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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Microwave-assisted synthesis and anticonvulsant activity of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives

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Abstract A series of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives were synthesized through microwave-promoted chemistry by condensation of the aromatic 1,2-diketones and thiosemicarbazide in a mixed green solvent. Subsequently, *S*-alkylation of 1,2,4-triazine-3-thiols afforded *S*-substituted derivatives. The anticonvulsant activity of the synthesized compounds was evaluated in vivo by electroshock and pentylenetetrazole (PTZ)-induced seizures tests. Among them, compound **4a** bearing 4-pyridylmethylthio moiety on the triazine ring showed the highest protection in both electroshock and PTZ-induced seizures tests. Compound **4a** showed no sign of neurotoxicity at the dose of 100 mg/kg in both rotarod and chimney tests.

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Introduction

Approximately 1 % of the world's population has epilepsy, the second most common neurologic disorder after stroke. Although standard therapy with antiepileptic drugs (AEDs) permits control seizures in 50 % of patients developing partial seizures and 60–70 % of those developing generalized seizures, rest are not responsive to the conventionally available AEDs. Moreover, the current drug therapy exhibits unfavorable side effects such as ataxia, hepatotoxicity, megaloblastic anemia, gingival hyperplasia, and hirsutism. These facts necessitate the search for new AEDs (Landmark, 2007; Siddiqui and Ahsan, 2010).

Several heterocycle containing compounds have been reported to have considerable antiepileptic activities. Among them, 1,2,4-triazines 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 (Kaushik *et al*., 2010). 1,2,4-Triazine template has also been associated with diverse pharmacological activities such as antiplatelet aggregation, anti-inflammatory (Ansari *et al*., 2010), potent neuroprotective (Irannejad *et al*., 2010), and antifungal (Sangshetti and Shinde, 2010) activities.

The reported structure–activity relationships studies on the 5,6-diaryl-3-amino-1,2,4-triazines revealed that the required pharmacophoric elements are lipophilic aryl rings attached to the 1,2,4-triazine as well as presence of hydrogen bonding capable moieties (Mallikarjuna *et al*.,

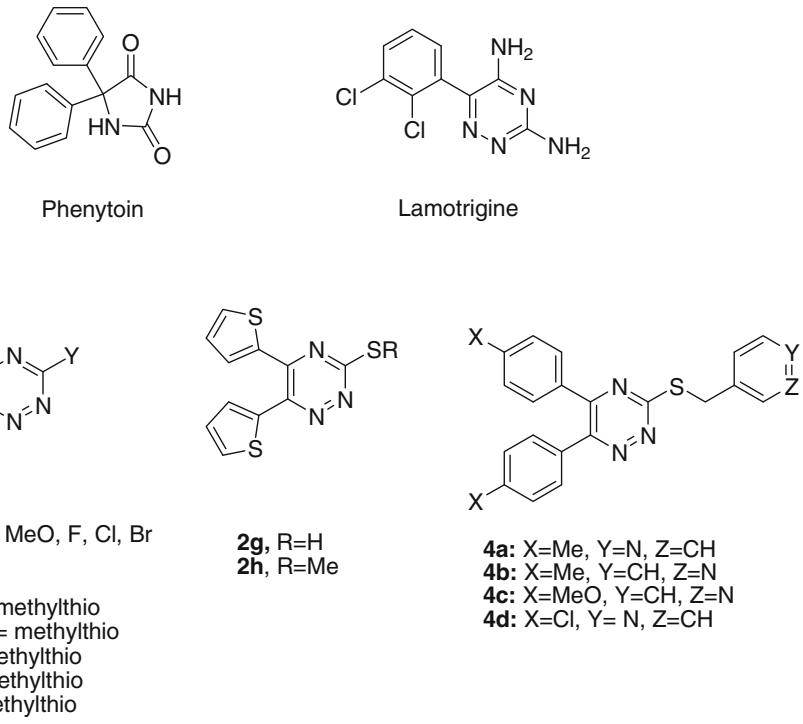


Fig. 1 Schematic representation of anticonvulsant drugs phenytoin and lamotrigine, and the synthesized compounds (**2a–g**, **3a–c**, and **4a–d**)

2007). These findings prompted us to the synthesis of some novel 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives **2–4** as anticonvulsant agents (Fig. 1).

Microwave-assisted organic synthesis (MAOS) has affected synthetic chemistry mainly by enabling rapid, reproducible, and scalable chemistry development. Microwave-enhanced organic synthesis can facilitate the discovery of new reactions and novel pharmaceutical lead compounds in drug discovery projects by reducing reaction time in optimization of reactions. In addition, it serves to expand chemical space and diversity in compound library design and synthesis. Synthesis of heterocycles has been significantly affected by the use of microwave in organic chemistry (Kappe and Dallinger, 2006); (Shipe *et al*., 2005).

Conventional methods for the synthesis of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives have been reported in the literature by condensation of aromatic 1,2-diketones with thiosemicarbazide in refluxing conditions in absolute ethanol (Bhalla *et al*., 1995) or in acetic acid for several hours (Khoshneviszadeh *et al*., 2013). Recently, we reported synthesis of some potent neuroprotective agents based on the 5,6-bisaryl-1,2,4-triazine-3-thiol substructure by refluxing of 1,2-diketones and thiosemicarbazide in water and 1,4-dioxane (Irannejad *et al*., 2010). Heravi *et al*. have reported solventless synthesis of only one derivative, 5,6-bisphenyl-1,2,4-triazine-3-thiol under microwave irradiation in a domestic microwave oven that is not applicable for laboratory synthesis and does not have reproducible results (Heravi *et al*., 2006).

Accordingly, herein we describe microwave-assisted green synthesis and anticonvulsant activity of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives (Fig. 1).

Materials and methods

Chemistry

Chemical reagents and starting materials were purchased from Sigma-Aldrich or Merck. Compounds **2a–e** and **3a–d** were known compounds and reported in literature (Ansari *et al*., 2010; Irannejad *et al*., 2010). ^{1}H NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Brucker DRX-500 MHz instrument. The chemical shifts (δ) were measured relative to TMS as an internal standard. Coupling constants (*J*) are expressed in Hz. Mass spectra were obtained with a Finnigan Mat TSQ-70 or Agilent 7890A spectrometers. TLC was conducted on 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 watt power.

General procedure for preparation of **2a–g**

To a mixture of the appropriate 1,2-diketone (1 eq) and thiosemicarbazide (1.2 eq) was added a few drops of concentrated hydrochloric acid in the reported solvent in Table 2 and then the mixture was irradiated under microwave conditions for 10 min at 120 °C (300 W). After

completion of the reaction, the precipitated solid was filtered and washed with water several times to give the desired product.

5,6-Diphenyl-1,2,4-triazine-3-thiol (**2a**)

M.p.: 89–91 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.32–7.45 (m, 6H, Ar), 7.51–7.56 (m, 4H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 128.42 (C-2 and C-6, phenyl), 128.73 (C-2 and C-6, phenyl), 128.82 (C-4, phenyl), 129.54 (C-4, phenyl), 129.61 (C-3 and C-5, phenyl), 130.57 (C-3 and C-5, phenyl), 135.53 (C-1, phenyl), 135.76 (C-1, phenyl), 155.91 (C-6, triazine), 156.31 (C-5, triazine), 173.62 (C-3, triazine). MS *m/z* (%): 265 (M^+, 34), 179 (100), 156 (33), 143 (21). Anal. Calcd for $C_{15}H_{11}N_3S$: C, 67.90; H, 4.18; N, 15.84. Found: C, 68.12; H, 4.34; N, 15.66.

5,6-Di-p-tolyl-1,2,4-triazine-3-thiol (**2b**)

M.p.: 156–157 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.35 (s, 3H, Ar-$\underline{CH_3}$), 2.36 (s, 3H, Ar–$\underline{CH_3}$), 7.11 (d, $J = 8.0$ Hz, 2H, Ar), 7.14 (d, $J = 8.0$ Hz, 2H, Ar), 7.40 (d, $J = 8.0$ Hz, 2H, Ar),7.46 (d, $J = 8.0$ Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 20.91 (CH_3), 21.44 (CH_3), 125.37 (C-2 and C-6, phenyl), 128.94 (C-2 and C-6, phenyl), 129.32 (C-3 and C-5, phenyl), 129.73 (C-3 and C-5, phenyl), 134.53 (C-1, phenyl), 135.51 (C-1, phenyl), 137.63 (C-4, phenyl), 138.21 (C-4, phenyl), 155.76 (C-6, triazine), 156.39 (C-5, triazine), 174.53 (C-3, triazine). MS *m/z* (%): 293 (M^+, 38), 207 (100), 191 (36), 170 (64), 133 (15). Anal. Calcd for $C_{17}H_{15}N_3S$: C, 69.59; H, 5.15; N, 14.32. Found: C, 69.34; H, 5.34; N, 14.76.

5,6-Bis(4-methoxyphenyl)-1,2,4-triazine-3-thiol (**2c**)

M.p.: 169 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 3.81 (s, 3H, OCH_3), 3.83(s, 3H, OCH_3), 6.80 (d, $J = 9.0$ Hz, 2H, Ar), 6.91 (d, $J = 9.0$ Hz, 2H, Ar), 7.52 (d, $J = 9.0$ Hz, 2H, Ar), 7.59 (d, $J = 9.0$ Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 55.31 (CH_3), 55.46 (CH_3), 114.33 (C-3 and C-5, phenyl), 114.54 (C-3 and C-5, phenyl), 127.30 (C-1, phenyl), 128.45 (C-1, phenyl), 134.80 (C-2 and C-6, phenyl), 135.55 (C-2 and C-6, phenyl), 155.86 (C-6, triazine), 156.77 (C-5, triazine), 159.71 (C-4, phenyl), 161.26 (C-4, phenyl), 174.22 (C-3, triazine). MS *m/z* (%): 325 (M^+, 22), 238 (73), 206 (100), 145 (46), 123 (16). Anal. Calcd. For $C_{17}H_{15}N_3O_2S$: C, 62.75; H, 4.65; N, 12.91. Found: C, 62.55; H, 4.29; N, 12.52.

5,6-Bis(4-fluorophenyl)-1,2,4-triazine-3-thiol (**2d**)

M.p.: 105–106 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.10 (t, $J = 8.5$ Hz, 2H, Ar), 7.15 (t, $J = 8.5$ Hz, 2H, Ar), 7.54 (dd, $J = 8.5$ Hz, 5.5 Hz, 2H, Ar), 7.59 (dd, $J = 8.5$ Hz, 5.5 Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 115.73 (C-3 and C-5, phenyl), 116.41 (C-3 and C-5, phenyl), 129.65 (C-1, phenyl), 130.55 (C-1, phenyl), 133.14 (C-2 and C-6, phenyl), 134.81 (C-2 and C-6, phenyl), 155.79 (C-6, triazine), 156.39 (C-5, triazine), 163.13 (C-4, phenyl), 163.90 (C-4, phenyl), 174.41 (C-3, triazine). MS *m/z* (%): 301 (M^+, 15), 214 (100), 173 (65), 160 (34). Anal. Calcd. For $C_{15}H_9F_2N_3S$: C, 59.79; H, 3.01; N, 13.95. Found: C, 59.42; H, 3.30; N,13.92.

5,6-Bis(4-chlorophenyl)-1,2,4-triazine-3-thiol (**2e**)

M.p.: 158–160 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.36 (d, $J = 9.0$ Hz, 2H, Ar), 7.38 (d, $J = 8.5$ Hz, 2H, Ar), 7.48 (d, $J = 9.0$ Hz, 2H, Ar), 7.58 (d, $J = 8.5$ Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 127.73 (C-2 and C-6, phenyl), 128.44 (C-2 and C-6, phenyl), 129.25 (C-3 and C-5, phenyl), 129.48 (C-3 and C-5, phenyl), 133.83 (C-1, phenyl), 134.36 (C-1, phenyl), 134.68 (C-4, phenyl), 135.63 (C-4, phenyl), 156.27 (C-6, triazine), 157.18 (C-5, triazine), 174.63 (C-3, triazine). MS *m/z* (%): 333 (M^+, 21), 246 (100), 223 (66), 209 (48), 150 (25), 147 (23). Anal. Calcd for $C_{15}H_9Cl_2N_3S$: C, 53.90; H, 2.71; N, 12.57. Found: C, 53.69; H, 2.84; N, 12.76.

5,6-Bis(4-bromophenyl)-1,2,4-triazine-3-thiol (**2f**)

M.p.: 147–148 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.61 (d, $J = 9.0$ Hz, 2H, Ar), 7.64 (d, $J = 9.0$ Hz, 2H, Ar), 7.70 (d, $J = 9.0$ Hz, 2H, Ar), 7.81 (d, $J = 8.8$ Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 121.55 (C-4, phenyl), 123.94 (C-4, phenyl), 127.68 (C-2 and C-6, phenyl), 129.73 (C-2 and C-6, phenyl), 132.02 (C-1, phenyl), 132.15 (C-1, phenyl), 134.81 (C-3 and C-5, phenyl), 136.62 (C-3 and C-5, phenyl), 154.66 (C-6, triazine), 159.45 (C-5, triazine), 176.42 (C-3, triazine). MS *m/z* (%): 425 (10), 423 (22), 421 (M^+, 14), 335 (100), 333 (57), 257 (32), 255 (19). Anal. Calcd for $C_{15}H_9Br_2N_3S$: C, 42.58; H, 2.14; N, 9.93. Found: C, 42.26; H, 2.37; N, 9.76.

5,6-Di(thiophen-2-yl)-1,2,4-triazine-3-thiol (**2g**)

M.p.: 215 °C; IR (KBr): 1289 (C=S), 1630 (C=N), 1503 (N=N). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 7.0 (t, $J = 5.0$ Hz, 1H, thienyl), 7,21 (t, $J = 5.0$ Hz, 1H, thienyl), 7.46 (d, $J = 3.5$ Hz, 1H, thienyl), 7.52 (d, $J = 3.5$ Hz, 1H, thienyl), 7.77 (d, $J = 5.0$ Hz, 1H, thienyl), 7.68 (d, $J = 5.0$ Hz, 1H, thienyl), ^{13}C NMR (125 MHz, $CDCl_3$) δ: 124.62 (C-5, thienyl), 126.90 (C-5, thienyl), 127.21 (C-3, thienyl), 127.65 (C-3, thienyl), 127.91 (C-4, thienyl), 128.30 (C-4, thienyl), 134.45 (C-2, thienyl), 138.21 (C-2, thienyl), 145.32 (C-6, triazine), 154.64 (C-5, triazine),

172.71 (C-3, triazine). MS *m/z* (%): 277 (M^+, 12), 191 (44), 161 (64), 149 (33), 91 (15). Anal. Calcd for $C_{11}H_7N_3S_3$: C, 47.63; H, 2.54; N, 15.15. Found: C, 47.44; H, 2.38; N, 15.32.

General procedure for preparation of **3a–e** and **2h**

To a stirring solution of compounds **2a–g** (1 mmol) in dry methanol (50 ml), methyl iodide (1.2 mmol) and triethylamine (0.1 ml) were added and the mixture was stirred for 2 h at room temperature. Then, the solvent was removed under reduced pressure. Water (20 ml) and dichloromethane (50 ml) were added to the mixture. The organic phase was separated, dried (sodium sulfate), filtered and evaporated under reduced pressure. The obtained residue was dissolved in chloroform and then precipitated by adding petroleum ether to give the desired product.

3-(Methylthio)-5,6-di p-tolyl-1,2,4-triazine (**3a**)

M.p.: 149–150 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.37 (s, 3H, Ar–$\underline{CH_3}$), 2.38 (s,3H, Ar–$\underline{CH_3}$), 2.74 (s, 3H, SCH_3), 7.13 (d, $J = 8.0$ Hz, 2H, Ar), 7.16 (d, $J = 8.0$ Hz, 2H, Ar), 7.42 (d, $J = 8.0$ Hz, 2H, Ar),7.47 (d, $J = 8.0$ Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 13.34 (SCH_3), 20.92 (Ar–$\underline{CH_3}$), 21.54 (Ar–$\underline{CH_3}$), 125.32 (C-2 and C-6, phenyl), 128.91 (C-2 and C-6, phenyl), 129.27 (C-3 and C-5, phenyl), 129.73 (C-3 and C-5, phenyl), 134.56 (C-1, phenyl), 135.54 (C-1, phenyl), 137.66 (C-4, phenyl), 138.26 (C-4, phenyl), 155.72 (C-6, triazine), 156.32 (C-5, triazine), 174.33 (C-3, triazine). MS *m/z* (%): 307 (M^+, 10), 206 (100), 176 (89), 190 (5), 119 (12). Anal. Calcd. For $C_{18}H_{17}NS$: C, 70.33; H, 5.57; N, 13.67. Found: C, 70.49; H, 5.19; N, 13.72.

5,6-Bis(4-methoxyphenyl)-3-(methylthio)-1,2,4-triazine (**3b**)

M.p.: 134 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.75 (s, 3H, SCH_3), 3.80 (s, 3H, OCH_3), 3.85(s, 3H, OCH_3), 6.85 (d, $J = 9.0$ Hz, 2H, Ar), 6.90 (d, $J = 9.0$ Hz, 2H, Ar), 7.50 (d, $J = 9.0$ Hz, 2H, Ar), 7.57 (d, $J = 9.0$ Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.73 (SCH_3), 55.32 (OCH_3), 55.45 (OCH_3), 114.35 (C-3 and C-5, phenyl), 114.56 (C-3 and C-5, phenyl), 127.28 (C-1, phenyl), 128.46 (C-1, phenyl), 134.82 (C-2 and C-6, phenyl), 135.54 (C-2 and C-6, phenyl), 155.80 (C-6, triazine), 156.76 (C-5, triazine), 159.70 (C-4, phenyl), 161.23 (C-4, phenyl), 174.13 (C-3, triazine). MS: *m/z* (%): 339 (M^+, 5), 237 (100), 222 (55), 194 (10), 152 (22), 118 (18), 54 (8). Anal. Calcd. For $C_{18}H_{17}N_3O_2S$: C, 63.70; H, 5.05; N, 12.38. Found: C, 63.92; H, 5.16; N, 12.42.

5,6-Bis(4-fluorophenyl)-3-(methylthio)-1,2,4-triazine (**3c**)

M.p.: 83 °C, ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.76 (s, 3H, SCH_3), 7.05 (t, $J = 8.5$ Hz, 2H, Ar), 7.08 (t, $J = 8.5$ Hz, 2H, Ar), 7.5 (dd, $J = 8.5$ Hz, 5 Hz, 2H, Ar), 7.56 (dd, $J = 8.5$ Hz, 5 Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.54 (SCH_3), 115.70 (C-3 and C-5, phenyl), 116.38 (C-3 and C-5, phenyl), 129.62 (C-1, phenyl), 130.57 (C-1, phenyl), 133.12 (C-2 and C-6, phenyl), 134.79 (C-2 and C-6, phenyl), 155.73 (C-6, triazine), 156.32 (C-5, triazine), 163.04 (C-4, phenyl), 163.89 (C-4, phenyl), 174.23 (C-3, triazine). MS: *m/z* (%): 315 (M^+, 5), 214 (100). Anal. Calcd. For $C_{16}H_{11}F_2N_3S$: C, 60.94; H, 3.52; N, 13.33. Found: C, 60.72; H, 3.36; N, 13.42.

5,6-Bis(4-chlorophenyl)-3-(methylthio)-1,2,4-triazine (**3d**)

M.p.: 137–139 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.70 (s, 3H, SCH_3), 7.34 (d, $J = 9.0$ Hz, 2H, Ar), 7.37 (d, $J = 8.5$ Hz, 2H, Ar), 7.45 (d, $J = 9.0$ Hz, 2H, Ar), 7.50 (d, $J = 8.5$ Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.33 (SCH_3), 127.66 (C-2 and C-6, phenyl), 128.37 (C-2 and C-6, phenyl), 129.15 (C-3 and C-5, phenyl), 129.46 (C-3 and C-5, phenyl), 133.79 (C-1, phenyl), 134.35 (C-1, phenyl), 134.66 (C-4, phenyl), 135.59 (C-4, phenyl), 156.17 (C-6, triazine), 157.04 (C-5, triazine), 174.43 (C-3, triazine). MS *m/z* (%): 347 (M^+, 17), 245 (100), 176 (45), 123 (15), 63 (42). Anal. Calcd for $C_{16}H_{11}Cl_2N_3S$: C, 55.09; H, 3.18; N, 12.05. Found: C, 55.39; H, 3.24; N, 11.89.

5,6-Bis(4-bromophenyl)-3-(methylthio)-1,2,4-triazine (**3e**)

M.p.: 122–124 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 2.60 (s, 3H, SCH_3), 7.59 (d, $J = 9.0$ Hz, 2H, Ar), 7.61 (d, $J = 9.0$ Hz, 2H, Ar), 7.67 (d, $J = 9.0$ Hz, 2H, Ar), 7.79 (d, $J = 8.5$ Hz, 2H, Ar). ^{13}C NMR (125 MHz, $CDCl_3$) δ: 14.33 (SCH_3), 121.50 (C-4, phenyl), 123.91 (C-4, phenyl), 127.67 (C-2 and C-6, phenyl), 129.62 (C-2 and C-6, phenyl), 131.90 (C-3 and C-5, phenyl), 132.10 (C-3 and C-5, phenyl), 134.75 (C-1, phenyl), 136.52 (C-1, phenyl), 154.60 (C-6, triazine), 159.40 (C-5, triazine), 176.14 (C-3, triazine). MS *m/z* (%): 439 (9), 437 (25), 435 (M^+, 12), 338 (49), 336 (100), 333 (52). Anal. Calcd for $C_{16}H_{11}Br_2N_3S$: C, 43.96; H, 2.54; N, 9.61. Found: C, 44.16; H, 2.27; N, 9.49.

3-(Methylthio)-5,6-di(thiophen-2-yl)-1,2,4-triazine (**2 h**):

M.p.: 135 °C; IR (KBr): 3086, 2842, 1671 (C=N), 1523 (N=N). ^{1}H NMR (500 MHz, $CDCl_3$) δ (ppm): 2.74 (s, 3H, SCH_3), 7.03 (t, $J = 5.0$ Hz, 1H, Ar), 7,14 (t, $J = 5.0$ Hz, 1H, Ar), 7.41 (d, $J = 3.5$ Hz, 1H, Ar), 7.47 (d, $J = 3.5$ Hz,

1H, Ar), 7.56 (d, $J = 5.0$ Hz, 1H, Ar), 7.60 (d, $J = 5.0$ Hz, 1H, Ar), ^{13}C NMR (125 MHz, $CDCl_3$) δ: 16.35 (SCH_3), 125.54 (C-5, thienyl), 126.88 (C-5, thienyl), 127.33 (C-3, thienyl), 127.67 (C-3, thienyl), 127.82 (C-4, thienyl), 128.34 (C-4, thienyl), 133.49 (C-2, thienyl), 137.82 (C-2, thienyl), 143.35 (C-6, triazine), 154.70 (C-5, triazine), 173.40 (C-3, triazine). MS *m/z* (%): 291 (M^+, 23), 204 (30), 189 (100), 149 (46), 95 (15), 67 (22). Anal. Calcd for $C_{12}H_9N_3S_3$: C, 49.46; H, 3.11; N, 14.42. Found: C, 49.74; H, 2.98; N, 14.62.

General procedure for preparation of compound **4a–d**

To a stirred solution of compound **2** (1.7 mmol) in ethanol (20 ml), sodium hydroxide solution (170 mg in 5 ml of water) and appropriate chloromethylpyridinium chloride (2 mmol) were added respectively. The resulting mixture was stirred for 3 h at room temperature. After completion of the reaction, it was concentrated under vacuum, neutralized with HCl 1N. The resulting aqueous phase was extracted with chloroform three times, dried over sodium sulfate and concentrated. The product was purified by column chromatography (silica gel, mesh 230–400) eluting with dichloromethane–methanol (9:1).

3-((Pyridin-4-ylmethyl)thio)-5,6-di-p-tolyl-1,2,4-triazine (**4a**)

M.p.: 180–181 °C; IR (KBr): 3195, 2898, 1668 (C=N), 1556 (N=N). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.28 (s, 3H, Ar–$\underline{CH_3}$), 2.29 (s, 3H, Ar–$\underline{CH_3}$), 3.41 (s, 2H, SCH_2), 7.15 (d, $J = 8.5$ Hz, 2H, phenyl), 7.33 (d, $J = 8.5$ Hz, 2H, phenyl), 7.48 (d, $J = 8.5$ Hz, 2H, phenyl), 7.52 (d, $J = 9.0$ Hz, 2H, phenyl), 7.70 (d, $J = 9.0$ Hz, 2H, pyridine), 8.1 (d, $J = 8.8$ Hz, 2H, pyridine). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 21.30 (CH_3), 21.72 (CH_3), 34.83 (CH_2), 122.65 (C-3 and C-5, pyridine), 125.32 (C-2 and C-6, phenyl), 128.24 (C-2 and C-6, phenyl), 129.54 (C-3 and C-5, phenyl), 130.33 (C-3 and C-5, phenyl), 134.55 (C-1, phenyl), 135.54 (C-1, phenyl), 138.35 (C-4, phenyl), 138.60 (C-4, phenyl), 139.06 (C-4, pyridine), 148.86 (C-2 and C-6, pyridine), 155.44 (C-6, triazine), 156.55 (C-5, triazine), 174.50 (C-3, triazine). MS *m/z* (%): 384 (M^+, 15), 326 (55), 281 (65), 221 (25), 206 (36), 147 (100). Anal. Calcd for $C_{23}H_{20}N_4S$: C, 71.85; H, 5.24; N, 14.57. Found: C, 72.15; H, 5.54; N, 14.45.

3-((Pyridin-3-ylmethyl)thio)-5,6-di-p-tolyl-1,2,4-triazine (**4b**)

M.p.: 220–221 °C; IR (KBr): 3185, 2970, 1664 (C=N), 1550 (N=N). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 2.30 (s, 3H, Ar–$\underline{CH_3}$), 2.31 (s, 3H, Ar–$\underline{CH_3}$), 3.32 (s, 2H, SCH_2), 7.15–7.23 (m, 4H, phenyl), 7.16 (d, $J = 8.5$ Hz, 2H, phenyl), 7.27 (d, $J = 8.5$ Hz, 2H, phenyl), 7.66 (d, $J = 8.0$ Hz, 1H, pyridine), 7.75 (d, $J = 8.0$ Hz, 1H, pyridine), 8.31 (d, $J = 7.5$ Hz, 1H, pyridine), 8.34 (s, 1H, pyridine). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 20.90 (CH_3), 21.55 (CH_3), 37.33 (CH_2), 123.55 (C-5, pyridine), 125.32 (C-2 and C-6, phenyl), 128.58 (C-2 and C-6, phenyl), 129.33 (C-3 and C-5, phenyl), 129.73 (C-3 and C-5, phenyl), 134.51 (C-3, pyridine), 134.76 (C-1, phenyl), 135.90 (C-1, phenyl), 136.43 (C-4, pyridine), 137.72 (C-4, phenyl), 138.60 (C-4, phenyl), 148.15 (C-6, pyridine), 150.41 (C-2, pyridine), 155.72 (C-6, triazine), 156.55 (C-5, triazine), 172.85 (C-3, triazine). MS *m/z* (%): 384 (M^+, 10), 326 (44), 281 (62), 221 (35), 206 (66), 147 (100). Anal. Calcd for $C_{23}H_{20}N_4S$: C, 71.85; H, 5.24; N, 14.57. Found: C, 72.19; H, 5.43; N, 14.39.

5,6-Bis(4-methoxyphenyl)-3-((pyridin-3-ylmethyl)thio)-1,2,4-triazine (**4c**)

M.p.: 265–266 °C; IR (KBr): 3181, 2835, 1673 (C=N), 1556 (N=N). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.76 (s, 3H, OCH_3), 3.77 (s, 3H, OCH_3), 3.78 (s, 2H, SCH_2), 6.90 (d, $J = 8.5$ Hz, 2H, phenyl), 6.91 (d, $J = 8.5$ Hz, 2H, phenyl), 7.21 (d, $J = 8.5$ Hz, 2H, phenyl), 7.37 (d, $J = 8.5$ Hz, 2H, phenyl), 7.57 (d, $J = 8.5$ Hz, 1H, pyridine), 7.70 (d, $J = 8.0$ Hz, 1H, pyridine), 8.84 (d, $J = 8.0$ Hz, 1H, pyridine), 8.27 (s, 1H, pyridine). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 37.26 (CH_2), 55.20 (OCH_3), 55.35 (OCH_3), 114.34 (C-3 and C-5, phenyl), 114.65 (C-3 and C-5, phenyl), 123.32 (C-5, pyridine), 127.34 (C-1, phenyl), 128.41 (C-1, phenyl), 134.55 (C-2 and C-6, phenyl), 134.89 (C-2 and C-6, phenyl), 135.55 (C-3, pyridine), 136.75(C-4, pyridine), 148.15 (C-6, pyridine), 150.31 (C-2, pyridine), 155.72 (C-6, triazine), 156.34 (C-5, triazine), 159.77 (C-4, phenyl), 161.15 (C-4, phenyl), 173.45 (C-3, triazine). MS *m/z* (%): 416 (M^+, 26), 326 (45), 281 (100), 252 (27), 206 (56), 147 (96), 118 (34). Anal. Calcd for $C_{23}H_{20}N_4O_2S$: C, 66.33; H, 4.84; N, 13.45. Found: C, 66.59; H, 4.64; N, 13.79.

5,6-Bis(4-chlorophenyl)-3-((pyridin-4-ylmethyl)thio)-1,2,4-triazine (**4d**)

M.p.: 205–206 °C; IR (KBr): 3125, 2888, 1672 (C=N), 1552 (N=N). ^{1}H NMR (500 MHz, DMSO-d_6) δ: 3.72 (s, 2H, SCH_2), 7.29 (d, $J = 8.5$ Hz, 2H, phenyl), 7.38 (d, $J = 8.5$ Hz, 2H, phenyl), 7.43 (d, $J = 8.5$ Hz, 2H, phenyl), 7.46 (d, $J = 8.5$ Hz, 2H, phenyl), 7.57 (d, $J = 9.0$ Hz, 2H, pyridine), 8.4 (d, $J = 9.0$ Hz, 2H, pyridine). ^{13}C NMR (125 MHz, DMSO-d_6) δ: 35.33 (CH_2), 122.70 (C-3 and C-5, pyridine), 126.5 (C-2 and C-6, phenyl), 128.27 (C-2 and C-6, phenyl), 128.92 (C-3 and C-5, phenyl), 129.34

(C-3 and C-5, phenyl), 133.72 (C-1, phenyl), 134.35 (C-1, phenyl), 134.73 (C-4, phenyl), 135.52 (C-4, phenyl), 137.34 (C-4, pyridine), 148.64 (C-2 and C-6, pyridine), 155.70 (C-6, triazine), 156.44 (C-5, triazine), 175.59 (C-3, triazine). MS *m/z* (%): 425 (M^+, 55), 326 (43), 281 (75), 206 (16), 147 (100), 94 (15). Anal. Calcd for $C_{21}H_{14}Cl_2N_4S$: C, 59.3; H, 3.32; N, 13.17. Found: C, 59.49; H, 3.54; N, 13.39.

Pharmacology

Male NMRI mice (Pasture Institute, Tehran, Iran) weighting 18–22 g were used for pharmacological study. Animals were allowed free access to food and water except during the experiment and housed at controlled room temperature with 12 h light/dark schedule (lights on at: 0700 h). All compounds were dissolved a vehicle consists of DMSO (10 %) in distilled water with the injection volume of 10 mL/kg body weight of mice. Control group received DMSO (10 %) in saline. Each test compound was administered to different groups of mice at the dose of 100 mg/kg. The antiseizure effect of test compounds was also compared with a standard antiepileptic drug (lamotrigine 12.5 mg/kg, i.p.), which has been shown to produce protective effect in both Electroshock and PTZ induced seizure. Each animal was used once throughout the experiments.

Electroshock-induced seizure test

The electroshock-induced seizure test was carried out by the methods described before. Seizures were elicited with a 50 Hz alternating current of 40 mA intensity in mice using a generator (Borjsanat, Tehran, Iran). The current was applied via corneal electrodes for 0.2 s. This current intensity could produce tonic–clonic seizures in mice. Protection against the spread of Electroshock-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure (Woodbury and Davenport, 1952; Woodbury and Swinyard, 1952). At 30 min after the administration of the test compounds, the activities were evaluated in electroshock-induced seizure test. Protection against seizures was expressed as the number of mice that did not exhibit hind-limb extension after applying electroshock to the animal.

PTZ-induced seizure test

PTZ-induced seizure test was performed 30 min after intraperitoneal (i.p.) injection (at a volume of 10 ml/kg) of therapeutic drugs or their respective vehicle. Seizures were induced by intravenous (i.v.) infusion of 1 % PTZ in saline at the constant rate of 0.25 ml/min using an infusion pump (model 53140, Stoelting, USA) through tail vein. A 30-gauge needle with flexible tube, allows infusion of PTZ to unrestrained animal. Infusion was stopped either when a tonic–clonic convulsion with loss of righting reflex was achieved or after the animal received a maximum amount of 1 ml PTZ solution. The dose of PTZ (mg/kg) administered to induce tonic–clonic seizures with loss of righting reflex was considered as seizures threshold (Löscher *et al*., 1991).

Neurotoxicity

The neurotoxicity of the compounds was measured in mice by the rotarod test as well as chimney test. In 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. injection of the test compounds and 30 min later were tested again on rotarod. 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. The effects of test compounds, at doses corresponding to their anticonvulsant effect from the PTZ and Electroshock-induced seizure tests, on motor performance in mice were also quantified with the chimney test (Naderi *et al*., 2011). In this test, animals had to climb backwards up a plastic tube (25-cm length, 3-cm inner diameter). Motor impairment was indicated by the inability of the animals to perform the test within 60 s. Motor impairment was defined as the number of mice who failed to climb the tube in 60 s.

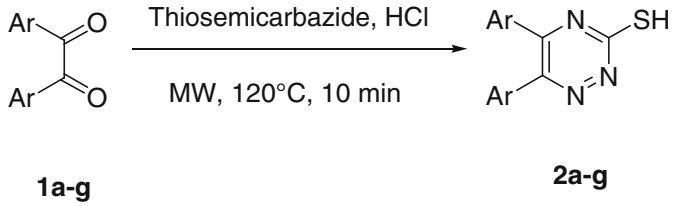
Statistical analysis

Data were analyzed using Graphpad Prism 5 (Graphpad Software Inc.). In electroshock, rotarod and chimney tests, the results of test drugs were compared with control group using Fisher's exact test. In PTZ-induced seizure, one-way ANOVA followed by Dunnett's post-test was used. Data were shown as mean ± SEM and a P value <0.05 was considered as statistically significant.

Computational study

Chemical structures were sketched in ChemSketch (ACD Lab 12.0, Advanced Chemistry Development, Inc.) and imported to ACD/3D Viewer version 12.0. The pharmacophore mapping studies, the distance between the various groups postulated as essential pharmacophoric elements for anticonvulsant activity, were performed on the 3D optimized structures. OSIRIS Property Explorer (OSIRIS Property Explorer, 2013) was used for the prediction of cLogP and solubility values reported in Table 1.

Table 1 Synthesis of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives

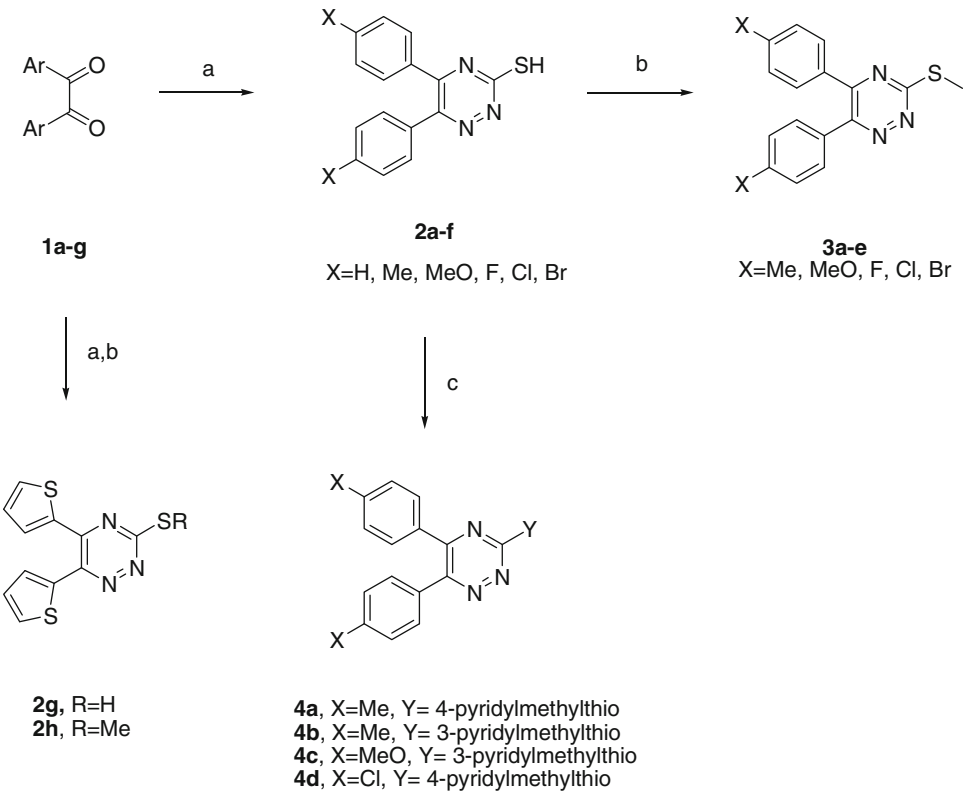


1a-g **2a-g**

Compound	Ar	Formula	MW	Solubility[a]	cLogP[b]	M.p. (°C)
2a	Phenyl	$C_{15}H_{11}N_3S$	265	−4.59	3.54	210
2b	*p*-Tolyl	$C_{17}H_{15}N_3S$	293	−5.28	4.17	182
2c	4-Methoxyphenyl	$C_{17}H_{15}N_3O_2S$	325	−4.63	3.33	235
2d	4-Fluorophenyl	$C_{15}H_9F_2N_3S$	301	−5.22	3.66	210
2e	4-Chlorophenyl	$C_{15}H_9Cl_2N_3S$	334	−6.06	4.77	205
2f	4-Bromophenyl	$C_{15}H_9Br_2N_3S$	423	−6.26	4.94	162
2g	2-Thienyl	$C_{11}H_7N_3S_3$	277	−4.43	3.78	215

[a] Estimated logS value is a logarithm (base 10) of the predicted solubility in mol/liter

[b] Logarithm of the partition coefficient between *n*-octanol and water $\log(C_{octanol}/C_{water})$



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* methyl iodide, triethylamine, methanol, rt; *c* 4-chloromethylpyridinium chloride or 3-chloromethylpyridinium chloride, NaOH, EtOH, rt

Results and discussion

Chemistry

The synthetic pathways leading to the formation of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives **2**–**4** are outlined in the Scheme 1. The 5,6-bisaryl-1,2,4-triazine-3-thiols **2a**–**g** were obtained by the condensation of 1,2-diarylketones **1a**–**g** with thiosemicarbazide and catalytic amount of hydrochloric acid under microwave irradiation at 120 °C for 10 min in the appropriate solvent system reported in Table 1. 5,6-Bisaryl-1,2,4-triazine-3-thiols **2a**–**g** were *S*-

Table 2 Optimization of reaction between 1,2-diketones and thiosemicarbazide in different solvent with their isolated production yield (%)

Compound	DMF	Acetonitrile	Water	Solvent systems			
				Water/ethanol (2:1)	Water/ethanol (1:1)	Water/ethanol (1:2)	Abs. ethanol
2a	50	33	30	**92**	54	62	55
2b	56	30	15	**88**	85	76	77
2c	55	20	10	**91**	47	54	49
2d	40	32	25	56	**83**	61	62
2e	45	nt[a]	23	15	**93**	60	65
2f	47	nt	12	26	25	67	**81**
2g	53	nt	16	40	**80**	75	65

The highest isolated production yield for each derivative is highlighted in bold

[a] Not tested

methylated by using methyl iodide to give 3-methylthio-5,6-bisaryl-1,2,4-triazine derivatives **3a–e** and **2h**. Compounds **4a–d** were obtained by reacting of 5,6-bisaryl-1,2,4-triazine-3-thiols **2a–f** with appropriate chloromethylpyridinium chloride in ethanol at room temperature.

Some physicochemical properties of thiol compounds **2a–g**, such as predicted solubility, clogP, melting point, and MW are given in Table 1. The formation of compounds was confirmed by ^{1}H NMR, ^{13}C NMR and MS spectral data. The ^{1}H NMR spectrum of the representative compound **2g** showed six doublets for the two thienyl rings containing six protons at δ 7.03, 7.14, 7.41, 7.47, 7.56, and 7.60 ppm. The IR spectrum showed bands at 1,559 and 1,503 cm^{-1} for C=N and N=N bonds at triazine ring. The spectral information of compound **3a** was identical to the previously reported data in the literature (Ansari *et al*., 2010). The ^{1}H NMR spectrum of the representative compound 5,6-bis(4-methoxyphenyl)-3-((pyridin-3-ylmethyl)-thio)-1,2,4-triazine (**4c**) showed two singlets at δ 3.76 and 3.77 ppm for the two methoxyl groups, and a singlet at 3.78 ppm for the methylene protons. Aromatic protons appeared as doublets at δ 6.90, 6.91, 7.21, and 7.37 ppm. The pyridyl protons of compound **4c** appeared at 7.57 (doublet), 7.70 (doublet), 8.44 (doublet), and 8.47 (singlet) ppm. Also, MS spectral data showed the correct molecular ion peak of the product.

Microwave-assisted green synthesis of **2a–g**

We explored the condensation of aromatic 1,2-diketones with thiosemicarbazide starting with the polar 4,4′-dimethoxybenzil in a different ratio of water and ethanol under MW irradiation for obtaining 5,6-bis(4-methoxyphenyl)-1,2,4-triazine-3-thiol (**2c**). After screening a range of various temperatures and reaction run times, we found that the highest yield could be accessible in 120 °C for 10 min and 300 watt power in closed vessel. The same protocol (reaction temperature and run time) was kept fixed for the preparation of all derivatives (Table 1). Afterward, choosing the best solvent among water, absolute ethanol, different ratio of water and ethanol (2:1, 1:1, and 1:2) was the crucial step for the optimization of reaction. Acetonitrile and dimethylformamide (DMF) have also been used as solvents. The results are summarized in Table 2.

The production yield in different ratio of water and ethanol strongly depends on the lipophilicity (LogP) or solubility of the starting material and final compound in the solvent. In the cases of compounds **2a** and **2c**, with more polar substituents and lower cLogP (3.33 and 3.54, respectively) and by using water and ethanol (2:1 ratio) as solvent, the higher yields (>90 %) were obtained. The most nonpolar substituent, *para*-bromo on the phenyl ring in compound **2f** with cLogP = 4.94 gave the highest yield in absolute ethanol (81 %). Eventually, the other compounds **2b**, **2d**, **2e**, and **2g**, dependent on their lipophilicity, performed well in either 2:1 or 1:1 ratio mixtures of water and ethanol with 88, 83, 93, and 80 % yields, respectively.

Performing reaction under microwave irradiation in organic solvents like acetonitrile and DMF produced low yields (40–50 % for DMF and 20–30 % for acetonitrile) as well as much more by-product and increased impurity of the product checked by TLC. Our observations showed that increasing percentage of water in the solvent system reduces the number of by-products and performs a clean reaction. However, hydrophobic nature of the starting material, 1,2-diketones bearing chloro, fluoro, bromo, and methyl at the *para*-position of phenyl ring, and also 2-thienyl group limits the high percentage usage of water in the reaction solvent. Usage of water alone as solvent did not give satisfactory yields due to insolubility problem of 1,2-diketones. Adding an environmentally friendly solvent with high safety such as ethanol for solubilizing the starting materials and also its high intrinsic ability to absorb microwave irradiation (dielectric loss = 22.86) makes ethanol a prominent solvent for MAOS (Hayes, 2002). Therefore, the best results were obtained in a mixture of

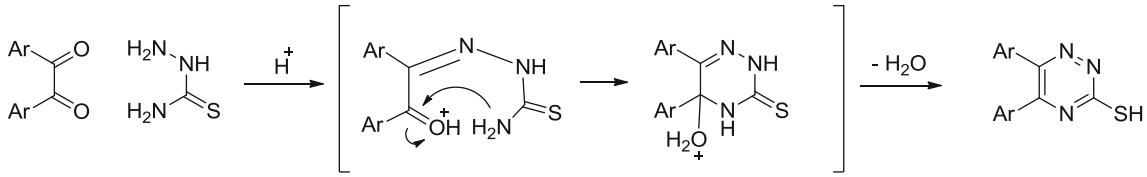
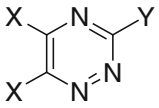


Fig. 2 Possible mechanism for the formation of the 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives

Table 3 The anticonvulsant effect of the test compounds (100 mg/kg) against electroshock-induced seizures, as well as muscle coordination in rotarod and Chimney tests



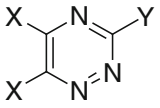
Compound	X	Y	Electroshock induced seizure (no. of animals protected/no. of animals tested)	Rotarod (no. of animal exhibiting toxicity/no. of animal tested)	Chimney (no. of animal exhibiting toxicity/no. of animal tested)
2b	*p*-Tolyl	–SH	1/6	1/6	1/6
2c	4-Methoxyphenyl	–SH	0/6	0/6	0/6
2e	4-Chlorophenyl	–SH	3/6	0/6	0/6
2g	2-thienyl	–SH	1/6	0/6	2/6
2h	2-thienyl	–SMe	2/6	1/6	1/6
3a	*p*-Tolyl	–SMe	3/6	0/6	0/6
3b	4-Methoxyphenyl	–SMe	3/6	0/6	1/6
3c	4-Chlorophenyl	–SMe	3/6	0/6	2/6
4a	*p*-Tolyl	4-Pyridylmethylthio	6/6	0/6	0/6
4b	*p*-Tolyl	3-Pyridylmethylthio	4/6	0/6	1/6
4c	4-Methoxyphenyl	3-Pyridylmethylthio	2/6	0/6	1/6
4d	4-Chlorophenyl	4-Pyridylmethylthio	5/6	1/6	0/6
Lamotrigine			6/6	0/6	0/6

water and ethanol with ratio of 2:1 and 1:1 for most various hydrophobic starting materials **1** with the exception of 4,4′-dibromobenzil.

Previous results have also revealed that simple alcohols like methanol and ethanol or alkanes such as heptane and hexane are environmentally preferable solvents, whereas the use of dioxane, acetonitrile, and tetrahydrofuran is not recommended from an environmental perspective. Additionally, in agreement with our finding, a case study indicated that methanol–water or ethanol–water mixtures are environmentally favorable compared to pure alcohol or propanol–water mixtures for solvolysis of *p*-methoxybenzoyl chloride (Capello *et al*., 2007).

Additionally, on the basis of the previous studies on the formation of triazine ring with thiosemicarbazide and phenylglyoxalaldoxime (Lalezari and Golgolab, 1970), a possible mechanism could be suggested as shown in Fig. 2. The first step is the feasible and easy formation of thiosemicarbazone intermediate in the presence of acid. This reaction involves nucleophilic attack of the hydrazine end of thiosemicarbazide as a strong nucleophile to one of the carbonyl groups. This open chain intermediate could be isolated and purified as a stable product. In the second step, the less and weak nucleophilic thioamide nitrogen atom attacks to the protonated carbonyl group to form the triazine ring. This step demands much more energy and driving force that is provided significantly by the high energy microwaves. Moreover, usage of a solvent that is capable to convert microwave electromagnetic energy into heat is favorable. The best parameter that provides an organic chemist with the coupling efficiency of a particular solvent is defined by dielectric loss. The more dielectric loss of a solvent, the higher converting ability of microwave energy is into heat. Therefore, using of a solvent with low dielectric loss such as DMF and acetonitrile with 6.07 and 2.32 is not recommended for performing a high energy

Table 4 Anticonvulsant effect of test compounds (100 mg/kg) against PTZ-induced seizures in mice



Compound	X	Y	PTZ dose (mg/kg)[a]
2b	*p*-Tolyl	–SH	40.4 ± 3.8
2c	4-Methoxyphenyl	–SH	nt[b]
2e	4-Chlorophenyl	–SH	nt
2g	2-thienyl	–SH	24.42 ± 4.63
2h	2-thienyl	–SMe	38.23 ± 2.34
3a	*p*-Tolyl	–SMe	39.4 ± 2.9
3b	4-Methoxyphenyl	–SMe	41.4 ± 4.2
3c	4-Chlorophenyl	–SMe	nt
4a	*p*-Tolyl	4-Pyridylmethylthio	49.4 ± 4.4
4b	*p*-Tolyl	3-Pyridylmethylthio	47.0 ± 4.7
4c	4-Methoxyphenyl	3-Pyridylmethylthio	41.5 ± 4.1
4d	4-Chlorophenyl	4-Pyridylmethylthio	43.0 ± 5.3
Lamotrigine			72.9 ± 10.6
Control[c]			31.5 ± 4.4

[a] PTZ dose (mg/kg) to induce clonic seizures (number of mice in each group = 6); a significant increase in required dose of PTZ to induce seizures shows significant protective effect of the test compound. Values are shown as mean ± SEM

[b] Not tested

[c] DMSO (10 %)

demanding reaction like the second step of triazine ring closure here. Usage of an appropriate solvent with high dielectric loss like ethanol (dielectric loss = 22.86) definitely proceeds the reaction toward completion (Hayes, 2002).

Anticonvulsant activity

The anticonvulsant effect of the test compounds (100 mg/kg) against electroshock-induced seizure, as well as muscle coordination in rotarod and chimney tests were listed in Table 3.

The results of electroshock-induced seizure test revealed that compounds **4a** and **d** bearing 4-pyridylmethylthio group at the position 3 of triazine ring had better antiseizure activity. Meanwhile, bis(*p*-tolyl)- analog **4a** showed 100 % protection against electroshock-induced seizure. Notably, the most effective compound **4a** showed no sign of neurotoxicity at the dose of 100 mg/kg in both rotarod and chimney tests. Moreover, in the rotarod test, other compounds with the exception of **2b** and **4d** did not show any sign of neurotoxicity. However, in the Chimney test

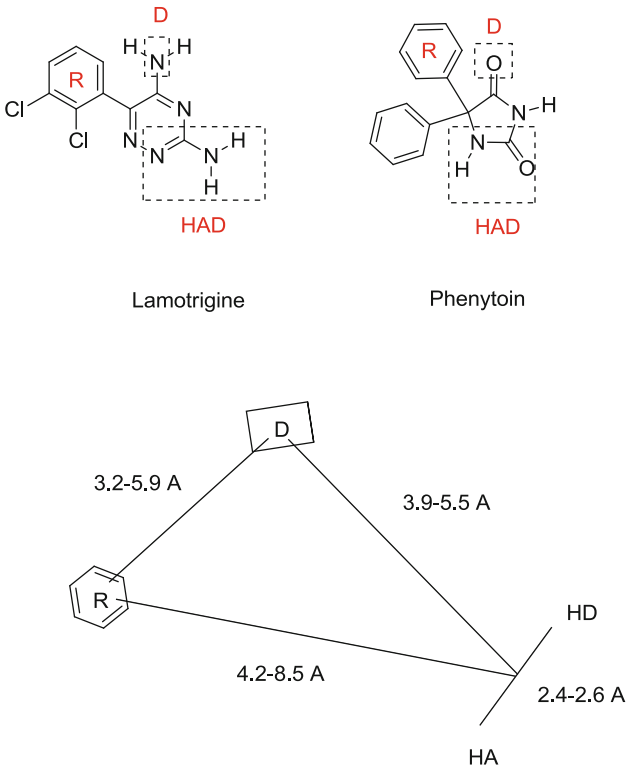


Fig. 3 Essential pharmacophoric elements in phenytoin and lamotrigine as voltage-gated sodium channel blockers. Distance ranges between the essential structural elements R, D, and HAD in pharmacophoric model proposed by Unverferth *et al.*, 1998

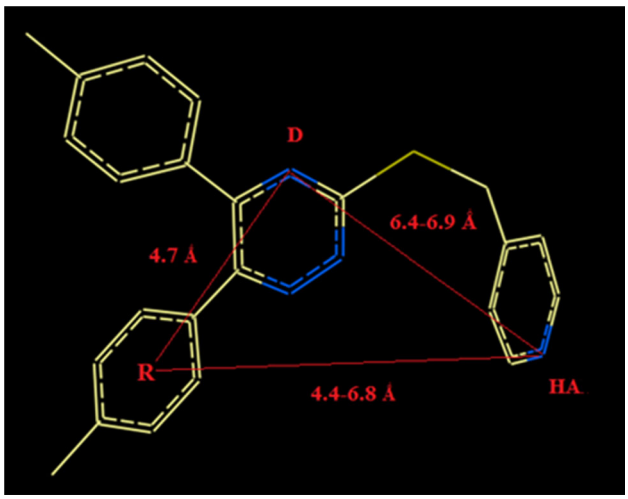


Fig. 4 Distance ranges between hydrophobic unit (R), electron-donor atom (D), and hydrogen bond acceptor (HA) in compound **4a**

some compounds exhibited maximum 33 % neurological toxicity (for example, compound **3c**). The results demonstrated that the target compounds had no significant neurotoxicity at the dose of 100 mg/kg.

The anticonvulsant activity of test compounds was also investigated by evaluating the protective potential of the compounds at the dose of 100 mg/kg against PTZ-induced

seizure in mice. The dose of PTZ (mg/kg) administered to induce tonic–clonic seizure with loss of righting reflex was considered as seizure threshold and the results were presented in Table 4. The control group receiving vehicle showed tonic–clonic seizures at the PTZ dose of 31.5 ± 4.4 mg/kg. A significant increase in required dose of PTZ to induce seizure shows significant protective effect of the test compounds. Again, compound **4a** having 4-pyridylmethylthio group showed the most protective effect against PTZ. The maximum required dose of PTZ (49.4 mg/kg) to induce seizure in mice among the compounds tested confirms the most protective effect of **4a**. Also, the regio-isomer of **4a** (compound **4b**) containing 3-pyridylmethylthio group showed significant increase in the required dose of PTZ to induce seizure in mice (47 mg/kg). In the case of standard drug lamotrigine, the higher dose of PTZ (72.9 mg/kg) was required for inducing tonic–clonic seizures.

Structurally, the obtained results demonstrated that presence of a lipophilic group on the vicinal bis-phenyl rings and a pyridylmethyl pendent group on sulfur atom connected to triazine core could increase anticonvulsant activity. It seems that the methyl or chloro-substituents at the *para*-position of the vicinal bis-phenyl rings which increased hydrophobic character of the molecule results in higher potency. The 4-methoxy derivative **4c** has relatively a lower hydrophobicity resulting in lowered anticonvulsant effect.

Computational study (distance mapping)

According to Unverferth *et al*. study (Unverferth *et al*., 1998) essential pharmacophoric elements that are necessary for good anticonvulsant activity through blocking of voltage-gated sodium channels are a hydrophobic unit (R), an electron-donor atom (D), and a hydrogen acceptor/donor unit (HAD). These structural features have to be positioned in an allowed specified distances to each other (Fig. 3).

Distance ranges between the essential structural features (R, D, and HAD) were measured for the most effective compound **4a** by ACD/3D Viewer software and are shown in Fig. 4. As shown, the measured distances are in the allowed and specified distance ranges defined by Unverferth *et al*. for the voltage-gated sodium channel blockers. Due to the flexibility of the 4-pyridylmethylthio side chain, distances between hydrophobic unit (R) and HAD and also between electron-donor atom (D) and HAD can be variable in a range shown in the figure.

Conclusion

In conclusion, we have described a rapid, simple and green procedure for the preparation of 5,6-bisaryl-1,2,4-triazine-3-thiol derivatives under MW irradiation that could be used in chemical library synthesis in combinatorial chemistry. Compared to the conventional synthetic methods, this approach has the advantage of using safe, nontoxic and environmentally benign solvents as well as being rapid, simple and easy purification procedure. The anticonvulsant activity evaluation of the synthesized compounds demonstrated that compound **4a** bearing 4-pyridylmethylthio moiety on the triazine ring showed the highest protection in both electroshock- and PTZ-induced seizures tests. The neurotoxicity study revealed that the target compound had no significant neurotoxicity at the dose of 100 mg/kg. Based on the promising pharmacological properties of compound **4a**, the 3-(pyridylmethylthio)triazine core structure with vicinal bis-aryl could be considered as a lead for discovery of novel antiseizure drugs.

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