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Synthesis and Anticonvulsant Evaluation of some New 6-(Substituted-phenyl)thiazolo[3,2-b][1,2,4]triazole Derivatives in Mice

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Abstract

Epilepsy is the most frequent neurological affliction and afflicts 1% about of the worlds population. Currently there is an urgent need for the development of novel anticonvulsants with higher levels of potency and lower levels of toxicity. In this paper, a series of new 6-(substituted-phenyl)thiazolo[3,2-b][1,2,4]triazole derivatives were synthesized and tested for their anticonvulsant activities using the maximal electroshock (MES) and subcutaneous pentylentetrazole (PTZ) screens, which are the most widely employed seizure models for early identification of candidate anticonvulsants. Their neurotoxicity was determined applying the rotarod test. In these compounds, 6-(4-fluorophenyl)thiazolo[3,2-b][1,2,4]triazole (3c) showed selective protection against the MES seizures with an \(ED_{50}\) value of 49.1 mg/Kg and a \(TD_{50}\) value of 94.1 mg/Kg, which provided compound 3c a protective index (PI = \(TD_{50}/ED_{50}\)) of 1.9 in the MES test. 6-(4-Propoxyphenyl)thiazolo[3,2-b][1,2,4]triazole (5b) was found to be active in both models, i.e. MES test and PTZ test. In the PTZ screen, compound 5b gave an \(ED_{50}\) of 63.4 mg/Kg and a \(TD_{50}\) of 105.6 mg/Kg, resulting in a PI value of 1.7 which is higher than carbamazepine.

Keywords: Synthesis; Anticonvulsant; Thiazolotriazole; Maximal electroshock; Pentylenetetrazole.

Introduction

Epilepsy is one of the most common neurological disorders, which is characterized by excessive temporary neuronal discharge resulting in recurrent unprovoked seizures (1, 2). It has been reported that about 1% of the world’s population (about 50 million people worldwide) are suffering with this neurological disorder at any one time (2). In recent years, significant efforts have been invested in the development of novel therapeutics, resulting in the emergence of several novel drugs as promising anticonvulsant agents (3, 4). However, the currently available anticonvulsants are effective in reducing the severity and frequency of seizures in less than 70% of patients. Up to 30% of patients are poorly treated with the available anticonvulsants (5, 6). Moreover, their usage is often associated with numerous undesirable side-effects (7-12). High levels of toxicity and intolerance, and a lack of efficacy also represent further limitations of the current anticonvulsant agents. With all of this in mind, there is an urgent need for the development of novel antiepileptic drugs (AEDs) with higher levels of potency and lower levels of toxicity.

1,2,4-Triazoles represent a key structure motif...
These compounds designed contained a hydrophobic unit (R), an electron donor group (D), and a hydrogen donor/acceptor unit (HAD), which are the major characteristics associated with good anticonvulsant activity for the currently used anticonvulsant agents (as shown in Figure 1) (27).

Experimental

The process of the synthesis and pharmacology

The target compounds (3a-3l and 5a-5l) were synthesized according to the route depicted in Scheme 1. Briefly, 6-phenylthiazolo[3,2-b][1,2,4]triazole derivatives were designed with an intention to synergize the anticonvulsant activity of 1,2,4-triazole and thiazole moiety in this paper. A benzene ring with substituents was introduced to the thiazole ring to increase the hydrophobicity of the whole structure.

These compounds designed contained a hydrophobic unit (R), an electron donor group (D), and a hydrogen donor/acceptor unit (HAD), which are the major characteristics associated with good anticonvulsant activity for the currently used anticonvulsant agents (as shown in Figure 1) (27).

Figure 1. Pharmacophoric characteristics of well known antiepileptics and target compounds with the vital structural features: (A) hydrophobic unit, (D) electron donor group, hydrogen bond acceptor/donor unit (HAD).
2l) in the presence of polyphosphoric acid (28). The intermediates 2a-2l were easily prepared by the reaction of various phenacyl bromide with [1,2,4]triazole-3-thione in boiling ethanol. Compounds 5a-5l was achieved by reacting compound 4 with halogenated hydrocarbon in acetonitrile in the presence of K₂CO₃. The compound 4 was smoothly got by treating the compound 3l with boron tribromide. The structures of the desired compounds were confirmed by IR, ¹H NMR, mass spectral and elemental analyses. The physicochemical properties of them are presented in the experimental section. Their anticonvulsant activities were all evaluated by maximal electroshock test (MES) and pentylenetetrazole (PTZ) model in mice, and their neurotoxicity were evaluated with the rotarod test.

**Materials and methods**

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a IRPrestige-21. ¹H-NMR spectra were measured on an AV-300 (Bruker, Fällanden, Switzerland), and all chemical shifts were given in ppm relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, Santa Clara, CA, USA). Elemental analyses were performed on a 204Q CHN (Perkin Elmer, Fremont, CA, USA). The major chemicals were purchased from Aldrich Chemical Corporation (Shanghai, China).

**Preparation of compounds** 3-(substituted-phenacyl)thio[1,2,4]triazoles (2a-2l).

[1,2,4]Triazole-3-thione (0.5 g, 0.005 mol) was added to a solution of substituted-bromoacetophenone 1a-1l (0.77 g, 0.005 mol) in ethanol and the mixture was stirred under reflux for 4 hours. After cooling, the precipitated product was filtered and recrystallized from water to give 3-(substituted-phenacyl)thio[1,2,4] triazoles (2a-2l) in good yield.

6-(substituted-phenyl)thiazolo[3,2-b][1,2,4]triazoles (3a-3l).

3-(Substituted-phenacyl)thio[1,2,4]triazoles 2a-2l (0.04 mol) and polyphosphoric acid (8 g) were heated at 120 °C for 2 hours. Then an aqueous solution of sodium hydrogen carbonate was added and the mixture was stirred under reflux for 4 hours. After cooling, the precipitated product was filtered and recrystallized from water to give 6-(substituted-phenyl)thiazolo[3,2-b][1,2,4] triazoles (3a-3l).

6-(4-hydroxy-phenyl)thiazolo[3,2-b][1,2,4]triazole (4)

6-(4-Methoxy-phenyl)thiazolo[3,2-b][1,2,4]triazole (3l) (2.3 g, 0.01 mol) was dissolved in 50 mL dichloromethane. BBr₃ (0.03 mol) was added dropwise to the solution and the mixture was stirred at room temperature. After 4 h the
mixture was added slowly 20 mL ice cold water and allowed to stir for half hour. After removing the dichloromethane under reduced pressure, the resulting white precipitate was obtained by filtration.

6-(4-(alkoxy)phenyl)thiazolo[3,2-b][1,2,4]triazoles (5a-5l).

K₂CO₃ (1.24 g, 0.009 mol) and 6-(4-hydroxy-phenyl)thiazolo[3,2-b][1,2,4]triazole 4 (0.003 mol) were dissolved in acetonitrile (50 mL) and refluxed for 30 min. Then alkyl bromide or benzyl chloride derivatives (0.0033 mol) were added into the mixture accompanied with some of benzyltriethylamine chloride (TEBA). The reaction mixture was stirred under reflux for 4-10 hours. After removing the solvent, 100 mL of water was added into the flask, which was extracted with dichloromethane (30 mL×3). The combined layer of dichloromethane was dried by anhydrous MgSO₄. Evaporation of the solvent gave a crude product, which was purified by silica gel column chromatography with CH₂Cl₂-CH₃OH (100:1) to a white solid. The yield, melting point and spectral data of each compound were given below.

6-phenylthiazolo[3,2-b][1,2,4]triazole (3a)
Mp 208-210 °C, yield 36.8 %, Mol. weight: 201.25. ¹H-NMR (CDCl₃, 300 MHz): δ 7.14 (s, 1H, thiazole-H), 7.47-7.56 (m, 3H, Ar-H), 8.06-8.23 (m, 2H, Ar-H), 8.24 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1634 1628 (C=N), 1175 (N-N). MS m/z 202 (M+1).

6-(2-fluorophenyl)thiazolo[3,2-b][1,2,4]triazole (3b)
Mp 100-102 °C, yield 62.2 %, Mol. weight: 235.69. ¹H-NMR (CDCl₃, 300 MHz): δ 7.20 (s, 1H, thiazole-H), 7.45-7.48 (m, 2H, Ar-H), 8.23 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1636 1619 (C=N), 1168 (N-N). MS m/z 236 (M+1). Anal. Calcd. for C₁₀H₆F₃N₃S: C, 54.78; H, 2.76; N, 19.17. Found: C, 54.54; H, 2.57; N, 19.34.

6-(4-fluorophenyl)thiazolo[3,2-b][1,2,4]triazole (3c)
Mp 100-102 °C, yield 62.2 %, Mol. weight: 235.69. ¹H-NMR (CDCl₃, 300 MHz): δ 7.10 (s, 1H, thiazole-H), 7.22 (d, 2H, J = 8.6 Hz, Ar-H), 8.09 (d, 2H, J = 8.6 Hz, Ar-H), 8.23 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1624 1611 (C=N), 1181 (N-N). MS m/z 220 (M+1). Anal. Calcd. for C₁₀H₁₂F₂N₃S: C, 54.78; H, 2.76; N, 19.17. Found: C, 54.54; H, 2.57; N, 19.34.

6-(2-bromophenyl)thiazolo[3,2-b][1,2,4]triazole (3d)
Mp 100-102 °C, yield 62.2 %, Mol. weight: 280.14. ¹H-NMR (CDCl₃, 300 MHz): δ 7.22 (s, 1H, thiazole-H), 7.37-7.48 (m, 2H, Ar-H), 7.70-7.77 (m, 2H, Ar-H), 8.17 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1634 1564 (C=N), 1179 (N-N). MS m/z 280 (M⁺). Anal. Calcd. for C₁₀H₁₀BrN₃S: C, 42.87; H, 2.16; N, 15.00. Found: C, 43.13; H, 2.02; N, 15.26.
6-(3-bromophenyl)thiazolo[3,2-b][1,2,4]triazole (3h)

Mp 156-158 °C, yield 64.6 %, Mol. weight: 280.14. 1H-NMR (CDCl3, 300 MHz): δ 7.20 (s, 1H, thiazole-H), 7.37-7.62 (m, 2H, Ar-H), 8.05-8.26 (m, 2H, Ar-H), 8.25 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1634 1570 (C=N), 1176 (N-N). MS m/z 280 (M⁺). Anal. Calcd. for C10H8BrN2S: C, 42.87; H, 2.16; N, 15.00. Found: C, 43.10; H, 2.07; N, 15.21.

6-(4-bromophenyl)thiazolo[3,2-b][1,2,4]triazole (3i)

Mp 184-186 °C, yield 60.4 %, Mol. weight: 280.14. 1H-NMR (CDCl3, 300 MHz): δ 7.17 (s, 1H thiazole-H), 7.65 (d, 2H, J = 8.6 Hz, Ar-H), 7.99 (d, 2H, J = 8.6 Hz, Ar-H), 8.23 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1630 1583 (C=N), 1167 (N-N). MS m/z 280 (M⁺). Anal. Calcd. for C10H8BrN2S: C, 42.87; H, 2.16; N, 15.00. Found: C, 42.74; H, 2.22; N, 15.13.

6-(4-nitrophenyl)thiazolo[3,2-b][1,2,4]triazole (3j)

Mp 224-226 °C, yield 67.4 %, Mol. weight: 246.25. 1H-NMR (CDCl3, 300 MHz): δ 7.42 (s, 1H thiazole-H), 8.28 (s, 1H, triazole-H), 8.38 (s, 4H, Ar-H). IR (KBr) cm⁻¹: 1642 1635 (C=N), 1180 (N-N). MS m/z 247 (M⁺). Anal. Calcd. for C10H6BrN2O2S: C, 48.78; H, 2.46; N, 22.75. Found: C, 48.55; H, 2.61; N, 22.89.

6-(4-methylphenoxy)thiazolo[3,2-b][1,2,4]triazole (3k)

Mp 176-178 °C, yield 42.3 %, Mol. weight: 215.27. 1H-NMR (CDCl3, 300 MHz): δ 2.42 (s, 3H, Ar-CH₃), 7.08 (s, 1H, thiazole-H), 7.32 (d, 2H, J = 8.1 Hz, Ar-H), 7.97 (d, 2H, J = 8.1 Hz, Ar-H), 8.22 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1611 1578 (C=N), 1179 (N-N). MS m/z 216 (M⁺). Anal. Calcd. for C₁₀H₁₀N₂S: C, 61.37; H, 4.21; N, 19.52. Found: C, 61.18; H, 4.34; N, 19.74.

6-(4-methoxyphenoxy)thiazolo[3,2-b][1,2,4]triazole (3l)

Mp 178-180 °C, yield 45.2 %, Mol. weight: 231.27. 1H-NMR (CDCl3, 300 MHz): δ 3.87 (s, 3H, Ar-CH₂O), 7.00 (s, 1H, thiazole-H), 7.04 (d, 2H, J = 8.8 Hz, Ar-H), 8.02 (d, 2H, J = 8.8 Hz, Ar-H), 8.22 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1607 1573 (C=N), 1182 (N-N). MS m/z 232 (M⁺). Anal. Calcd. for C₁₀H₁₀N₂O₂S: C, 57.13; H, 3.92; N, 18.17. Found: C, 57.35; H, 4.10; N, 18.33.

6-(4-hydroxy-phenyl)thiazolo[3,2-b][1,2,4]triazole (4)

Mp 232-234 °C, yield 79.8 %, Mol. Weight: 217.25. 1H-NMR (DMSO-d₆, 300 MHz): δ 4.83 (s, 1H, Ar-ΟΗ), 6.99 (s, 1H, thiazole-H), 7.04 (d, 2H, J = 8.7 Hz, Ar-H), 7.94 (d, 2H, J = 8.7 Hz, Ar-H), 8.21 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1614 1566 (C=N), 1173 (N-N). MS m/z 218 (M⁺). Anal. Calcd. for C₁₀H₈N₂O₂S: C, 55.29; H, 3.25; N, 19.34. Found: C, 55.04; H, 3.18; N, 19.61.

6-(4-ethoxyphenyl)thiazolo[3,2-b][1,2,4]triazole (5a)

Mp 122-124 °C, yield 87.4 %, Mol. Weight: 245.30. 1H-NMR (CDCl3, 300 MHz): δ 1.45 (t, 3H, J = 6.9 Hz, CH₃), 4.10 (q, 2H, J = 6.9 Hz, OCH₂), 7.00 (s, 1H, thiazole-H), 7.02 (d, 2H, J = 8.8 Hz, Ar-H), 8.01 (d, 2H, J = 8.8 Hz, Ar-H), 8.22 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1606 1569 (C=N), 1181 (N-N). MS m/z 246 (M⁺). Anal. Calcd. for C₁₀H₁₀N₂O₂S: C, 58.76; H, 4.52; N, 17.13. Found: C, 58.98; H, 4.40; N, 17.01.

6-(4-propoxyphenyl)thiazolo[3,2-b][1,2,4]triazole (5b)

Mp 82-84 °C, yield 89.3 %, Mol. Weight: 259.33. 1H-NMR (CDCl3, 300 MHz): δ 1.06 (t, 3H, J = 7.3 Hz, CH₃), 1.81-1.88 (m, 2H, CH₂), 3.99 (q, 2H, J = 6.5 Hz, OCH₂), 6.99 (s, 1H, thiazole-H), 7.02 (d, 2H, J = 8.5 Hz, Ar-H), 8.01 (d, 2H, J = 8.5 Hz, Ar-H), 8.22 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1602 1569 (C=N), 1158 (N-N). MS m/z 260.3 (M⁺). Anal. Calcd. for C₁₀H₁₁N₂O₃S: C, 60.21; H, 5.05; N, 16.20. Found: C, 60.05; H, 5.16; N, 16.46.

6-(4-butoxyphenyl)thiazolo[3,2-b][1,2,4]triazole (5c)

Mp 102-104 °C, yield 87.1 %, Mol. Weight: 273.35. 1H-NMR (CDCl3, 300 MHz): δ 1.00 (t, 3H, J = 7.4 Hz, CH₃), 1.51-1.81 (m, 4H, (CH₂)₂), 4.03 (q, 2H, J = 6.5 Hz, OCH₂), 7.00 (s, 1H, thiazole-H), 7.03 (d, 2H, J = 8.9 Hz, Ar-H).
6-(4-(pentyloxy)phenyl)thiazolo[3,2-b][1,2,4]triazole (5d)

Mp 86-88 °C, yield 92.3 %, Mol. Weight: 315.43. 1H-NMR (CDCl3, 300 MHz): δ 0.94 (t, 3H, J = 6.6 Hz, Ar-H), 1.77-1.85 (m, 2H, CH2), 4.02 (q, 2H, J = 6.6 Hz, OCH2), 6.99 (s, 1H, triazole-H). IR (KBr) cm−1: 1597, 1143 (N-N). MS m/z 316 (M+1). Anal. Calcd. for C15H11N3O: C, 62.42; H, 6.12; N, 14.86. Found: C, 61.75; H, 5.44; N, 15.59.

6-(4-heptyloxy)phenyl)thiazolo[3,2-b][1,2,4]triazole (5e)

Mp 76-78 °C, yield 90.7 %, Mol. Weight: 316.38. 1H-NMR (CDCl3, 300 MHz): δ 0.90 (t, 3H, J = 7.0 Hz, CH3), 1.41-1.49 (m, 4H, (CH2)2), 1.77-1.85 (m, 2H, CH2), 4.02 (q, 2H, J = 6.6 Hz, OCH2), 6.99 (s, 1H, triazole-H). IR (KBr) cm−1: 1587, 1172 (N-N). MS m/z 317 (M+1). Anal. Calcd. for C16H21N3O: C, 64.73; H, 6.63; N, 13.51. Found: C, 64.95; H, 6.71; N, 13.32.

6-(4-(benzyloxy)phenyl)thiazolo[3,2-b][1,2,4]triazole (5f)

Mp 102-104 °C, yield 76.5 %, Mol. weight: 325.36. 1H-NMR (CDCl3, 300 MHz): δ 5.13 (s, 2H, OCH2), 7.01 (s, 1H, thiazole-H), 7.12 (m, 4H, Ar-H), 8.01 (d, 2H, J = 8.9 Hz, Ar-H), 8.22 (s, 1H, triazole-H). IR (KBr) cm−1: 1575, 1164 (N-N). MS m/z 326 (M+1). Anal. Calcd. for C17H16FN3O: C, 66.29; H, 4.35; N, 13.67. Found: C, 66.20; H, 4.35; N, 13.89.

6-(4-(2-chlorobenzyloxy)phenyl)thiazolo[3,2-b][1,2,4]triazole (5g)

Mp 98-100 °C, yield 80.9 %, Mol. Weight: 341.81. 1H-NMR (CDCl3, 300 MHz): δ 5.12 (s, 2H, OCH2), 7.01 (s, 1H, thiazole-H), 7.12 (d, 2H, J = 8.9 Hz, Ar-H), 7.26-7.57 (m, 4H, Ar-H), 8.22 (s, 1H, triazole-H). IR (KBr) cm−1: 1594, 1171 (N-N). MS m/z 342 (M+1). Anal. Calcd. for C18H16ClN3O: C, 59.73; H, 3.54; N, 12.29. Found: C, 59.61; H, 3.59; N, 12.47.

6-(4-(2-fluorobenzyloxy)phenyl)thiazolo[3,2-b][1,2,4]triazole (5h)

Thiazolo[3,2-b][1,2,4]triazoles as new anticonvulsants

6-(4-(4-chlorobenzyloxy)phenyl) thiazolo[3,2-b][1,2,4]triazole (5l)

M.p 141-143°C, yield 69.5%, Mol. Weight: 341.81. 'H-NMR (CDCl₃, 300 MHz): δ 5.10 (s, 2H, OCH₂), 7.01 (s, 1H, thiazole-H), 7.09 (d, 2H, J = 8.6 Hz, Ar-H), 7.38 (s, 4H, Ar-H), 8.03 (d, 2H, J = 8.6 Hz, Ar-H), 8.22 (s, 1H, triazole-H). IR (KBr) cm⁻¹: 1598 1582 (C=N), 1175 (N-N). MS m/z 342.3 (M+1).

Anticonvulsant effects in the MES test (29, 30)

The MES test was carried out using the methods described in the anticonvulsant drug development (ADD) program of the National Institutes of Health (USA). Seizures were elicited with a 60 Hz alternating current of 50 mA intensity in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. Animals were given intraperitoneal injection (i.p.) of the test compounds in the MES test. At 30 min after the administration of the compounds, the activities were evaluated in the MES test. In phase-I screening, each compound was administered at the dose levels of 30, 100, and 300 mg/Kg for evaluating the preliminary anticonvulsant activity. For determination of the median effective dose (ED₅₀) and the median toxic dose (TD₅₀), the phase-II screening was prepared. Several groups (each group of 10 mice) were given various intraperitoneal doses of the tested compound until at least three points were established in the range of 10-90% seizure protection or neurotoxicity. The number of animals per group protected against MES (or neurotoxic in the rotarod test) is converted to a percentage, and a dose–response curve can be constructed. Then the respective ED₅₀ and TD₅₀ values, 95% confidence intervals were calculated by the statistics software SPSS 13.0 with probit analysis.

Neurotoxicity (NT) screening (29, 30)

The neurotoxicity of the compounds was measured in mice by the rotarod test. The mice were trained to stay on a rotarod of diameter 3.2 cm that rotates at 10 rpm. Trained animals were given i.p. of the test compounds. Neurotoxicity was indicated by the inability of the animal to maintain equilibrium on the rod for at least 1 min in each of the trials.

PTZ-induced seizures (29, 30)

The PTZ test utilizes a dose of pentylenetetrazole (85 mg/kg). PTZ can produce clonic seizures lasting for at least five seconds in 97 percent of animals tested. At 30 min after the administration (i.p.) of the test compound, 85 mg/Kg PTZ dissolved in saline was administered subcutaneously. Animals are observed over a 30 minute period. Absence of clonic spasms in the observed time period indicates that the compound has the ability to abolish the effect of pentylenetetrazole on seizure threshold.

Log P calculation

The calculated Log P (miLog P) values were calculated using the Molinspiration online property calculation toolkit (31).

Results and Discussion

Anticonvulsant activity

A very important step in antiepileptic drug discovery is the choice of an appropriate animal model for the initial screening. At present, there are three models in-vivo - the MES, the PTZ, and the kindling model - which are routinely used by most AEDs discovery programs. Of these, the MES and PTZ seizure models represent...
the two animal seizure models most widely used in the search for new AEDs (32, 33). The MES test is thought to predict drugs effective against generalized seizures of the tonic-clonic (grand mal) type, whereas the PTZ test is used to find drugs effective against the generalized seizures of the petit mal (absence) type. In this study, the two models were used for screening the anticonvulsant activity of target compounds.

In the preliminary evaluation of anticonvulsant activities (Phase I), doses of 30, 100, and 300 mg/Kg were used in both models, and the results were presented in Table 1.

According to the results of the anticonvulsant activity studies, 6-(4-fluorophenyl)thiazolo[3,2-b][1,2,4]triazole (3c) was highly selective and found to be the most active compound in MES test with the complete protection at the dose of 100 mg/Kg and partial protection (one-third) at the dose of 30 mg/Kg. In the same test, compound 3a, 3b, 3k, 3l, and 5c were protective at the dose of 100 mg/Kg with the proportion of one-third, compound 5b showed protection in two-thirds at the same dose. Compounds 3a-3d, 3k-3l, and 5a-5d showed activities against MES at 300 mg/Kg in varying degrees.

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In the PTZ model the most active compound of tested compounds was 6-(4-propoxyphenyl) thiazolo[3,2-b][1,2,4]triazole (5b), which showed the complete protection at the dose of 100 mg/Kg and partial protection (one-third) at the dose of 30 mg/Kg. Compound 3i, with a 4-bromo moiety, was found to have anticonvulsant activity at 100 mg/Kg dose with protection in two-thirds. While derivatives with 3-chloro (3e), 4-methoxyl (3l) and 4-butoxy (5c) substitution exhibited protection against PTZ in one-third at 100 mg/Kg dose. At the dose of 300 mg/Kg, 3e, 3f, 3h, 3i, 3l, and 5b-5c showed activities against PTZ in varying degrees.

It is well accepted that blood-brain barrier (BBB) is an important selective barrier on the drug’s way to the central nervous system. Overcoming the difficulty of delivering therapeutic agents to specific regions of the brain presents a major challenge to treatment of most brain disorders. According to Kaliszan et al. (34), lipophilicity (logP) and molecular weight (MW) of the compound are the main factors affecting drug delivery across the BBB. From the calculated logP parameters of the prepared compounds (3a-3l, and 5a-5l), it can be observed that all compounds exhibited a nice logP ranging from 2.1 to 5.1, which would enable the compounds to penetrate the BBB (Table 1). In group 5a-5l, however, only four compounds (5a-5d) with relatively small substituents showed anticonvulsant activities in MES or PTZ test, as the sizes of substituents increased, the anticonvulsant activity of them disappeared (5e-5l). This may be due to the big lipophilicity of the molecules, which interrupted the absorption and distribution of these compounds sequentially reduced bioavailability (35). The above considerations were also in agreement with the theory that there was an optimum Log P for the drugs acting on the central nervous system, and the drugs with this optimum Log P will be least inhibited in their movement through the aqueous and lipophilic phases of living tissue (36). Another contributor for the non-activity of compounds 5e-5l may be the steric hindrance formed by big size of the substitution, which may drop their affinity to some assumed target receptors.

From the rotarod test results, it seems that compounds, with anticonvulsant activities in MES/PTZ test, exhibited neurotoxicity at the same doses. For example, compounds 3c and 5b with high activity also displayed serious neurotoxicity.

Compounds 3c and 5b were selected for quantification of the pharmacological parameters (ED\textsubscript{50} and TD\textsubscript{50}). Results of the quantitative test for the compounds, along with the data of the standard drugs carbamazepine, are reported in Table 2. In the MES screen, 6-(4-fluorophenyl)thiazolo[3,2-b][1,2,4]triazole (3c) showed an ED\textsubscript{50} and protective index (PI) value of 49.1 and 1.9. In the PTZ screen, 6-(4-propoxyphenyl)thiazolo[3,2-b][1,2,4]triazole 5b gave an ED\textsubscript{50} of 63.4 mg/Kg and a TD\textsubscript{50} of 105.6 mg/Kg, resulting in a high pi-value of 1.7 when compared to carbamazepine (PI < 0.44).

<table>
<thead>
<tr>
<th>Compound</th>
<th>ED\textsubscript{50} (MES)\textsuperscript{a}</th>
<th>ED\textsubscript{50} (PTZ)\textsuperscript{b}</th>
<th>TD\textsubscript{50} \textsuperscript{d}</th>
<th>PI (TD\textsubscript{50}/ ED\textsubscript{50})</th>
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<tr>
<td>3c</td>
<td>49.1 (44.4-54.3)</td>
<td>-</td>
<td>94.7 (86.1-104.2)</td>
<td>1.9</td>
</tr>
<tr>
<td>5b</td>
<td>-</td>
<td>63.4 (55.0-73.1)</td>
<td>105.6 (92.4-120.7)</td>
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<tr>
<td>Carbamazepine</td>
<td>9.8 (8.9-10.8)</td>
<td>&gt;100</td>
<td>44.0 (40.2-48.1)</td>
<td>4.5 &lt;0.44</td>
</tr>
</tbody>
</table>

\textsuperscript{a}: All animals was administered intraperitoneal injection.
\textsuperscript{b}: The median effective dose (ED\textsubscript{50}) was measured in maximal electroshock seizure test, confidence intervals given in the bracket and the unit is mg/Kg.
\textsuperscript{c}: The median effective dose (ED\textsubscript{50}) was measured in pentylenetetrazole-induced seizure test, confidence intervals given in the bracket and the unit is mg/Kg.
\textsuperscript{d}: The median neurotoxic dose (TD\textsubscript{50}) was measured in the rotarod test, confidence intervals given in the bracket and the unit is mg/Kg.
\textsuperscript{e}: Not tested.
Conclusion

A series of new 6-(substituted-phenyl)thiazolo[3,2-b][1,2,4]triazole derivatives were synthesized and studied for their anticonvulsant activity using MES and PTZ tests. Among the compounds synthesized, two compounds (3c and 5b) were found to have promising anticonvulsant activities in the models employed for anticonvulsant evaluation. 6-(4-Fluorophenyl)thiazolo[3,2-b][1,2,4]triazole (3c) was highly selective and found to be the most active compound against MES seizures. 6-(4-Propoxyphenyl)thiazolo[3,2-b][1,2,4]triazole (5b) was active in both models. In the PTZ screen, compound 5b gave an ED$_{50}$ of 63.4 mg/Kg and a TD$_{50}$ of 105.6 mg/Kg, resulting in a high PI value of 1.7 when compared to carbamazepine (PI < 0.44). High Neurotoxicity is the main problem of this series of compounds, which resulted in the narrow safety margin. Further modifications of the thiazolo-triazole fragment will be the focus of our next efforts with the aim of reducing the neurotoxicity of these compounds.

Acknowledgment

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References

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