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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and anticonvulsant activity evaluation of 7-alkoxy-2,4-dihydro-1*H*-benzo[*b*][1,2,4]triazolo[4,3-*d*][1,4]thiazin-1-ones in various murine experimental seizure models

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Abstract A novel series of 7-alkoxy-2,4-dihydro-1*H*-benzo[*b*][1,2,4]triazolo[4,3-*d*][1,4]-thiazin-1-ones have been synthesized and tested for their anticonvulsant activity using the maximal electroshock (MES) method. The majority of the compounds prepared were effective in the MES screens at a dose level of 100 mg/kg. Of the compounds tested, the most promising was 7-[(4-fluorobenzyl)oxy]-2,4-dihydro-1*H*-[1,2,4]-triazolo[4,3-*d*][1,4]-benzothiazin-1-one (**6m**), which showed an ED_{50} value of 9.2 mg/kg in the MES test in mice. Furthermore, the compound exhibited a PI value of 15.4 which was superior to the standard drug carbamazepine (PI value of 6.4). As well as demonstrating the anti-MES efficacy of compound **6m**, its potency against seizures induced by pentylenetetrazole, 3-mercaptopropionic acid, and bicuculline were also established, with the results suggesting that several different mechanisms of action might be involved in its anticonvulsant activity, including the inhibition of voltage-gated ion channels and the modulation of GABAergic activity.

Keywords Synthesis · Anticonvulsant · Triazolothiazinone · Maximal electroshock · Neurotoxicity

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Introduction

Epilepsy is one of the most common neurological disorders, characterized by excessive temporary neuronal discharge resulting in recurrent unprovoked seizures (Strine *et al*., 2005; Mc Namara *et al*., 2006). There have been reports suggesting that around 1 % of the world's population at any time (about 50 million people worldwide) is afflicted with this neurological disorder. In the recent years, extensive efforts have been devoted to the development of novel therapeutics, and these efforts have resulted in the development of several new drug candidates as promising anticonvulsants (Stefan and Feuerstein, 2007; Donner and Snead, 2006). The currently available anticonvulsants, however, are effective only in reducing the severity and number of seizures in less than 70 % of patients. Furthermore, their usage is often associated with numerous undesirable side effects (Bootsma *et al*., 2009; Kennedy and Lhatoo, 2008; Penovich and James Willmore, 2009). Although many of the antiepileptic drugs (AEDs) currently available, including phenytoin, carbamazepine, ethosuximide, valproic acid and the barbiturates, are widely prescribed, they can induce a number of adverse side effects, including ataxia, hepatotoxicity, gingival hyperplasia, and megaloblastic anemia (Bialer *et al.*, 2007; Bialer and Yagen, 2007). With this in mind, it is clear that a number of the current AEDs are limited by their toxicity, intolerance, and lack of efficacy. There is, therefore, an urgent need for the development of novel AEDs with greater levels of efficacy and lower levels of toxicity.

In our previous work, we synthesized a series of 6-alkoxy-3,4-dihydro-2(1*H*)-quinolinone derivatives (**I**) with anticonvulsant activities. Of these compounds, 6-hexyloxy-3,4-dihydro-2(1*H*)-quinolinone showed the strongest activity, with an ED_{50} value of 24.0 mg/kg in the maximal electroshock test (MES) and a PI value of 2.4 (Quan *et al.*, 2005). In a subsequent

study, a second series of compounds **II** was designed and synthesized according to a strategy involving the incorporation of a triazolone moiety into the initial series of compounds **I** (Jin *et al.*, 2006). In our laboratory, we have reported the synthesis and anticonvulsant activity evaluation of some compounds containing triazolone, the majority of them exhibited potent anticonvulsant activity (Shu *et al.*, 2013; Zheng *et al.*, 2013). This new design was based on the research reported by Gitto, *et al.* (2003), in which the introduction of a triazolone to the precursor compounds **III** provided a remarkable increase in their anticonvulsant activity. The pharmacological results achieved with series **II** were also in agreement with those reported by *Gitto* et al. Following the incorporation of the triazolone moiety into series **I**, the anticonvulsant activity of compounds **II** were markedly increased. Consequently, in the current study, a series of 7-alkoxy-2,4-dihydro-1*H*-benzo[*b*][1,2,4]triazolo[4,3-*d*][1,4]thiazin-1-one derivatives were designed and synthesized according to a strategy involving the introduction of a triazolone moiety to compound series **IV**, with the latter having already been reported as a potent anticonvulsant in our previous research (Zhang *et al.*, 2010), in which, 7-(pentyloxy)-2*H*-benzo[*b*][1,4]thiazin-3(4*H*)-one (**IV-e**) was the most active compound from series **IV** (leading structures) with an ED_{50} value of 34.1 mg/kg, TD_{50} value of 305.5 mg/kg and PI of 8.9 (Fig. 1).

The structures of target compounds were characterized using IR, ^{1}H NMR, MS, and ^{13}C NMR techniques. The anticonvulsant activities of the newly synthesized compounds were evaluated using MES-induced seizure in mice as an experimental epilepsy model. The rotarod assay was performed in mice to evaluate their neurotoxicity. To elucidate the possible mechanism of action, the most active compound **6m** was tested in pentylenetetrazole (PTZ), 3-mercaptopropionic acid (3-MP), and bicuculline (BIC)-induced seizure tests.

Materials and methods

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on IRPrestige-21. ^{1}H NMR and ^{13}C NMR spectra were measured on an AV-300(Bruker, Switzerland), and all chemical shifts were given in ppm relative to tetramethysilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). The major chemicals were purchased from Aldrich Chemical Corporation.

Chemistry

*Synthesis of 2-amino-5-methoxy-thiophenol (**1**)*

2-Amino-6-methoxybenzothiazole (36.0 g; 206.4 mmol) was suspended in 50 % KOH (180 g KOH dissolved in 180 ml water). The suspension was heated to reflux for 20 h. Upon cooling to room temperature, concentrated hydrochloric acid was added to adjust the pH to 6 to give a large number of pale yellow solid. Filter cake was obtained by vacuum-filtration and it was dried rapidly.

*Synthesis of 7-methoxy-2H-benzo[b][1,4]thiazin-3(4H)-one (**2**)*

Compound **1** (24.54 g; 158 mmol), sodium bicarbonate (53.20 g; 633 mol), and benzyltriethylammonium chloride (TEBA) (25.18 g; 110 mmol) were placed into a round-bottomed flask containing 500 ml of acetonitrile. The mixture was put into an ice–salt bath to keep the temperature at 0–5 °C. 2-Chloroacetyl-chloride (23.26 g; 21 mmol) was added into the mixture at a moderate speed and kept for 40 min. Replacing the ice–salt bath with an oil bath, the mixture was refluxed for 2 h, then the solvent was removed under reduced pressure. The resultant residue was purified by recrystallization with ethanol–water (1:1) to obtain pure compound **2**.

*Synthesis of 7-hydroxy-2H-benzo[b][1,4]thiazin-3(4H)-one (**3**)*

To a stirred solution of boron tribromide (9 mmol) in anhydrous CH_2Cl_2, a CH_2Cl_2 solution of compound **2**

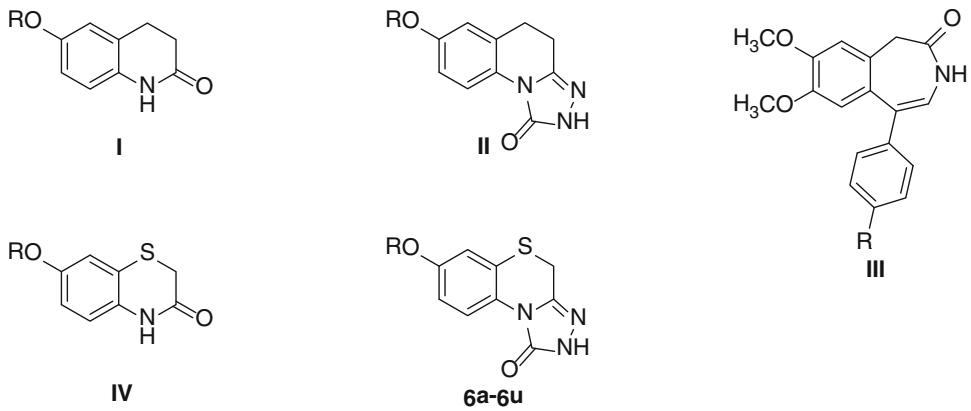


Fig. 1 The structures of compound series **I**, **II**, **III**, and **IV**, and the target compounds

(0.6 g; 3 mmol) was added drop-wise and the reaction continued for 1 h at 0 °C and an additional 2 h at 20 °C. Following the addition of ice-cold water, the organic layer was separated and the aqueous layer extracted with ethyl acetate. The combined organic layers were dried over with anhydrous magnesium sulfate and evaporated under reduced pressure to give a brown solid.

*Synthesis of 7-alkoxy-2H-benzo[b][1,4]thiazin-3 (4H)-ones (**4a–4t**)*

K_2CO_3 (0.42 g; 3 mmol), acetonitrile (40 ml), and 7-hydroxy-2*H*-benzo[*b*][1,4] thiazin-3(4*H*)-one **3** (0.53 g; 3 mmol) were mixed in a 100 ml round-bottomed flask equipped with reflux condenser. After refluxing the mixture 30 min, alkyl bromide or benzyl chloride derivative (4 mmol) was added drop-wise into the mixture. The mixture was heated under reflux for 4–24 h, cooled down and then poured into 100 ml of water. Aqueous layer was extracted with dichloromethane (30 ml × 3). The combined layers of dichloromethane were dried by anhydrous $MgSO_4$. Evaporation of the solvent gave a crude product, which was purified by silica gel column chromatography with petroleum ether: ethyl acetate (3:2) to a light-yellow solid.

*Synthesis of 7-alkoxy-2H-benzo[b][1,4]thiazin-3(4H)-thiones (**5a–5t**)*

To a toluene solution in a three-necked round-bottomed flask in an oil bath, Lawesson's reagent (0.6 eq) and **4a–4t** was added, and the solution was refluxed for 3–6 h under nitrogen. After removing the solvent under reduced pressure, the residue was dissolved in dichloromethane (30 ml), washed with water (30 ml × 3) and dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product, which was purified by silica gel column chromatography with dichloromethane to a light-yellow solid.

*Synthesis of target compounds (**6a–6t**)*

A solution of **5a–5t** (3 mmol) in appropriate *n*-butanol and methyl hydrazinecarboxylate (9 mmol) was refluxed for 2–3 days (TLC monitoring), the solvent was evaporated to dryness under reduced pressure, and the residue was extracted twice with dichloromethane (60 ml). The dichloromethane layer was washed three times with saturated aqueous NaCl (60 ml × 3) and dried over anhydrous $MgSO_4$. After removing the solvents, products were purified by silica gel column chromatography with CH_2Cl_2–CH_3OH (70:1). The yield, melting point and spectral data of each compound were given below.

*7-Ethoxy-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4]benzothiazin-1-one (**6a**)* White solid; mp. 184–186 °C. IR (KBr) cm^{-1}: 3189 (NH), 1708 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.42 (t, 3H, $J = 6.0$ Hz, –CH_3), 3.83 (s, 2H, –SCH_2–), 4.03 (q, 2H, $J = 7.0$ Hz, –OCH_2–), 6.85–8.27 (m, 3H, Ar–H), 9.91 (s, 1H, –NHCO–). ^{13}C–NMR ($CDCl_3$, 300 MHz): δ 14.71, 24.12 (ethyoxy-C), 63.95 (–SCH_2–), 113.79, 114.25, 120.40, 124.39, 125.54, 141.49 (Ar–C), 152.99 (triazolone, C=N), 156.86 (triazolone, C=O). MS-EI *m/z* 250 (M+1).

*7-Propoxy-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4]benzothiazin-1-one (**6b**)* White solid; mp. 160–162 °C. IR (KBr) cm^{-1}: 3178 (NH), 1704 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 1.04 (t, 3H, $J = 7.5$ Hz, –CH_3), 1.76–1.87 (m, 2H, –CH_2–), 3.83 (s, 2H, –SCH_2–), 3.92 (t, 2H, $J = 6.0$ Hz, –OCH_2–), 6.86–8.27 (m, 3H, Ar–H), 9.85 (s, 1H, –NHCO–). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 10.47, 22.47, 24.13 (propoxy-C), 69.96 (–SCH_2–), 113.79, 114.28, 120.40, 124.38, 125.54, 141.19 (Ar–C), 153.33 (triazolone, C=N), 157.04 (triazolone, C=O). MS-EI *m/z* 264 (M+1).

*7-Butoxy-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4]benzothiazin-1-one (**6c**)* White solid; mp. 154–156 °C. IR (KBr) cm^{-1}: 3190 (NH), 1704 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.98 (t, 3H, $J = 6.0$ Hz, –CH_3), 1.43–1.55 (m, 2H, –CH_2–), 1.72–1.81 (m, 2H, –CH_2–), 3.83 (s, 2H, –SCH_2–), 3.96 (t, 2H, $J = 6.0$ Hz, –OCH_2–), 6.85–8.27 (m, 3H, Ar–H), 10.16 (s, 1H, –NHCO–). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 13.83, 19.18, 24.11, 31.16 (butoxy-C), 68.16 (–SCH_2–), 113.79, 114.26, 120.40, 124.39, 125.51, 141.33 (Ar–C), 153.34 (triazolone, C=N), 157.06 (triazolone, C=O). MS-EI *m/z* 278 (M+1).

*7-(Pentyloxy)-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4]benzothiazin-1-one (**6d**)* White solid; mp. 120–122 °C. IR (KBr) cm^{-1}: 3186 (NH), 1701 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.94 (t, 3H, $J = 7.0$ Hz, –CH_3), 1.35–1.47 (m, 4H, –CH_2–), 1.74–1.83 (m, 2H, –CH_2–), 3.83 (s, 2H, –SCH_2–), 3.95 (t, 2H, $J = 6.5$ Hz, –OCH_2–), 6.85–8.26 (m, 3H, Ar–H), 9.95 (s, 1H, –NHCO–). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 14.03, 22.43, 24.09, 28.13, 28.82 (pentyloxy-C), 68.45 (–SCH_2–), 113.73, 114.23, 120.39, 124.41, 125.53, 141.11 (Ar–C), 153.50 (triazolone, C=N), 157.01 (triazolone, C=O). MS-EI *m/z* 292 (M+1).

*7-(Hexyloxy)-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4]benzothiazin-1-one (**6e**)* White solid; mp. 118–120 °C. IR (KBr) cm^{-1}: 3185 (NH), 1702 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.91 (t, 3H, $J = 6.5$ Hz, –CH_3), 1.33–1.47 (m, 6H, –CH_2–), 1.73–1.82 (m, 2H, –CH_2–), 3.83 (s, 2H, –SCH_2–), 3.95 (t, 2H, $J = 6.5$ Hz, –OCH_2–), 6.84–8.26 (m, 3H, Ar–H), 10.23 (s, 1H, –NHCO–). ^{13}C NMR

($CDCl_3$, 300 MHz): δ 14.04, 22.60, 24.12, 25.66, 29.09, 31.54 (hexyloxy-C), 68.48 (–SCH_2–), 113.78, 114.25, 120.39, 124.39, 125.52, 141.18 (Ar–C), 153.32 (triazolone, C=N), 157.04 (triazolone, C=O). MS-EI *m/z* 306 (M+1).

7-(Heptyloxy)-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4] benzothiazin-1-one ***(6f)*** White solid; mp. 110–112 °C. IR (KBr) cm^{-1}: 3180 (NH), 1704 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.90 (t, 3H, $J = 6.6$ Hz, –CH_3), 1.31–1.47 (m, 8H, –CH_2–), 1.73–1.82 (m, 2H, –CH_2–), 3.83 (s, 2H, –SCH_2–), 3.95 (t, 2H, $J = 6.5$ Hz, –OCH_2–), 6.85–8.26 (m, 3H, Ar–H), 9.76 (s, 1H, –NHCO–). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 14.09, 22.61, 24.11, 25.94, 29.02, 29.13, 31.76 (heptyloxy-C), 68.47 (–SCH_2–), 113.77, 114.24, 120.39, 124.39, 125.53, 141.13 (Ar–C), 153.35 (triazolone, C=N), 157.03 (triazolone, C=O). MS-EI *m/z* 320 (M+1).

7-(Octyloxy)-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4] benzothiazin-1-one ***(6g)*** White solid; mp. 94–96 °C. IR (KBr) cm^{-1}: 3181 (NH), 1709 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.89 (t, 3H, $J = 6.0$ Hz, –CH_3), 1.29–1.44 (m, 10H, –CH_2–), 1.73–1.82(m, 2H, –CH_2–), 3.83 (s, 2H, –SCH_2–), 3.94 (t, 2H, $J = 7.5$ Hz, –OCH_2–), 6.84–8.26 (m, 3H, Ar–H), 10.53 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 14.41, 22.54, 23.51, 25.91, 29.13, 31.70, 37.42, 51.76 (octyloxy-C), 68.35 (–SCH_2–), 113.91, 114.20, 120.06, 124.85, 125.10, 141.05 (Ar–C), 152.75 (triazolone, C=N), 156.58 (triazolone, C=O). MS-EI *m/z* 334 (M+1).

7-(Dodecyloxy)-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4] benzothiazin-1-one ***(6h)*** White solid; mp. 92–94 °C. IR (KBr) cm^{-1}: 3179 (NH), 1711 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, 3H, $J = 6.0$ Hz, –CH_3), 1.26–1.45 (m, 18H, –CH_2–), 1.70–1.83 (m, 2H. –CH_2–), 3.83 (s, 2H, –SCH_2–), 3.95 (t, 2H, $J = 6.0$ Hz, –OCH_2–), 6.83–8.24 (m, 3H, Ar–H), 10.52 (s, 1H, –NHCO–). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 14.13, 22.69, 24.09, 25.98, 29.12, 29.36, 29.49, 29.60, 29.64, 29.66, 31.92, 50.75 (dodecyloxy-C), 68.47 (–SCH_2–), 113.75, 114.23, 120.36, 124.41, 125.48, 141.16 (Ar–C), 153.14 (triazolone, C=N), 157.04 (triazolone, C=O). MS-EI *m/z* 390 (M+1).

7-(Tetradecyloxy)-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4] benzothiazin-1-one ***(6i)*** White solid; mp. 112–114 °C. IR (KBr) cm^{-1}: 3177 (NH), 1709 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.88 (t, 3H, $J = 6.2$ Hz, –CH_3), 1.26–1.47 (m, 22H, –CH_2–), 1.73–1.82 (m, 2H, –CH_2–), 3.83 (s, 2H, –SCH_2–), 3.95 (t, 2H, $J = 6.0$ Hz, –OCH_2–), 6.85–8.27 (m, 3H, Ar–H), 10.19 (s, 1H, –NHCO–). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 14.14, 22.71, 24.10, 25.98, 29.13, 29.38, 29.48, 29.50, 29.58, 29.61, 29.67, 29.78, 31.94, 50.74 (tetradecyloxy-C), 68.47 (–SCH_2–), 113.76, 114.25, 120.40, 124.40, 125.50, 141.18 (Ar–C), 153.43 (triazolone, C=N), 157.05 (triazolone, C=O). MS-EI *m/z* 418 (M+1).

7-(Benzyloxy)-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4] benzothiazin-1-one ***(6j)*** White solid; mp. 178–180 °C. IR (KBr) cm^{-1}: 3183 (NH), 1721 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 4.02 (s, 2H, –SCH_2–), 5.12 (s, 2H, –OCH_2–), 7.14–8.16 (m, 8H, Ar–H), 12.00 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 23.90 (–OCH_2–), 70.15 (–SCH_2–), 113.88, 114.52, 120.05, 124.58, 126.36, 127.65, 127.65, 128.14, 128.64, 128.64, 136.63, 140.37 (Ar–C), 152.8 (triazolone, C=N), 156.13 (triazolone, C=O). MS-EI *m/z* 312 (M+1).

7-[(2-Fluorobenzyl)oxy]-2,4-dihydro-1H-[1,2,4]triazolo [4,3-d][1,4]benzothiazin-1-one ***(6k)*** White solid; mp. 196–198 °C. IR (KBr) cm^{-1}: 3188 (NH), 1720 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 3.84 (s, 2H, –SCH_2–), 5.14 (s, 2H, –OCH_2–), 6.96–8.29 (m, 7H, Ar–H), 9.47 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 28.60 (–OCH_2–), 68.98 (–SCH_2–), 118.56, 119.28, 120.12, 120.40, 124.83, 128.32, 128.51, 129.32, 129.47, 131.33, 135.18, 145.15 (Ar–C), 157.54 (triazolone, C=N), 160.68 (triazolone, C=O). MS-EI *m/z* 330 (M+1).

7-[(3-Fluorobenzyl)oxy]-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4]benzothiazin-1-one ***(6l)*** White solid; mp. 132–134 °C. IR (KBr) cm^{-1}: 3178 (NH), 1718 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 3.83 (s, 2H, –SCH_2–), 5.07 (s, 2H, –OCH_2–), 6.92–8.29 (m, 7H, Ar–H), 9.19 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 23.82 (–OCH_2–), 69.24 (–SCH_2–), 113.86, 114.07, 114.55, 114.70, 114.98, 120.07, 123.19, 124.68, 126.51, 130.47, 139.54, 140.41 (Ar–C), 155.86 (triazolone, C=N), 159.38 (triazolone, C=O). MS-EI *m/z* 330 (M+1).

7-[(4-Fluorobenzyl)oxy]-2,4-dihydro-1H-[1,2,4]triazolo [4,3-d][1,4]benzothiazin-1-one ***(6m)*** White solid; mp. 202–204 °C. IR (KBr) cm^{-1}: 3184 (NH), 1715 (C=O). ^1H NMR ($CDCl_3$, 300 MHz): δ 3.84 (s, 2H, –SCH_2–), 5.03 (s, 2H, –OCH_2–), 6.93–8.29 (m, 7H, Ar–H), 9.42 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 23.86 (–OCH_2–), 69.41 (–SCH_2–), 113.83, 114.51, 115.32, 115.60, 120.05, 124.61, 129.66, 129.77, 132.63, 132.67, 139.06, 140.39 (Ar–C), 152.82 (triazolone, C=N), 155.98 (triazolone, C=O). MS-EI *m/z* 330 (M+1).

7-[(2-Chlorobenzyl)oxy]-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4]benzothiazin-1-one ***(6n)*** White solid; mp. 212–214 °C. IR (KBr) cm^{-1}: 3179 (NH), 1701 (C=O); ^1H NMR (DMSO-d_6, 300 MHz): δ 4.04 (s, 2H, –SCH_2–), 5.17 (s, 2H, –OCH_2–), 7.19–8.18 (m, 7H, Ar–H), 12.00 (s, 1H,

–NHCO–). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 23.50 (–OCH_2–), 67.81 (–SCH_2–), 114.25, 114.66, 120.13, 125.05, 126.66, 127.87, 129.91, 130.50, 130.78, 133.18, 134.43, 141.06 (Ar–C), 152.79 (triazolone, C=N), 156.05 (triazolone, C=O). MS-EI *m/z* 346 (M+1).

*7-[(3-Chlorobenzyl)oxy]-2,4-dihydro-1H-[1,2,4]triazolo [4,3-d][1,4]benzothiazin-1-one (**6o**)* White solid; mp. 194–196 °C. IR (KBr) cm^{-1}: 3182 (NH), 1721 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.84 (s, 2H, –SCH_2–), 5.04 (s, 2H, –OCH_2–), 6.92–8.29 (m, 7H, Ar–H), 9.18 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 23.85 (–OCH_2–), 69.18 (–SCH_2–), 113.85, 114.54, 120.06, 124.69, 125.84, 126.53, 127.40, 128.09, 130.22, 134.02, 139.06, 140.38 (Ar–C), 152.80 (triazolone, C=N), 155.82 (triazolone, C=O). MS-EI *m/z* 346 (M+1).

*7-[(4-Chlorobenzyl)oxy]-2,4-dihydro-1H-[1,2,4]triazolo [4,3-d][1,4]benzothiazin-1-one (**6p**)* White solid; mp. 198–200 °C. IR (KBr) cm^{-1}: 3179 (NH), 1705 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.83 (s, 2H, –SCH_2–), 5.04 (s, 2H, –OCH_2–), 6.91–8.29 (m, 7H, Ar–H), 9.45 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 23.95 (–OCH_2–), 69.35 (–SCH_2–), 113.84, 114.54, 120.08, 124.56, 126.45, 128.68, 128.68, 129.01, 129.01, 133.52, 135.23, 140.30 (Ar–C), 152.87 (triazolone, C=N), 155.89 (triazolone, C=O). MS-EI *m/z* 346 (M+1).

*7-[(2,6-Dichlorobenzyl)oxy]-2,4-dihydro-1H-[1,2,4]triazolo [4,3-d][1,4]benzothiazin-1-one (**6q**)* White solid; mp. 220–222 °C. IR (KBr) cm^{-1}: 3184 (NH), 1709 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.85 (s, 2H, –SCH_2–), 5.29 (s, 2H, –OCH_2–), 7.00–8.23 (m, 6H, Ar–H), 9.21 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 23.89 (–OCH_2–), 69.60 (–SCH_2–), 113.89, 114.56, 120.10, 124.73, 126.72, 128.70, 128.70, 131.18, 131.67, 136.74, 136.74, 140.39 (Ar–C), 152.82 (triazolone, C=N), 156.22 (triazolone, C=O). MS-EI *m/z* 381 (M+1).

*7-[(4-Methylbenzyl)oxy]-2,4-dihydro-1H-[1,2,4]triazolo [4,3-d][1,4]benzothiazin-1-one (**6r**)* White solid; mp. 164–166 °C. IR (KBr) cm^{-1}: 3187 (NH), 1717 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 2.37 (s, 3H, –CH_3), 3.83 (s, 2H, –SCH_2–), 5.03 (s, 2H, –OCH_2–), 6.92–8.27 (m, 7H, Ar–H), 9.66 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 21.26 (–CH_3), 23.80 (–OCH_2–), 70.03 (–SCH_2–), 113.95, 114.52, 120.03, 124.62, 126.32, 127.91, 127.91, 129.30, 129.30, 133.74, 137.58, 140.49 (Ar–C), 152.78 (triazolone, C=N), 156.17 (triazolone, C=O). MS-EI *m/z* 326 (M+1).

*7-{[3-(Trifluoromethyl)benzyl]oxy}-2,4-dihydro-1H-[1,2,4] triazolo[4,3-d][1,4]benzothiazin-1-one (**6s**)* White solid; mp. 176–178 °C. IR (KBr) cm^{-1}: 3189 (NH), 1710 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.84 (s, 2H, –SCH_2–), 5.11 (s, 2H, –OCH_2–), 6.92–8.30 (m, 7H, Ar–H), 9.44 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, $CDCl_3$, 300 MHz): δ 23.91 (–OCH_2–), 69.30 (–SCH_2–), 113.81, 114.58, 120.10, 122.36, 124.05, 124.68, 126.60, 129.34, 130.20, 130.63, 131.06, 137.89, 140.31 (Ar–C), 152.85 (triazolone, C=N), 155.82 (triazolone, C=O). MS-EI *m/z* 380 (M+1).

*2-{[(1-Oxo-2,4-dihydro-1H-[1,2,4]triazolo[4,3-d][1,4] benzothiazin-7-yl)oxy]methyl}benzonitrile (**6t**)* White solid; mp. 212–214 °C. IR (KBr) cm^{-1}: 3188 (NH), 1721 (C=O). ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.85 (s, 2H, –SCH_2–), 5.27 (s, 2H, –OCH_2–), 6.96–8.32 (m, 7H, Ar–H), 9.25 (s, 1H, –NHCO–). ^{13}C NMR (DMSO-d_6, 300 MHz): δ 23.48 (–OCH_2–), 68.46 (–SCH_2–), 111.89, 114.33, 114.73, 117.65 (–CN), 120.13, 125.08, 125.82, 129.71, 130.24, 133.84, 133.94, 140.09, 141.05 (Ar–C), 152.74 (triazolone, C=N), 155.90 (triazolone, C=O). MS-EI *m/z* 337 (M+1).

Biological assay

Anticonvulsant activity

All the target compounds were screened for their anticonvulsant activity using the MES test, which is the most frequently used method for evaluating the anticonvulsant activity of compounds. Neurotoxicity was assessed using the rotarod test. The MES and rotarod tests were carried out according to the anticonvulsant drug development (ADD) program protocol. All of compounds, which were dissolved in DMSO, were evaluated for their anticonvulsant activity and neurotoxicity using KunMing mice in the 18–22 g weight range. KunMing mice were purchased from the Laboratory of Animal Research of the College of Pharmacy at Yanbian University. Several compounds possessed good activities when they were quantified for their anticonvulsant activity and neurotoxicity, and compound **6m**, which was the most potent, was tested in the PTZ, 3-MP, and BIC-induced seizure tests.

Maximal electroshock seizure (MES) test

The MES test was carried out by the methods described in the ADD of the National Institutes of Health (USA) (Krall *et al.*, 1978; Poter *et al.*, 1984). 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 absence of tonic extension of the hind leg. After 0.5 h of

drug administration, the activities were evaluated in MES test. In phase-I screening, each compound was administered at the dose levels of 100, 30, 15, and 7.5 mg/kg for evaluating the preliminary anticonvulsant activity. For determination of the median effective dose (ED_{50}) and the median toxic dose (TD_{50}), the phase-II screening was prepared. Groups of 5 mice were given a range of intraperitoneal doses of the tested compound until at least three points were established in the range of 10–90 % seizure protection or minimal observed neurotoxicity. From these data, the respective ED_{50}, TD_{50} values, and 95 % confidence intervals were calculated by probit analysis.

Neurotoxicity screening (NT)

The neurotoxicity of the compounds was measured in mice by the rotarod test. The mice were trained to stay on an accelerating rotarod of diameter an inch that rotates at 10 rpm. Trained animals were given i.p. injection 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 (Krall *et al.*, 1978; Poter *et al.*, 1984).

Chemical induced seizsure tests

In chemically induced seizures (see Table 3), mice were given doses of convulsant drugs that could induce seizures at least 97 % of animals. The doses used were: PTZ, 85 mg/kg; 3-mercaptopropionic acid, 40 mg/kg; and BIC, 5.4 mg/kg. The test compounds and standard AEDs were administered i. p. in a volume of 30 mg/kg to groups of ten mice 30 min before injection of PTZ (s. c.), 3-MP (i. p.) and BIC (s. c.). The mice were placed in individual cages and observed for 30 min, the number of clonic seizures, tonic seizures, and the lethality were recorded (Arnoldi *et al.*, 1990; Löscher and Schmidt, 1988).

Results and discussion

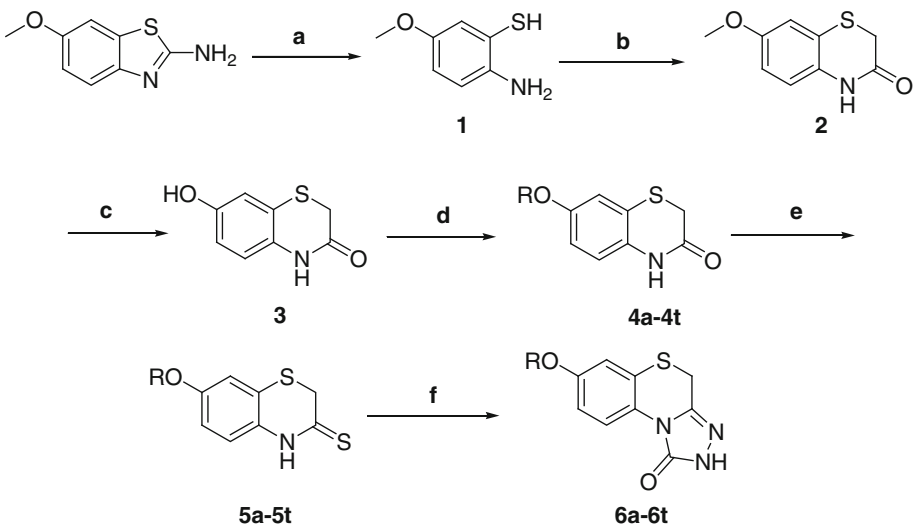
Chemistry

6-Methoxybenzo[*d*]thiazol-2-amine was used as a starting material for the preparation of all compounds described in the current paper. All of the compounds were prepared as outlined in Scheme 1. 6-Methoxybenzo[*d*]thiazol-2-amine was hydrolyzed in a 50 % KOH solution to give 2-amino-5-methoxybenzenethiol (**1**), which was treated with chloroacetyl chloride to give compound **2** in accordance with a procedure reported in the literature (Shridhar *et al.*, 1984). Compound **2** was successfully demethylated by treatment with boron tribromide to give the corresponding phenolic species 7-hydroxy-2*H*-benzo[*b*][1,4]thiazin-3(4*H*)-one (**3**). This material was then alkylated with a variety of different alkylating agents to give the 7-alkoxy-2*H*-benzo[*b*][1,4]thiazin-3(4*H*)-ones (**4a–t**). These compounds **4a–t** were then treated with Lawesson's reagent to give the corresponding intermediate 7-alkoxy-2*H*-benzo[*b*][1,4]thiazin-3(4*H*)-thiones (**5a–t**) (Zhang *et al.*, 2010), which were converted to the target compounds **6a–t** by treatment with methyl hydrazinecarboxylate in refluxing *n*-butanol. Their chemical structures were characterized using IR, ^{1}H NMR, MS, and ^{13}C NMR techniques. A detailed overview of their physical and analytical data has been provided in Synthesis of target compounds (**6a–6t**).

Pharmacology

Phase I evaluation of the anticonvulsant activity

The choice of an appropriate animal model for the initial compound screening process represents a very important step in antiepileptic drug discovery. There are currently three models available for the in vivo



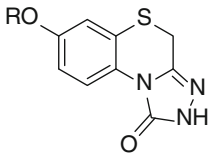
Scheme 1 The synthesis of the target compounds **6a–6t** **a** 50 % KOH, reflux, 20 h. **b** $NaHCO_3$, TEBA, $ClCOCH_2Cl$, CH_3CN, reflux, 2 h. **c** BBr_3, CH_2Cl_2, 0 °C, 3 h. **d** K_2CO_3, alkyl bromide or benzyl chloride derivative, CH_3CN, reflux, 4–24 h **e** Lawesson's reagent, toluene, reflux, 3–6 h. **f** $NH_2NHCO_2CH_3$, *n*-Butanol, reflux, 2–3d

evaluation of a drug candidate's anticonvulsant activity, namely the MES, subcutaneous pentylenetetrazole (scPTZ), and kindling models, which are routinely used by most AED discovery programs. Of these tests, the MES and scPTZ seizure models represent the two animal seizure models most widely used in the search for new AEDs (White, 2003; Levy *et al.*, 1995). The MES test is believed to provide an effective prediction of drugs that will be effective against generalized seizures of the tonic–clonic (grand mal) type, whereas the scPTZ test is used to find drugs that are effective against the generalized seizures of the petit mal (absence) type. In the current study, the MES seizure model was used for screening the anticonvulsant activity of target compounds. The calculated Log P (Clog P) values were calculated using the ChemOffice 12.0 software (CambridgeSoft, USA).

During the preliminary screening process, compounds were administered as an intraperitoneal (i.p.) injection at dose levels of 100, 30, 15, and 7.5 mg/kg. Their anticonvulsant effects were assessed at 0.5 h intervals following administration in mice. The following observations were obtained and listed in Table 1.

In the MES test, most of the compounds were active at a dose of 100 mg/kg, indicating their potent anticonvulsant properties. Of the compounds tested, compounds **6a–6e**, **6j–6m**, **6o**, and **6r** exhibited absolute protection, whereas compounds **6f**, **6p**, **6q**, and **6s** showed partial protection (1/3 or 2/3) at a dose of 100 mg/kg. At a dose of 30 mg/kg, compounds **6b–6f**, **6j–6m**, and **6o–6r** showed different degrees of protection against the MES-induced seizures. At a dose of 15 mg/kg, compounds **6b–6f**, **6j–6m**, and **6r** still exhibited activity, with compounds **6c–6e** in particular providing complete protection for all of the mice tested

Table 1 Phase-I preliminary evaluation of the anticonvulsant activity in mice (i.p.)



Compound	R	MES[a] mg/kg				CLog P[b]
		100	30	15	7.5	
6a	$-C_2H_5$	3/3	0/5	–[c]	–	1.44
6b	n $-C_3H_7$	3/3	5/5	3/5	2/5	1.97
6c	n $-C_4H_9$	3/3	5/5	5/5	3/5	2.50
6d	n $-C_5H_{11}$	3/3	5/5	5/5	3/5	3.03
6e	n $-C_6H_{13}$	3/3	5/5	5/5	3/5	3.56
6f	n $-C_7H_{15}$	2/3	2/5	1/5	0/5	4.09
6g	n $-C_8H_{17}$	0/3	–	–	–	4.62
6h	n $-C_{12}H_{25}$	0/3	–	–	–	6.73
6i	n $-C_{14}H_{29}$	0/3	–	–	–	7.79
6j	$-CH_2C_6H_5$	3/3	4/5	3/5	3/5	2.68
6k	$-CH_2C_6H_4$(o-F)	3/3	3/5	1/5	0/5	2.83
6l	$-CH_2C_6H_4$(m-F)	3/3	2/5	1/5	0/5	2.83
6m	$-CH_2C_6H_4$(p-F)	3/3	5/5	3/5	2/5	2.83
6n	$-CH_2C_6H_4$(o-Cl)	0/3	–	–	–	3.40
6o	$-CH_2C_6H_4$(m-Cl)	3/3	2/5	0/5	–	3.40
6p	$-CH_2C_6H_4$(p-Cl)	2/3	2/5	0/5	–	3.40
6q	$-CH_2C_6H_3$(2,6-Cl_2)	1/3	1/5	0/5	–	4.11
6r	$-CH_2C_6H_4$(p-CH_3)	3/3	5/5	3/5	0/5	3.18
6s	$-CH_2C_6H_4$(m-CF_3)	1/3	0/5	–	–	3.57
6t	$-CH_2C_6H_4$(o-CN)	0/3	–	–	–	2.26

[a] Maximal electroshock seizure test (number of animals protected/number of animals tested)

[b] CLog P was calculated using software ChemOffice 12.0

[c] Not tested

(5/5). Six compounds (**6b–6e, 6j,** and **6m**) exhibited moderate protection at the lower dose of 7.5 mg/kg.

Phase II evaluation of the anticonvulsant activity

Based on the considerable anticonvulsant activity demonstrated during the phase I testing, compounds **6b–6e**, **6j**, and **6m** were subjected to phase II trials for quantification of their anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}) in mice. Results of the quantitative testing of selected compounds, together with data collected for the current antiepileptic drug carbamazepine, are shown in Table 2. The six compounds tested gave ED_{50} values in the range of 7.1–12.2 mg/kg and TD_{50} values in the range of 141.4–81.8 mg/kg, leading to protective index values in the range of 7.0–15.4. All the compounds tested showed a higher protective index (PI) than carbamazepine, which gave a PI value of 6.4. Of the six compounds tested, compound **6d** was the most active possessing strong anti-MES activity with an ED_{50} value of 7.1 mg/kg. Unfortunately, however, it also showed a high level of neurotoxicity with a TD_{50} value of 81.8 mg/kg that consequently resulted in a PI value of 11.5. With all these factors in mind (i.e., efficacy, toxicity, and safety), compound **6m** was considered as the most promising compound in this study because of its efficacy and low neurotoxicity (ED_{50} = 9.2 mg/kg and TD_{50} = 141.4 mg/kg, resulting in a PI value of 15.4). Compound **IV-e** was the most active compound from series **IV** (leading structures) with an ED_{50} value of 34.1 mg/kg, TD_{50} value of 305.5 mg/kg and PI of 8.9. As shown in Table 2, all of the tested compounds (**6b–6e**, **6j**, and **6m**) showed better levels of anticonvulsant activity than compound **IV-e**, and compounds **6c–6e** and **6m** also showed a higher safety margin than compound **IV-e**.

Structure–Activity Relationships

In the current study, we have synthesized a series of 7-alkoxy-2,4-dihydro-1*H*-benzo[*b*][1,2,4]triazolo[4,3-*d*][1,4]thiazin-1-one derivatives (**6a–6t**). Following an analysis of the activities of the synthesized compounds, the following structure–activity relationships (SARs) were obtained. Of all of the compounds tested, compounds **6a–6i** possessed different alkoxy groups attached to the core triazolobenzothiazine fragment ranging from an ethoxy group to a tetradecyloxy group. The length of the alkyl chain appeared to have an impact on the anticonvulsant activity of the derivatives. For example, as the alkyl chain length increased from **6a** to **6d**, the anticonvulsant activity gradually increased with compound **6d** (with the *n*-pentane chain) being the most active compound. From **6d** to **6i**, although the alkyl chain length increased further, the anticonvulsant activity decreased and ultimately disappeared. Compound **6d** was the most active compound with an ED_{50} value of 7.1 mg/kg, indicating the optimal partition coefficient associated with the easiest crossing of the biological membranes in the congeners. Compound **6j** was substituted with a benzyloxy group at the 7-position of the triazolobenzothiazine core and F, Cl, Br, CH_3, CF_3, and CN groups were subsequently added on to the benzyloxy of **6j** in different position providing compounds **6k–6t**. Unfortunately, the introduction of the halogens and other groups to the phenyl ring led to a reduction in the anticonvulsant activity relative to **6j**, with the exception of compound **6m** (*p*-F substituent). Compounds **6g**, **6h**, and **6i** did not show activity in the MES test, and their Clog *P* values were in the range of 4.62–7.79, and therefore considered too high for the absorption and distribution of drugs that act on the central nervous system (Lien *et al.*, 1979). These results provided an indication of the importance of the lipophilicity as well as electronic

Table 2 Phase-II quantitative anticonvulsant evaluation in Mice (i.p.)

Compound	R	ED_{50}^{a} (mg/kg)	TD_{50}^{b} (mg/kg)	PI[c] (TD_{50}/ED_{50})
6b	*n* $-C_3H_7$	12.2 (4.1–36.1)[d]	84.9 (64.9–110.9)	7.0
6c	*n* $-C_4H_9$	7.9 (6.9–9.1)	117.8 (103.1–134.7)	14.9
6d	*n* $-C_5H_{11}$	7.1 (6.2–8.2)	81.8 (62.2–106.9)	11.5
6e	*n* $-C_6H_{13}$	7.9 (6.8–9.2)	89.6 (50.6–158.8)	11.3
6j	$-CH_2C_6H_5$	12.2 (4.1–36.1)	94.7 (71.1–126.0)	7.8
6m	$-CH_2C_6H_4$(*p*-F)	9.2 (7.0–12.2)	141.4 (123.7–161.6)	15.4
Compound IVe	–	34.08 (23.9–48.6)	305.5 (217.3–29.6)	8.9
Carbamazepine	–	11.8 (8.5–16.4)	76.1 (55.8–103.7)	6.4

[a] ED_{50} median effective dose affording anticonvulsant protection in 50 % of animals, the dose is measured in mg/kg

[b] TD_{50} median toxic dose eliciting minimal neurological toxicity in 50 % of animals, the dose is measured in mg/kg

[c] PI protective index (TD_{50}/ED_{50})

[d] 95 % confidence intervals given in parentheses

Table 3 Effects of compound **6m** on chemically-induced seizures in mice (i.p.)

Chemical substances	Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
Pentylenetetrazole	DMSO	–	0.5	100	100	100
	Carbamazepine	30	0.5	100	0	10
	6m	30	0.5	100	10	80
3-Mercaptopropionic acid	DMSO	–	0.5	100	100	100
	Carbamazepine	30	0.5	100	0	0
	6m	30	0.5	100	0	60
Bicuculline	DMSO	–	0.5	100	100	100
	Carbamazepine	30	0.5	100	0	60
	6m	30	0.5	90	10	30

properties of the substituents on the activity of these compounds.

Speculation of mechanism

In this study, the majority of synthesized compounds were highly potent in the MES test, and the MES test is known to be sensitive to sodium channel inhibitors (e.g., phenytoin, carbamazepine), which suggested that the tested compounds may inhibit voltage-gated ion channels (particularly sodium channels). To further investigate the effects of the anticonvulsant activity in several different models and speculate about the possible mechanism action of anticonvulsant, compound **6m** was tested against convulsions induced by chemical substances, including PTZ, 3-MP, and BIC. Compound **6m** was administered to mice at 30 mg/kg i.p., which was higher than its ED_{50} value and far below its TD_{50} value. The reference drug carbamazepine was also administered at 30 mg/kg i.p.

In the sc-PTZ model, carbamazepine inhibited the clonic seizures, tonic seizures and death at the rates of 0, 100, and 90 %, respectively. Compound **6m** inhibited the clonic seizures, tonic seizures, and lethality at the rates of 0, 90, and 20 % induced by sc-PTZ, respectively (Table 3). From these data we can see that compound **6m** can inhibit the tonic seizures induced by PTZ. PTZ has been reported to produce seizures by inhibiting γ-aminobutyric acid (GABA) neurotransmission (Okada *et al.*, 1989; Olsen 1981). GABA is the main inhibitory neurotransmitter in the brain, and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures (Gale, 1992), while GABAergic enhancement is known to inhibit or attenuate seizures. The inhibition of compound **6m** upon the PTZ-induced tonic seizures might be related to the enhancing of GABAergic neurotransmission or activity.

In the 3-MP induced seizure model, carbamazepine inhibited the clonic seizures, tonic seizures and death at rates of 0, 100, and 100 %, respectively. By comparison, compounds **6m** showed an anticonvulsant effect similar to that of carbamazepine in inhibiting the clonic and tonic seizures. While its protection for the death induced by 3-MP cannot compare with carbamazepine, with inhibition of death induced by 3-MP at rate of 40 % (Table 3). 3-MP is competitive inhibitor of the GABA synthesis enzyme glutamate decarboxylase (GAD), and it inhibits the synthesis of GABA resulting in decrease of GABA levels in the brain (Loscher, 1979). Compound **6m** exhibited the efficacy to inhibit the tonic seizures induced by 3-MP though the protection for death is barely satisfactory, which suggesting that the increase of GABA levels might be involved in its anticonvulsant mechanisms.

In the BIC-induced seizure model, both carbamazepine and **6m** inhibited tonic seizures and death, but did not inhibit clonic seizures. Carbamazepine showed inhibition of clonic and tonic seizures and death at rates of 0, 100, and 40 %, respectively. Compound **6m** showed inhibition of clonic and tonic seizures and death at rates of 10, 90, and 70 %, respectively (Table 3). BIC is a competitive antagonist of the $GABA_A$ receptor (Macdonald and Barker, 1978). As compound **6m** inhibited the seizures induced by BIC, it likely exerts anticonvulsant activity, at least partially, through $GABA_A$-mediated mechanisms.

Conclusion

In summary, the current study has demonstrated that a series of 7-alkoxy-2,4-dihydro-1*H*-benzo[*b*][1,2,4]triazolo[4,3-*d*][1,4]thiazin-1-one derivatives **6a–t** were effective in the MES screens. The most promising of these compounds was 7-[(4-fluorobenzyl)oxy]-2,4-dihydro-1*H*-[1,2,4]triazolo[4,3-*d*][1,4]benzothiazin-1-one (**6m**), which showed an ED_{50} value of 9.2 mg/kg and a PI value of 15.4. These values were superior to those provided by carbamazepine (ED_{50} and PI values of 11.8 and 6.4, respectively) in the

MES test in mice. As well as its anti-MES efficacy, the potencies of compound **6m** against seizures induced by PTZ, 3-MP, and BIC were also established, with the results suggesting that several different mechanisms of action might be involved in its anticonvulsant activity, including the inhibition of voltage-gated ion channels and the modulation of GABAergic activity.

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Conflict of interest We declare that we have no conflict of interest with respect to this study.

References

Arnoldi A, Bonsignori A, Melloni P, Merlini L, Luisa Quisadri M, Rossi AC, Valsecchi M (1990) Synthesis and anticonvulsant and sedative-hypnotic activity of 4-(alkylimino)-2,3-dihydro-4H-1-benzopyrans and benzothiopyrans. J Med Chem 33:2865–2869

Bialer M, Yagen B (2007) Valproic acid: second generation. Neurotherapeutics 4:130–137

Bialer M, Johannessen SI, Kupferberg HJ, Levy RH, Perucca E, Tomson T (2007) Progress report on new antiepileptic drugs: a summary of the Eigth Eilat Conference (EILAT VIII). Epilepsy Res 73:1–52

Bootsma HP, Ricker L, Hekster YA, Hulsman J, Lambrechts D, Majoie M, Schellekens A, Krom M, Aldenkamp AP (2009) The impact of side effects on long-term retention in three new antiepileptic drugs. Seizure 18:327–331

Donner EJ, Snead OC (2006) New generation anticonvulsants for the treatment of epilepsy in children. NeuroRX 3:170–180

Gale K (1992) GABA and epilepsy: basic concepts from preclinical research. Epilepsia 33:S3–S12

Gitto R, Orlando V, Quartarone S, De Sarro G, De Sarro A, Russo E, Ferreri G, Chimirri A (2003) Synthesis and evaluation of pharmacological properties of novel annelated 2,3-benzodiazepine derivatives. J Med Chem 46:3758

Jin HG, Sun XY, Chai KY, Piao HR, Quan ZS (2006) Anticonvulsant and toxicity evaluation of some 7-alkoxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline-1(2H)-ones. Bioorg Med Chem 14:6868–6873

Kennedy GM, Lhatoo SD (2008) CNS adverse events associated with antiepileptic drugs. CNS Drugs 22:739–760

Krall RJ, Penry JK, White BG, Kupferberg HJ (1978) Antiepileptic drug development: II Anticonvulsant drug screening. Epilepsia 19:409–428

Levy RH, Mattson RH, Meldrum BS (eds) (1995) Antiepileptic drugs, 4th edn. Raven Press, New York, pp 99–110

Lien EJ, Liao RC, Shinouda HG (1979) Quantitative structure-activity relationships and dipole moments of anticonvulsants and CNS depressants. J Pharm Sci 68:463–465

Löscher W, Schmidt D (1988) Which animal models should be used in the search for new antiepileptic drugs? A proposal based on experimental and clinical considerations. Epilepsy Res 2: 145–181

Loscher W (1979) 3-Mercaptopropionic acid: convulsant properties, effects on enzymes of the γ-aminobutyrate system in mouse brain and antagonism by certain anticonvulsant drugs, aminooxyacetic acid and gabaculine. Biochem Pharmacol 28:1397–1407

Macdonald RL, Barker JL (1978) GABA and glycine may share the some conductance channel on cultured mammalian neurons. Neurology 28:325–330

Mc Namara OJ, Brunton LL, Lazo JS, Parker KL (eds) (2006) The Pharmacological Basis of Therapeutics. McGraw-Hill, New York, pp 501–526

Okada R, Negishi N, Nagaya H (1989) The role of the nigrotegmental GABAergic pathway in the propagation of pentylenetetrazol-induced seizures. Brain Res 480:383–387

Olsen RW (1981) GABA-Benzodiazepine-Barbiturate Receptor Interactions. J Neurochem 37:1–13

Penovich PE, James Willmore L (2009) Use of a new antiepileptic drug or an old one as first drug for treatment of absence epilepsy. Epilepsia 50:37–41

Poter RJ, Cereghino JJ, Gladding GD, Hessie BJ, Kupferberg HJ, Scoville B (1984) Antiepileptic drug development program. Cleve Clin Q 51:293–305

Quan ZS, Wang JM, Rho JR, Kwak KC, Kang HC, Qun CS, Chai KY (2005) Synthesis of 6-alkyloxyl-3,4-dihydro-2(1H)-quinoliones and their anticonvulsant activities. Bull Korean Chem Soc 26:1757

Shridhar DR, Jogibhukta M, Vishwakarma LC, Joshi PP, Narayan GKASS (1984) Synthesis and biological activity of some substituted 4H-[1,2,4]triazolo[3,4-c][1,4]benzoxazines and 4H-[1,2,4]triazolo[3,4-c][1,4]benzothiazines. Indian J Chem 23B:445–448

Shu B, Zheng Y, Wang SB, Deng XQ, Quan ZS (2013) Design, synthesis, and anticonvulsant activity evaluation of 4-(3-alkoxy-phenyl)-2,4-dihydro-[1,2,4]triazol-3-ones. Arch Pharm 346:127–133

Stefan H, Feuerstein T (2007) Novel anticonvulsivant drugs. Pharmacol Ther 113:165–183

Strine TW, Kobau R, Chapman DP, Thurman DJ, Price P, Balluz LS (2005) Psychological distress, comorbidities, and health behaviors among U.S. adults with seizures: results from the 2002 National Health Interview Survey. Epilepsia 46:1133–1139

White HS (2003) Preclinical development of antiepileptic drugs: past, present, and future directions. Epilepsia 44:2–8

Zhang LQ, Guan LP, Wei CX, Deng XQ, Quan ZS (2010) Synthesis and anticonvulsant activity of some 7-Alkoxy-2*H*-1,4-benzothiazin-3(4*H*)-ones and 7-Alkoxy-4*H*-[1,2,4]triazolo[4,3-*d*]benzo[*b*][1,4]thiazines. Chem Pharm Bull 58:326–331

Zheng Y, Deng XQ, Shu B, Wang SB, Cao X, Quan ZS (2013) Synthesis and evaluation of the anticonvulsant activity of 5-alkylthio-4-phenyl-2,4-dihydro-3*H*-1,2,4-triazol-3-one derivatives. Lett Drug Des Discovery 10:543–549