electroshock (MES) and pentylenetetrazole (PTZ)-induced seizures tests (Irannejad *et al.*, 2014). Motivated by these observations and in continuation of our previous ongoing research studies on novel neuroprotectives and anticonvulsants, we synthesized some new 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives (Fig. 2) and evaluated their anticonvulsant and 15-LOX inhibition activities. Evaluation of anticonvulsant activity was performed by two conventional and well-established methods, MES and PTZ-induced seizures tests. Neurotoxicity of synthesized compounds was assessed by rota-rod test, and the ability of compounds to inhibit soybean 15-LOX was also evaluated. To find out their possible anticonvulsant mechanism of action, molecular docking was performed on the two main targets of conventional antiseizure drugs, Na + channel and $GABA_A$ receptor.

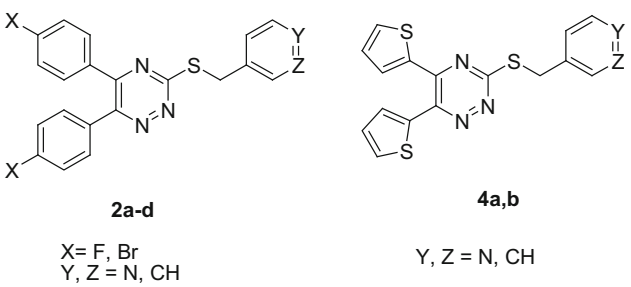


Fig. 2 New compounds (**2a–d** and **4a–b**) as novel anticonvulsant and neuroprotective agents

Materials and methods

Chemistry

Chemical reagents and solvents were purchased from Sigma-Aldrich or Merck. NMR spectra were recorded in DMSO-d_6 on a Brucker DRX400 MHz instrument. TMS was used as an internal standard in reporting chemical shifts (δ). Mass spectra were obtained with Agilent 7890A spectrometer. Progress of reaction and purity of compounds were monitored on TLC silica gel F_{254} plates. The microwave irradiation was carried out using MicroSYNTH Laboratory System (MILESTONE S.r.l) in Teflon closed vessel at 300 W power.

General procedure for preparation of **3** and **1a**,**b**

Preparation of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives was performed by a rapid, simple, and green procedure reported previously by us (Scheme 1). In general, a few drops of concentrated hydrochloric acid were added to a mixture of the appropriate 1,2-diketone (1 eq) and thiosemicarbazide (1.2 eq) in an appropriate ratio of water and ethanol and then the mixture was irradiated under microwave conditions for 10 min at 120 °C (300 W). At the end of this time, the precipitated solid was filtered and washed with water several times to give the desired product (Irannejad *et al.*, 2014).

General procedure for preparation of compound **2a**–**d** and **4a**,**b**

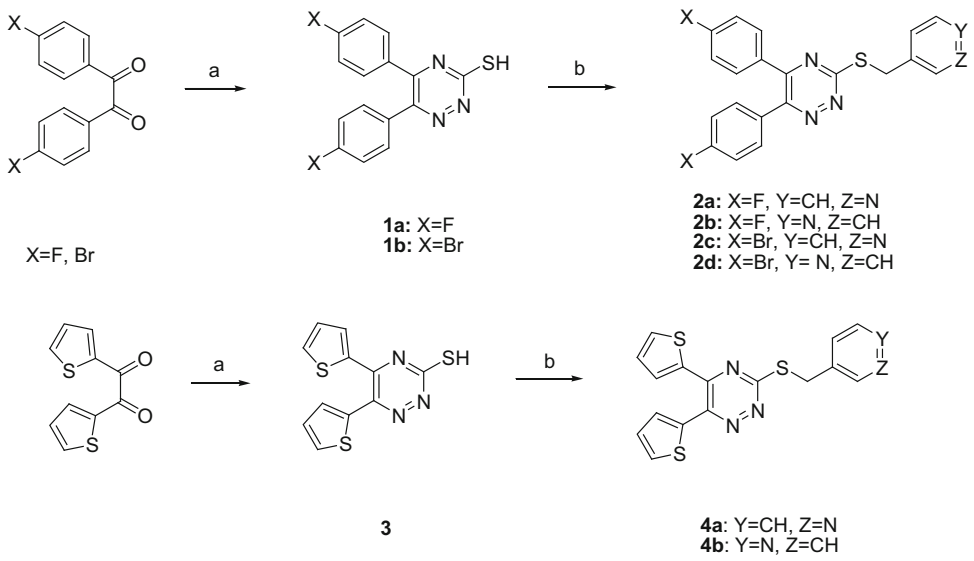
To a stirred solution of compound **3** or **1a**,**b** (1.7 mmol) in 20 ml of ethanol, sodium hydroxide solution (170 mg in 5 ml of water) and appropriate chloromethylpyridinium chloride (2 mmol) were added, and the resulting mixture was stirred at room temperature for 3 h. At the end of this time, it was concentrated under vacuum and neutralized with HCl 1 N, and the resulting aqueous phase was extracted with chloroform, dried with sodium sulfate. Upon concentrating the organic phase under vacuum, the resulting material was purified by column chromatography (silica gel, mesh 230–400) eluting with dichloromethane–methanol (9:1) (Irannejad *et al.*, 2014).

*5,6-Bis(4-fluorophenyl)-3-(pyridin-3-ylmethylthio)-1,2,4-triazine (**2a**)*

M.p.: 188–190 °C; IR (KBr): 3125, 2870, 1667 (C=N), 1560 (N=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.73 (s, 2H, SCH_2), 7.18 (d, $J = 7.24$ Hz, 2H, phenyl), 7.22 (d, $J = 7.28$ Hz, 2H, phenyl), 7.32 (dd, $J = 6.76$ Hz, 4.36 Hz, 2H, phenyl), 7.43 (dd, $J = 6.8$ Hz, 4.36 Hz, 2H, phenyl), 7.70 (d, $J = 6.12$ Hz, 1H, pyridine), 8.44 (d, $J = 3.6$ Hz, 1H, pyridine), 8.47 (s, 1H, pyridine). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 32.31, 115.10 (d, $J = 6.24$ Hz), 115.27 (d, $J = 6.24$ Hz), 123.49, 131.12 (d, $J = 6.79$ Hz), 131.96 (d, $J = 6.79$ Hz), 132.30, 136.30, 141.50, 148.05, 149.71, 154.32, 161.20, 162.38, 163.15, 164.36, 165.33. Anal. Calcd for $C_{21}H_{14}F_2N_4S$: C, 64.27; H, 3.60; N, 14.28. Found: C, 64.41; H, 3.53; N, 14.39.

*5,6-Bis(4-fluorophenyl)-3-(pyridin-4-ylmethylthio)-1,2,4-triazine (**2b**)*

M.p.: 172–174 °C; IR (KBr): 3,122, 2,884, 1,665 (C=N), 1,569 (N=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.78 (s, 2H, SCH_2), 7.11 (t, $J = 7.5$ Hz, 2H, Ar), 7.14 (t,



Scheme 1 Synthesis of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives. Reagents and conditions: *a* thiosemicarbazide, HCl, MW, 120 °C, 10 min; *b* 4-chloromethylpyridinium chloride or 3-chloromethylpyridinium chloride, NaOH, EtOH, rt

$J = 7.5$ Hz, 2H, Ar), 7.56 (dd, $J = 6.8$ Hz, 4.5 Hz, 2H, Ar), 7.59 (dd, $J = 6.8$ Hz, 4.5 Hz, 2H, Ar), 7.82 (d, $J = 4.7$ Hz, 2H, pyridine), 8.44 (d, $J = 4.8$ Hz, 2H, pyridine). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 34.33, 117.45 (d, $J = 6.29$ Hz), 117.56 (d, $J = 6.29$ Hz), 122.42, 133.57 (d, $J = 6.75$ Hz), 134.12 (d, $J = 6.75$ Hz), 134.77, 136.16, 137.29, 142.46, 147.28, 149.63, 157.51, 160.53, 162.07. Anal. Calcd for $C_{21}H_{14}F_2N_4S$: C, 64.27; H, 3.60; N, 14.28. Found: C, 64.32; H, 3.48; N, 14.12.

*5,6-Bis(4-bromophenyl)-3-(pyridin-3-ylmethylthio)-1,2,4-triazine (**2c**)*

M.p.: 212–214 °C; IR (KBr): 3,093, 2,897, 1,680 (C=N), 1,564 (N=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 3.72 (s, 2H, SCH_2), 7.22 (d, $J = 6.72$ Hz, 2H, phenyl), 7.31 (d, $J = 6.72$ Hz, 2H, phenyl), 7.51 (t, $J = 6.34$ Hz, 1H, pyridine), 7.55 (d, $J = 6.68$ Hz, 2H, phenyl), 7.59 (d, $J = 6.84$ Hz, 2H, phenyl), 7.70 (d, $J = 6.08$ Hz, 1H, pyridine), 8.44 (d, $J = 2.84$ Hz 1H, pyridine), 8.47 (s, 1H, pyridine). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 32.32, 122.26, 123.50, 124.57, 130.24, 130.92, 131.18, 131.24, 131.37, 133.90, 134.99, 136.30, 141.35, 148.04, 149.71, 154.53, 165.06. Anal. Calcd for $C_{21}H_{14}Br_2N_4S$: C, 49.05; H, 2.74; N, 10.90. Found: C, 49.28; H, 2.68; N, 10.54 s.

*5,6-Bis(4-bromophenyl)-3-(pyridin-4-ylmethylthio)-1,2,4-triazine (**2d**)*

M.p.: 209–211 °C; IR (KBr): 3,104, 2,833, 1,684 (C=N), 1,562 (N=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ: 4.6 (s, 2H, SCH_2), 7.40 (d, $J = 6.72$ Hz, 2H, phenyl), 7.42 (d, $J = 6.72$ Hz, 2H, phenyl), 7.63 (d, $J = 6.72$ Hz, 4H, phenyl), 7.51 (d, $J = 4.72$ Hz, 2H, pyridine), 8.51 (d, $J = 4.8$ Hz, 2H, pyridine). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 32.66, 123.17, 123.98, 124.85, 131.38, 131.53, 131.74, 133.92, 134.16, 146.70, 149.61, 153.37, 154.89, 169.24. Anal. Calcd for $C_{21}H_{14}Br_2N_4S$: C, 49.05; H, 2.74; N, 10.90. Found: C, 49.23; H, 2.64; N, 10.69.

*3-(Pyridin-3-ylmethylthio)-5,6-di(thiophen-2-yl)-1,2,4-triazine (**4a**)*

M.p.: 175 °C; IR (KBr): 3,086, 2,852, 1,671 (C=N), 1,523 (N=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.62 (s, 2H, SCH_2), 7.02 (d, $J = 3.2$ Hz, 1H, Ar), 7.14 (t, $J = 3.6$ Hz, 1H, Ar), 7.24 (t, $J = 3.6$ Hz, 1H, Ar), 7.34 (d, $J = 3.3$ Hz, 1H, Ar), 7.57 (d, $J = 4.0$ Hz, 1H, Ar), 7.87 (d, $J = 4.0$ Hz, 1H, Ar), 7.51 (t, $J = 4.5$ Hz, 1H, pyridine), 7.81 (d, $J = 4.0$ Hz, 1H, pyridine), 8.31 (s, 1H, pyridine), 8.43 (d, $J = 4.5$ Hz, 1H, pyridine). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 36.82, 125.93, 127.69, 128.45, 129.11, 129.27, 132.51, 132.59, 133.20, 134.62, 136.38, 138.63, 147.07, 148.03, 149.08, 150.51, 168.54. Anal. Calcd for $C_{17}H_{12}N_4S_3$: C, 55.41; H, 3.28; N, 15.20. Found: C, 55.69; H, 3.16; N, 15.49.

*3-(Pyridin-4-ylmethylthio)-5,6-di(thiophen-2-yl)-1,2,4-triazine (**4b**)*

M.p.: 178–180 °C; IR (KBr): 3,091, 2,899, 1,681 (C=N), 1,556 (N=N). ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 3.68 (s, 2H, SCH_2), 6.96 (d, $J = 3.0$ Hz, 1H, Ar), 7.08 (t, $J = 3.96$ Hz, 1H, Ar), 7.18 (t, $J = 4.08$ Hz, 1H, Ar), 7.30 (d, $J = 2.48$ Hz, 1H, Ar), 7.80 (d, $J = 4.16$ Hz, 1H, Ar), 7.95 (d, $J = 4.16$ Hz, 1H, Ar), 7.37 (d, $J = 4.6$ Hz, 2H, pyridine), 8.49 (d, $J = 4.6$ Hz, 2H, pyridine). ^{13}C NMR (100 MHz, DMSO-d_6) δ: 38.48, 126.82, 127.43, 128.06, 129.22, 129.31, 131.58, 132.49, 134.82, 135.25, 145.67, 147.71, 148.73, 148.86, 166.72. Anal. Calcd for $C_{17}H_{12}N_4S_3$: C, 55.41; H, 3.28; N, 15.20. Found: C, 55.53; H, 3.41; N, 15.38.

Docking study

The homology model of the Diazepam bound $GABA_A$ receptor developed by Richter et al. (2012) and lamotrigine bound Na+ channel by Lipkind and Fozzard (2010) was retrieved from the supplementary material of their published paper.

Protein structure was prepared with Pymol 1.6.0 (The PyMOL Molecular Graphics System, Schrödinger, LLC). HyperChem v.8, with AM1 force field was used to generate the lowest energy conformations. Docking study has been performed with AutoDock 4.2 software, using AutoDock Tools (ADT) in MGLTools 1.5.6. For the protein and the ligand, Kollman and Gasteiger charges were computed, respectively. The Lamarckian genetic algorithm (LGA) was used as a search engine. AutoGrid 4.2 was utilized for defining active site, and the grid size was set to $20 \times 20 \times 20$ points with a grid spacing of 1.0 on the center of the X-ray ligand in the crystal structure complex. The other parameters were as follows:

Maximum number of energy evaluations: 2,500,000, independent GA runs: 20, maximum number of LGA operations: 27,000, population size: 150, crossover: 0.80, mutation: 0.02, elitism: 1.

Pharmacology

In vivo seizure test was performed on male NMRI mice weighting 20–30 g (Pasteur Institute, Tehran, Iran). Mice were kept in animal facility at controlled temperature (22 ± 2 °C) with 12–12 h light–dark cycle and free access to food and water. The behavioral experiments were done between 9 a.m. and 4 p.m. Each mouse was used only once

in all experiments. Solutions were prepared in a vehicle consisted of polyethyleneglycol (PEG) 30 % v/v in distilled water and were administered at the dose of 100 mg/kg to mice. All compounds (or vehicle in control group) were administered through intraperitoneal (*i.p.*) injection 30 min before performing seizure test. Procedures involving animals were conducted in accordance with the Shahid Beheshti University Guidelines for Care and Use of Laboratory Animals.

Pentylenetetrazole (PTZ)-induced seizure test

Thirty minutes after intraperitoneal injection (at a volume of 10 ml/kg) of synthesized compounds or vehicle (PEG 30 % v/v in distilled water), animals were subject to PTZ-induced seizure test. The threshold for clonic seizures was determined by infusion of a 1 % solution of PTZ in distilled water through the tail vein of unrestricted freely moving mouse at a constant rate of 0.25 ml/min with an infusion pump (model 53140, Stoelting, USA). The required dose of PTZ (in mg/kg) that caused generalized clonic seizure with loss of righting reflex was considered as the threshold for the clonic seizure (Naderi *et al.*, 2011).

Maximal electroshock (MES)-induced seizure test

Electroshock was induced by generating an alternating current (intensity 40 mA, pulse duration 0.2 s, frequency 50 Hz, pulse width 0.5 ms) through ear clip electrodes by a stimulator (Borj Sanat, Iran). In order to improve electrode conduction, electrodes were moistened by saline before attaching to the ear of mouse. The end-point for the occurrence of seizure was the observation of hind-limb tonic extension (HLTE) in mice (Woodbury and Davenport, 1952); (Woodbury and Swinyard, 1952). To select proper current intensity for electroconvulsions, 5 groups of mice consisting of five animals per group were challenged with electroshocks of various intensities (25, 30, 35, 40, and 45 mA) that yield seizure in minimum of 20 % and maximum of 100 % of animals in a current-dependent manner. After plotting a current intensity-seizure curve, the CS97 (40 mA), which was a current intensity that produced HLTE in 97 % of animals, was determined according to log-Probit method by Litchfield and Wilcoxon (1949) and was applied in all electroshock experiments. Data were shown as percent protection, which is the percentage of animals in each group that did not have HLTE after receiving electroshock.

Rota-rod test

To evaluate the effect of compounds on motor coordination, rota-rod test was conducted before seizure test. The animals were placed on rods (rotating at the speed of 10 rounds per minute). Failure to keep staying on the rotating rod for a period of 90 s was considered as drug-induced motor impairment.

Statistical analysis

Statistical analysis was done using GraphPad Prism 5.0 (GraphPad Software Inc., CA, USA). In PTZ test, the results were shown as mean ± SEM. One-way analysis of variance (ANOVA) followed by Dunnett's post-test was used to compare various groups with the control group. In MES test, data were analyzed using Fisher's exact test. p values less than 0.05 were considered as statistically significant.

15-Lipoxygenase inhibition assay

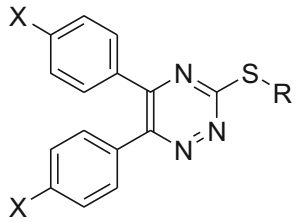
LOX inhibitory activity of target compounds was studied by a spectrophotometric assay. The tested compounds were dissolved in DMSO (1 ml) and phosphate buffer (9 ml, 0.1 M, pH 8). This stock solution was added to test solution containing enzyme (final concentration: 167 U/ml) and phosphate buffer (pH 8) to achieve concentrations in range of 10^{-3}–10^{-6} M. After incubation of test solution for 4 min, substrate (linoleic acid, final concentration: 134 μM) was added, and change in the absorbance was measured for 60 s at 234 nm. The enzyme solution was kept in ice, and controls were measured at intervals throughout the experimental periods to ensure that the enzyme activity was constant (Assadieskandar *et al.*, 2012).

Results and discussion

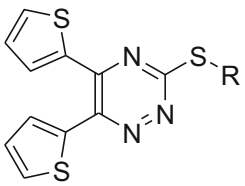
Antiseizure effects of compounds in PTZ and MES-induced seizure test in mice

Protective effect of new compounds against convulsion was evaluated in MES and PTZ-induced seizures in mice. The required dose of PTZ to induce seizure, 30 min after the intraperitoneal administration of drugs, through continuous *i.v.* infusion for each compound is shown in Table 1. One-way ANOVA revealed a significant change in the dose of PTZ required for seizure induction between different groups [$F(7,41) = 2.567$; $p = 0.027$]. Further analysis using Dunnett's test revealed a significant increase in PTZ dose for seizure induction in mice pretreated with compound **2c** (70 mg/kg, $p < 0.05$) compared with the control group. Compound **2c** bearing bromine atom at para position of phenyl rings and 3-pyridylmethylthio side chain had the highest protective effect in PTZ-induced seizure

Table 1 Effects of *i.p.* administration of compounds in protection against PTZ and MES-induced seizures in mice



2a-d



4a,b

Compound	X	R	% protection in MES	PTZ dose (mg/kg)
2a	F	3-Pyridylmethyl	20	41
2b	F	4-Pyridylmethyl	58**	52
2c	Br	3-Pyridylmethyl	60**	70*
2d	Br	4-Pyridylmethyl	80**	59
4a	–	3-Pyridylmethyl	71**	56
4b	–	4-Pyridylmethyl	40	66
1a	F	H	40	43
1b	Br	H	34	47
3	–	H	0	33
Control	–	–	0	45
Lamotrigine	–	–	100**	82***

p values are related to each group compared with the control group

* $p < 0.05$ significant difference compared with the control group; ** $p < 0.01$ significant difference compared with the control group; *** $p < 0.005$ significant difference compared with the control group

test among all compounds. The required dose of PTZ to induce seizure in control group was 45 mg/kg and in group receiving the reference drug lamotrigine was 82 mg/kg. Compound **4b** was in the second order of protective effect and required 66 mg/kg of PTZ to induce seizure. The other compounds had less anticonvulsive effect especially compounds **3** and **1a**,**b** without the pyridylmethyl side chain on thiol group.

The results of MES-induced seizure test are also shown in Table 1. Control group (receiving PEG 30 % in water) did not show any protective effect against MES-induced seizures, while group receiving the reference drug lamotrigine revealed 100 % protection. Among compounds tested, compound **2d** with bromine atom at phenyl rings had the highest protective effect up to 80 % with *p* value less than 0.01. Compounds **4a**, **2c,** and **2b** could protect animals up to 71, 60, and 58 %, respectively (*p* value <0.01). Similar to PTZ-induced seizure test, compounds **3** and **1a**,**b** without the pyridylmethyl side chain on thiol group had the least protective effect against MES-induced seizures. Obtained results clearly indicated that the pyridylmethyl side chain is necessary for optimal anticonvulsive effect, and a lipophilic group such as bromine atom at para positions of phenyl rings could improve the protective effect of compounds. The results of rota-rod test revealed no drug-induced motor impairment at the administered dose of 100 mg/kg (data not shown).

The results of anticonvulsant evaluation of compounds revealed that presence of a lipophilic atom such as bromine at *para* position of phenyl rings could improve the antiseizure ability of compounds **2c** and **2d**. Presence of thiophene ring instead of 4-bromophenyl moiety has also increased the anticonvulsant property in compound **4a** due to its lipophilic character. Similar results were obtained in our previous study on 1,2,4-triazine-substituted derivatives in which lipophilic substituents such as chloro atom and methyl group at para position of phenyl ring showed higher anticonvulsant action (Irannejad *et al.*, 2014). Presence of a suitable substitution at thiol group is necessary for optimum antiseizure property. According to our previous study on new antiepileptic compounds, 3-pyridylmethyl or 4-pyridylmethyl groups could increase antiepileptic character of compounds and are preferred over methyl group or non-substituted compounds at thiol groups (compounds **1a**,**b** and **3**). Investigation of structure–activity relationship of 5,6-diaryl-1,2,4-triazine derivatives as new anticonvulsant compounds has shown that incorporation of groups capable of constituting polar interactions such as hydrogen bonds (in this case, nitrogen atom of pyridine ring) can substantially increase the anticonvulsant effect.

Molecular docking study

In order to find out their possible mechanism of anticonvulsion, molecular docking was performed on the two main targets of conventional anticonvulsant drugs, Na+ channel and $GABA_A$ receptor. Compound **2d** was chosen for this purpose due to its high anticonvulsive property.

Na+ channel is a multisubunit complex. Channel's pore and especially the principal drug-binding site are composed of α-subunit with approximately 2,000 amino acids. Four highly homologous domains (I–IV) make the α-subunit, and each domain is composed of six presumably transmembrane α-helical segments (S1–S6), assembled in a clockwise pattern around the central pore. This pore has a large outer vestibule, formed by the four extracellular P loop segments. These P loops fold back into the membrane to make a shallow funnel so that in deeper part becomes narrow and makes the selectivity filter with 4 × 6 Å size.

The large outer vestibule of this pore was used as binding site for docking of anticonvulsant drugs. After docking calculation, compound **2d** was located in the middle of sodium channel and could occlude the inner pore

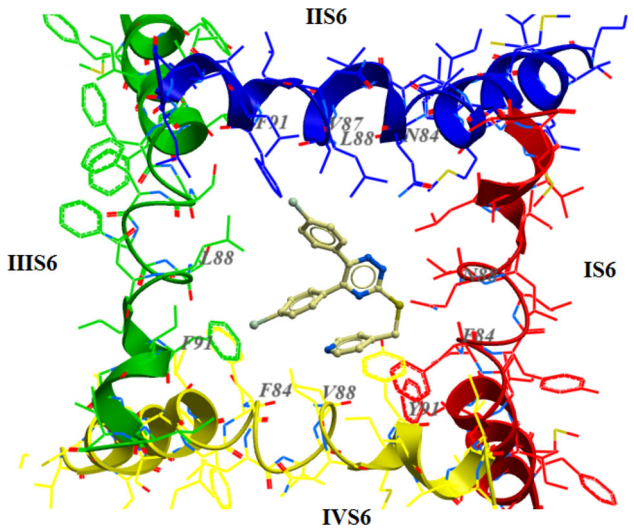


Fig. 3 Location of compound **2d** inside the inner pore of the Na+ Channel (*top view*). The backbones of S6 α-helices of domains I–IV are shown by *red*, *blue*, *green*, and *yellow ribbons*, respectively. The *same colors* are used for amino acid residues in each domain (Color figure online)

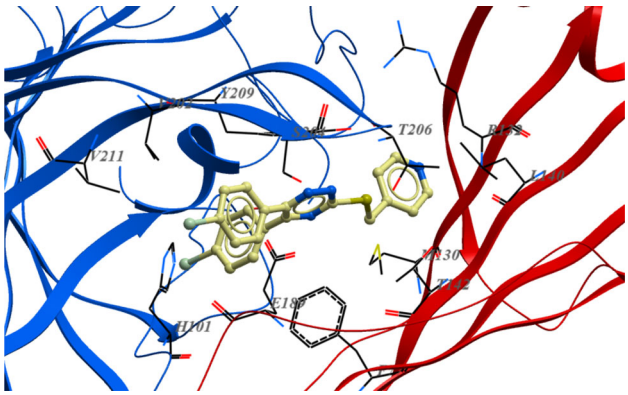


Fig. 4 Binding mode of compound **2d** in α1–γ2 subunits interface. The α-subunit is shown in *blue* and γ-subunit in *red* (Color figure online)

as shown in Fig. 3. Binding mode of compound **2d** showed that 4-bromophenyl moieties of this compound are interacting with Phe91 and Leu88 of IIS6 and IIIS6 and Phe84 and Val88 in IVS6 through hydrophobic and π–π interactions. Pyridyl ring has twisted toward Val87, Val88, and Tyr91 of IVS6 and Phe84 from IS6 and constitutes van der waals interactions mainly by its aromatic π electrons. Finally, triazine ring is hydrogen bonded to Asn88 side chain in IS6. The estimated free energy of binding to sodium channel was −6.38 kcal/mol.

$GABA_A$ receptor is one of the main targets for anticonvulsive drugs such as benzodiazepines. The majority of $GABA_A$ receptors are composed of α, β, and γ-subunits. The benzodiazepine-binding site of these receptors is located extra-cellularly at α1–γ2 interface. Molecular docking was performed on the benzodiazepine-binding site of $GABA_A$ receptor.

Binding mode of compound **2d** is visualized in Fig. 4. As shown, one 4-bromophenyl part of ligand is interacted with Tyr159 and His101 at α1-subunit, while the other 4-bromophenyl group is in contact with α1-Val202 and Phe77 at δ2-subunit. Triazine ring has π–π stacking with α1-Tyr209, and the protonated nitrogen atom of pyridine ring at physiological pH is ionic bonded to δ2-Arg132. The free energy of binding was estimated to be −11.74 kcal/mol, in benzodiazepine-binding site in $GABA_A$ receptor. The higher free energy of binding for compound **2d** in $GABA_A$ receptor than Na+ channel may propose its possible mechanism of anticonvulsant action through binding to benzodiazepine-binding site on $GABA_A$ receptor. On the other hand, compound **2d** and its congeners (compounds **2a–d** and **4a**,**b**) possibly bind to the benzodiazepine-binding site on $GABA_A$ receptor to exert their antiseizure action as revealed by the binding free energy calculation. However, other important anticonvulsant targets such as glutamate receptors and calcium channels are not evaluated here, but it remains to be cleared in our next study.

In vitro 15-LOX inhibition assay

As discussed earlier in this section, 15-lipoxygenase (15-LOX) is a key enzyme in initiation of oxidative stress in neurodegenerative diseases. Thus, inhibition of 15-LOX by synthesized 1,2,4-triazine-substituted derivatives was assessed, and the results are shown in Table 2. All compounds showed mild to moderate inhibition of 15-LOX at about 100 μM, while quercetin as a reference drug inhibited the 15-LOX at 18 μM. Compound **2c** with highest anticonvulsive property in PTZ test could inhibit 15-LOX at 89 μM which was again the best one among compounds. There was not a significant difference in potency among compounds against 15-LOX, and the IC50 values were

Table 2 The IC_{50} values of the target compounds against soybean 15-lipoxygenase

Compound	15-LOX (IC_{50} μM)
2a	119.8 ± 7.33
2b	97.88 ± 7
2c	89.3 ± 6.5
2d	105 ± 6.69
4a	98.12 ± 6.98
4b	112 ± 5.57
1a	NA
1b	NA
3	NA
Quercetin	18 ± 0.9

NA not-active

ranging from 89 to 119 μM due to their high structural similarity protective effect of compounds against MES and PTZ-induced seizures along with their moderate inhibition of 15-LOX may provide synergistic effect in prevention of neurodegenerative diseases progression and to introduce new generation of anticonvulsant agents.

Prediction of blood–brain barrier permeability

Blood–brain barrier (BBB) permeability is one of the most important challenges in the pharmacology of CNS active drugs. The extent of CNS penetration has limited usage of many drugs whose target is located in the central nervous system and high percentage of CNS-acting drugs fail to pass the clinical trials annually due to the CNS penetration failure. This drawback has imposed high expenses at pharmaceutical and drug development companies nowadays. Therefore, evaluating the capability of new compounds to pass the BBB by in silico methods would be inevitable and helpful.

Several "rules of thumb" have been developed for prediction of passive BBB permeability. Compounds are more likely to penetrate the BBB if the following criteria meet. (1) Molecular weight (MW) less than 400–500 Da. (2) LogD (logarithm of distribution coefficient) between −1 and 4. (3) Number of total hydrogen bonds (nHB) less than 10. (4) Polar surface area (TPSA) less than 90 $Å^2$. Increasing the number of rules which are not obeyed by one compound decreases the possibility of its BBB permeability (Waterbeemd *et al.*, 1998).

The calculated physiochemical parameters for compounds **2a**–**d** and **4a**,**b** are shown in Table 3 along with their predicted values of logBB (logarithm of brain/plasma concentration) and CNS activity by ACD/Lab. As shown, values of MW are almost in the defined range (<500 Da), but with a minor and negligible increase for compounds **2c** and **2d**, but predicted log*D* values for these two compounds (5.96 and 5.94, respectively) are located a bit far from the maximum allowed value of 4. As seen in this table, number of total hydrogen bonds for all compounds is less than 10. However, some predicted values such as log*D* of compounds **2c**,**d** and TPSA for **4a**,**b** fall a bit out of the range, but it does not mean that these compounds have lack of CNS activity and only may reduce their extent of passage through BBB. Moreover, this fact was further confirmed by the predicted values of logBB and CNS activity. Finally, considering the *pred.* logBB values of compounds **2a**–**d** and **4a**,**b** reported in Table 3, all of them were predicted to be CNS active.

Table 3 Calculated physicochemical parameters for compounds **2a**–**d** and **4a**,**b** and prediction of their CNS activity

Compound	MW	log*D*[a] (pH 7.4)	nHB[a]	TPSA[a]	*pred.* logBB[a]	*pred.* CNS activity[a]
2a	392.4	4.11	4	76.8	−0.25	Active
2b	392.4	4.09	4	76.8	−0.26	Active
2c	514.2	5.96	4	76.8	−0.25	Active
2d	514.2	5.94	4	76.8	−0.26	Active
4a	368.5	4.3	4	133.3	−0.41	Active
4b	368.5	4.28	4	133.3	−0.41	Active

nHB number of total hydrogen bonds (acceptors + donors), *logD* logarithm of distribution coefficient, *logBB* logarithm of brain/plasma concentration

[a] Calculated or predicted by ACD/Lab (www.acdlab.com)

Conclusion

Development of novel anticonvulsive drugs has become a challenge of the new century. Available anticonvulsive drugs are not effective and responsive in most cases. Therefore, introduction of novel and multitargeted anticonvulsive agents offers new opportunity to overcome convulsions. In this study, some new 1,2,4-triazine derivatives were evaluated as dual anticonvulsive and 15-lipoxygenase inhibitors. Molecular docking study revealed their possible antiseizure mechanism of action through $GABA_A$ receptor. Finally, in silico assessment of their BBB permeability indicated them as CNS active agents. Future studies of these novel neuroprotective inhibitors of 15-LOX may provide new therapeutic opportunities to combat convulsion and neurodegenerative diseases.

Acknowledgments We gratefully acknowledge the financial support from the Research Council of Mazandaran University of Medical Sciences, Sari, Iran.

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