

RESEARCH ARTICLE

Synthesis and anticonvulsant activity evaluation of 6-substituted-[1,2,4]triazolo[3,4-a](tetrazolo[5,1-a])phthalazine derivatives

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Abstract

With the aim of finding new anticonvulsant drugs, new 6-substituted-[1,2,4]triazolo[3,4-a](tetrazolo[5,1-a])phthalazine derivatives (**1–34**) have been designed and synthesized. All the compounds were evaluated for their anticonvulsant activities using the maximal electroshock test (MES). Most of the synthesized compounds exhibited potent anticonvulsant activities in the MES. The most promising compound **14** showed significant anticonvulsant activity in MES test with ED₅₀ value of 9.3 mg/kg. It displayed a wide margin of safety with protective index much higher than the standard drug Carbamazepine. And the potency of compound **14** against seizures induced by Pentylentetrazole, Isoniazid, Thiosemicarbazide and 3-Mercaptopropionic acid in the chemical-induced seizure tests suggested that compound **14** displayed wide spectrum of activity in several models.

Keywords: Triazole, Tetrazole, Phthalazine, Anticonvulsant, Maximal electroshock

Introduction

Epilepsy, a ubiquitous disease characterized by recurrent seizures, afflicts more than 60 million people worldwide according to epidemiological studies¹. For epilepsy treatment, nearly 95% of clinically available drugs were approved before 1985 and they could provide satisfactory seizure control for 60–70% of patients. These drugs, however, also cause notable adverse side effects such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anemia^{2–4}, and even life threatening conditions⁵. Research to find more effective and safer antiepileptic drugs is, therefore, imperative and challenging in medicinal chemistry.

We previously described the synthesis and anticonvulsant activity evaluation of some compounds containing triazole, which exhibited potent activity in maximal electroshock test (MES) and in several chemical models such as Pentylentetrazole- and Isoniazid-induced

seizure tests (Figure 1)^{6–12}. From the currently used AEDs, the major characteristics important in newly synthesized compounds are the inclusion of a hydrophobic unit (R), an electron donor group (D), a hydrogen donor/acceptor unit (HAD). With respect to the compounds mentioned above, obviously the R is the phenyl group and the substituents on it, the D is the oxygen atom and the HAD is the triazole. As part of our continuous efforts to find better anticonvulsant agents in this area, a series of 6-substituted-[1,2,4]triazolo[3,4-a](tetrazolo[5,1-a])phthalazine derivatives (**1–34**) were designed and synthesized in this study, which also possessed the three parts mentioned above.

All the 34 new compounds were synthesized and evaluated as anticonvulsant agents in experimental epilepsy models. The rotarod assay was performed in mice to evaluate the neurotoxicity of the compounds. The anti-MES activity and the neurotoxicity of the marketed agent carbamazepine were evaluated in our

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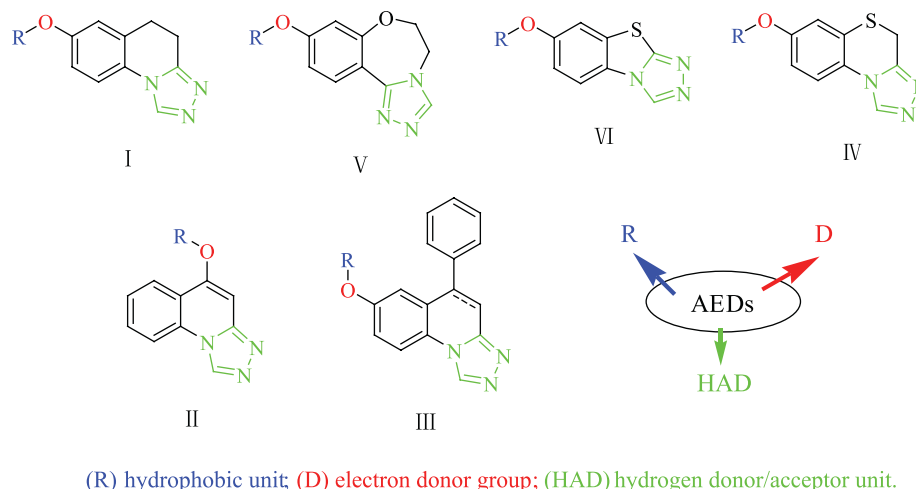


Figure 1. Structures of some compounds in containing triazole and the sketch map. (R) hydrophobic unit; (D) electron donor group; (HAD) hydrogen donor/acceptor unit.

laboratory under the same conditions for the purpose of comparison.

Experimental protocols

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on IR Prestige-21. ^1H NMR spectra were measured on an AV-300 (Bruker, Switzerland), and all chemical shifts were given in parts per million relative to tetramethylsilane. Mass spectra were measured on a HP1100LC (Agilent Technologies, USA). Elemental analyses were performed on a 204Q CHN (Perkin Elmer, USA). The chemicals were purchased from Aldrich Chemical Corporation.

Chemistry

Synthesis of 1,4-dichlorophthalazine (36)

Compound **35** (8.5 g, 52.5 mmol) was dissolved in phosphorus oxychloride (45 mL) and stirred under reflux for 4 h. Then the solvent was removed under vacuum. The residue was dissolved in dichloromethane (200 mL) and stirred rapidly, and the solution was neutralized by the addition of solid and aqueous sodium hydrogen carbonate (cautiously). When effervescence had ceased, the organic layer was separated and the aqueous layer was extracted with dichloromethane (200 mL twice). The combined organic layers were dried over MgSO_4 , filtered and evaporated. The residue was purified by silica gel column chromatography with ethyl acetate: petroleum ether (1:8) and gained 9.0 g.

Synthesis of 1-hydrazine-4-chlorophthalazine (37)

A solution of compound **36** (5 g, 25.1 mmol) in THF (60 mL) was added dropwise to a solution of hydrazine hydrate (6.28 g, 125.6 mmol) in THF (10 mL) at room temperature. The mixture was stirred and heated at 60°C for 1 h, then half of the solvent was removed under reduced pressure and the solution was poured into petroleum ether. The precipitate was filtered and washed with

petroleum ether, then kept below 0°C . The compound **37** obtained was pure enough for the following step.

Synthesis of 6-chloro-[1,2,4]triazolo[3,4-a]phthalazine (38)

In a round-bottomed flask, compound **37** (2.2 g, 11.3 mmol) was dissolved in Triethoxymethane (60 mL), and the solution was refluxed for 2 h, then the solvent was removed under reduced pressure. The residue was purified by silica gel chromatography using dichloromethane.

The general procedure for the synthesis of compounds 1–25

Compound **38** (0.65 g, 3.2 mmol), K_2CO_3 (0.44 g, 3.2 mmol), benzyltrietethylammonium chloride (TEBA) (0.23 g, 1 mmol) and corresponding alcohol (0.70 mmol) were suspended in acetonitrile (50 mL) reacted with stirring and refluxing for about 3 h. After the acetonitrile was removed under reduced pressure, the solid residue was purified by silica gel chromatography with ethyl acetate: petroleum ether (2:3) to obtain compounds **1–25**. The yield, melting point and spectral data of each compound were given below.

6-(Benzyloxy)-[1,2,4]triazolo[3,4-a]phthalazine (1)

Yield: 87%, m.p. $170\text{--}172^\circ\text{C}$. ^1H -NMR (CDCl_3): δ 8.93 (s, 1H, 3-H), 8.90 (d, 1H, $J = 9.0$ Hz, 10-H), 8.27 (d, 1H, $J = 9.0$ Hz, 7-H), 8.00 (t, 1H, $J = 9.0$ Hz, 9-H), 7.85 (t, 1H, $J = 9.0$ Hz, 8-H), 7.55 (d, 2H, $J = 9.0$ Hz, 2'6'-H), 7.49–7.37 (m, 3H, 3', 4', 5'-H), 5.57 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1532 (C=N). MS (APCI) m/z 277 ($\text{M}+1$). Anal. Calc. for $\text{C}_{16}\text{H}_{12}\text{N}_4\text{O}$: C, 69.55; H, 4.38; N, 20.28. Found: C, 69.32; H, 4.41; N, 20.33.

6-(2-Fluorobenzyloxy)-[1,2,4]triazolo[3,4-a]phthalazine (2)

Yield: 89%, m.p. $177\text{--}179^\circ\text{C}$. ^1H -NMR (CDCl_3): δ 8.87 (s, 1H, 3-H), 8.62 (d, 1H, $J = 9.0$ Hz, 10-H), 8.17 (d, 1H, $J = 9.0$ Hz, 7-H), 7.89 (t, 1H, $J = 9.0$ Hz, 9-H), 7.78 (t, 1H, $J = 9.0$ Hz, 8-H), 7.58 (t, 1H, $J = 9.0$ Hz, 5'-H), 7.37–7.42 (m, 1H, 4'-H), 7.25–7.16 (m, 2H, 3'-H), 5.60 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1519 (C=N). MS (APCI) m/z 295 ($\text{M}+1$). Anal.

Calc. for $C_{16}H_{11}FN_4O$: C, 65.30; H, 3.77; N, 19.04. Found: C, 65.28; H, 3.82; N, 19.22.

6-(3-Fluorobenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (3)

Yield: 88%, m.p. 169–171°C. 1H -NMR ($CDCl_3$): δ 8.86 (s, 1H, 3-H), 8.63 (d, 1H, $J = 9.0$ Hz, 10-H), 8.20 (d, 1H, $J = 9.0$ Hz, 7-H), 7.96 (t, 1H, $J = 9.0$ Hz, 9-H), 7.81 (t, 1H, $J = 9.0$ Hz, 8-H), 7.43–7.38 (m, 1H, 2'-H), 7.32–7.24 (m, 2H, 4' and 5'-H), 7.12–7.09 (m, 1H, 6'-H), 5.53 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1512 (C=N). MS (APCI) m/z 295 (M+1). *Anal.* Calc. for $C_{16}H_{11}FN_4O$: C, 65.30; H, 3.77; N, 19.04. Found: C, 65.31; H, 3.82; N, 19.15.

6-(4-Fluorobenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (4)

Yield: 89%, m.p. 200–202°C. 1H -NMR ($CDCl_3$): δ 8.92 (d, 1H, $J = 9.0$ Hz, 10-H), 8.90 (s, 1H, 3-H), 8.27 (d, 1H, $J = 9.0$ Hz, 7-H), 8.02 (t, 1H, $J = 9.0$ Hz, 9-H), 7.88 (t, 1H, $J = 9.0$ Hz, 8-H), 7.70 (s, 1H, 2' or 6'-H), 7.55 (d, 1H, $J = 9.0$ Hz, 3' or 5'-H), 7.47 (d, 1H, $J = 9.0$ Hz, 3' or 5'-H), 7.36–7.31 (m, 1H, 2' or 6'-H), 5.32 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1499 (C=N). MS (APCI) m/z 295 (M+1). *Anal.* Calc. for $C_{16}H_{11}FN_4O$: C, 65.30; H, 3.77; N, 19.04. Found: C, 65.22; H, 3.91; N, 19.17.

6-(2-Chlorobenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (5)

Yield: 91%, m.p. 166–167°C. 1H -NMR ($CDCl_3$): δ 8.89 (s, 1H, 3-H), 8.72 (d, 1H, $J = 9.0$ Hz, 10-H), 8.24 (d, 1H, $J = 9.0$ Hz, 7-H), 7.95 (t, 1H, $J = 9.0$ Hz, 9-H), 7.80 (t, 1H, $J = 9.0$ Hz, 8-H), 7.62 (t, 1H, $J = 9.0$ Hz, 5'-H), 7.50–7.47 (m, 1H, 4'-H), 7.39–7.33 (m, 2H, 3' and 6'-H), 5.66 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1532 (C=N). MS (APCI) m/z 311 (M+1). *Anal.* Calc. for $C_{16}H_{11}ClN_4O$: C, 61.84; H, 3.57; N, 18.03. Found: C, 61.81; H, 3.65; N, 18.11.

6-(3-Chlorobenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (6)

Yield: 90%, m.p. 176–178°C. 1H -NMR ($CDCl_3$): δ 8.85 (s, 1H, 3-H), 8.58 (d, 1H, $J = 9.0$ Hz, 10-H), 8.18 (d, 1H, $J = 9.0$ Hz, 7-H), 7.89 (t, 1H, $J = 9.0$ Hz, 9-H), 7.77 (t, 1H, $J = 9.0$ Hz, 8-H), 7.53 (s, 1H, 2'-H), 7.44–7.29 (m, 2H, 4', 5' and 6'-H), 5.49 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1511 (C=N). MS (APCI) m/z 311 (M+1). *Anal.* Calc. for $C_{16}H_{11}ClN_4O$: C, 61.84; H, 3.57; N, 18.03. Found: C, 61.90; H, 3.67; N, 18.04.

6-(4-Chlorobenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (7)

Yield: 89%, m.p. 210–212°C. 1H -NMR ($CDCl_3$): δ 8.88 (s, 1H, 3-H), 8.78 (d, 1H, $J = 9.0$ Hz, 10-H), 8.22 (d, 1H, $J = 9.0$ Hz, 7-H), 7.98 (t, 1H, $J = 9.0$ Hz, 9-H), 7.82 (t, 1H, $J = 9.0$ Hz, 8-H), 7.49 (d, 2H, $J = 9.0$ Hz, 2' and 6'-H), 7.42 (d, 2H, $J = 9.0$ Hz, 3' or 5'-H), 5.52 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1524 (C=N). MS (APCI) m/z 311 (M+1). *Anal.* Calc. for

$C_{16}H_{11}ClN_4O$: C, 61.84; H, 3.57; N, 18.03. Found: C, 61.77; H, 3.49; N, 18.07.

6-(2-Bromobenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (8)

Yield: 78%, m.p. 177–179°C. 1H -NMR ($CDCl_3$): δ 8.90 (s, 1H, 3-H), 8.55 (d, 1H, $J = 9.0$ Hz, 10-H), 8.17 (d, 1H, $J = 9.0$ Hz, 7-H), 7.88 (t, 1H, $J = 9.0$ Hz, 9-H), 7.74 (t, 1H, $J = 9.0$ Hz, 8-H), 7.64–7.59 (m, 1H, 3' and 6'-H), 7.38 (d, 1H, $J = 9.0$ Hz, 4'-H), 7.28–7.23 (m, 1H, 5'-H), 5.56 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1509 (C=N). MS (APCI) m/z 355 (M+1). *Anal.* Calc. for $C_{16}H_{11}BrN_4O$: C, 54.10; H, 3.12; N, 15.77. Found: C, 54.01; H, 3.23; N, 15.81.

6-(3-Bromobenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (9)

Yield: 84%, m.p. 186–187°C. 1H -NMR ($CDCl_3$): δ 8.89 (s, 1H, 3-H), 8.78 (d, 1H, $J = 9.0$ Hz, 10-H), 8.22 (d, 1H, $J = 9.0$ Hz, 7-H), 7.98 (t, 1H, $J = 9.0$ Hz, 9-H), 7.82 (t, 1H, $J = 9.0$ Hz, 8-H), 7.56–7.51 (m, 2H, 2' and 4'-H), 7.18–7.12 (m, 2H, 5' and 6'-H), 5.53 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1525 (C=N). MS (APCI) m/z 355 (M+1). *Anal.* Calc. for $C_{16}H_{11}BrN_4O$: C, 54.10; H, 3.12; N, 15.77. Found: C, 54.04; H, 3.21; N, 15.79.

6-(2-Methoxybenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (10)

Yield: 78%, m.p. 187–189°C. 1H -NMR ($CDCl_3$): δ 8.87 (s, 1H, 3-H), 8.59 (d, 1H, $J = 9.0$ Hz, 10-H), 8.19 (d, 1H, $J = 9.0$ Hz, 7-H), 7.87 (t, 1H, $J = 9.0$ Hz, 9-H), 7.76 (t, 1H, $J = 9.0$ Hz, 8-H), 7.53 (d, 1H, $J = 9.0$ Hz, 6'-H), 7.37 (t, 1H, $J = 9.0$ Hz, 5'-H), 7.05 (t, 1H, $J = 9.0$ Hz, 4'-H), 6.97 (d, 1H, $J = 9.0$ Hz, 3'-H), 5.56 (s, 2H, CH_2), 3.88 (s, 3H, $-OCH_3$). IR (KBr) cm^{-1} : 1512 (C=N). MS (APCI) m/z 307 (M+1). *Anal.* Calc. for $C_{17}H_{14}N_4O_2$: C, 66.66; H, 4.61; N, 18.29. Found: C, 66.71; H, 4.69; N, 18.21.

6-(3-Methoxybenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (11)

Yield: 80%, m.p. 156–158°C. 1H -NMR ($CDCl_3$): δ 8.86 (s, 1H, 3-H), 8.63 (d, 1H, $J = 9.0$ Hz, 10-H), 8.22 (d, 1H, $J = 9.0$ Hz, 7-H), 7.96 (t, 1H, $J = 9.0$ Hz, 9-H), 7.80 (t, 1H, $J = 9.0$ Hz, 8-H), 7.37 (t, 1H, $J = 9.0$ Hz, 5'-H), 7.13–7.08 (m, 2H, 2' and 6'-H), 6.95 (d, 1H, 4'-H), 5.56 (s, 2H, CH_2), 3.90 (s, 3H, $-OCH_3$). IR (KBr) cm^{-1} : 1541 (C=N). MS (APCI) m/z 307 (M+1). *Anal.* Calc. for $C_{17}H_{14}N_4O_2$: C, 66.66; H, 4.61; N, 18.29. Found: C, 66.72; H, 4.65; N, 18.22.

6-(4-Methoxybenzoyloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (12)

Yield: 88%, m.p. 198–200°C. 1H -NMR ($CDCl_3$): δ 8.86 (d, 1H, $J = 9.0$ Hz, 10-H), 8.58 (s, 1H, 3-H), 8.17 (d, 1H, $J = 9.0$ Hz, 7-H), 7.90 (t, 1H, $J = 9.0$ Hz, 9-H), 7.74 (t, 1H, $J = 9.0$ Hz, 8-H), 7.47 (d, 2H, $J = 9.0$ Hz, 2' and 6'-H), 6.96 (d, 2H, $J = 9.0$ Hz, 3' and 5'-H), 5.46 (s, 2H, CH_2), 3.85 (s, 3H, $-OCH_3$). IR (KBr) cm^{-1} : 1519 (C=N). MS (APCI) m/z

307 (M+1). *Anal.* Calc. for $C_{17}H_{14}N_4O_2$: C, 66.66; H, 4.61; N, 18.29. Found: C, 66.71; H, 4.59; N, 18.19.

6-(2-(Trifluoromethyl)benzyloxy)-[1,2,4]triazolo[3,4-a]phthalazine (13)

Yield: 89%, m.p. 188–190°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.88 (s, 1H, 3-H), 8.71 (d, 1H, $J = 9.0$ Hz, 10-H), 8.24 (d, 1H, $J = 9.0$ Hz, 7-H), 7.94 (t, 1H, $J = 9.0$ Hz, 9-H), 7.81 (t, 1H, $J = 9.0$ Hz, 8-H), 7.62 (t, 1H, $J = 9.0$ Hz, 5'-H), 7.51–7.49 (m, 1H, 4'-H), 7.40–7.33 (m, 2H, 3' and 6'-H), 5.64 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1518 (C=N). MS (APCI) m/z 345 (M+1). *Anal.* Calc. for $C_{17}H_{11}F_3N_4O$: C, 59.31; H, 3.22; N, 16.27. Found: C, 59.29; H, 3.19; N, 16.32.

6-(3-(Trifluoromethyl)benzyloxy)-[1,2,4]triazolo[3,4-a]phthalazine (14)

Yield: 87%, m.p. 164–166°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.86 (d, 1H, $J = 9.0$ Hz, 10-H), 8.62 (s, 1H, 3-H), 8.19 (d, 1H, $J = 9.0$ Hz, 7-H), 7.95 (t, 1H, $J = 9.0$ Hz, 9-H), 7.81 (s, 1H, 2'-H), 7.79 (t, 1H, $J = 9.0$ Hz, 8-H), 7.76 (d, 1H, $J = 9.0$ Hz, 4'-H), 7.68 (d, 1H, $J = 9.0$ Hz, 6'-H), 7.58 (d, 1H, $J = 9.0$ Hz, 5'-H), 5.59 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1517 (C=N). MS (APCI) m/z 345 (M+1). *Anal.* Calc. for $C_{17}H_{11}F_3N_4O$: C, 59.31; H, 3.22; N, 16.27. Found: C, 59.35; H, 3.23; N, 16.38.

6-(4-(Trifluoromethyl)benzyloxy)-[1,2,4]triazolo[3,4-a]phthalazine (15)

Yield: 87%, m.p. 210–212°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.89 (s, 1H, 3-H), 8.79 (d, 1H, $J = 9.0$ Hz, 10-H), 8.23 (d, 1H, $J = 9.0$ Hz, 7-H), 7.99 (t, 1H, $J = 9.0$ Hz, 9-H), 7.83 (t, 1H, $J = 9.0$ Hz, 8-H), 7.50 (d, 2H, $J = 9.0$ Hz, 2' and 6'-H), 7.45 (d, 2H, $J = 9.0$ Hz, 3' or 5'-H), 5.54 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1503 (C=N). MS (APCI) m/z 345 (M+1). *Anal.* Calc. for $C_{17}H_{11}F_3N_4O$: C, 59.31; H, 3.22; N, 16.27. Found: C, 59.33; H, 3.29; N, 16.34.

6-(4-Methylbenzyloxy)-[1,2,4]triazolo[3,4-a]phthalazine (16)

Yield: 88%, m.p. 196–198°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.87 (s, 1H, 3-H), 8.72 (d, 1H, $J = 9.0$ Hz, 10-H), 8.21 (d, 1H, $J = 9.0$ Hz, 7-H), 7.94 (t, 1H, $J = 9.0$ Hz, 9-H), 7.78 (t, 1H, $J = 9.0$ Hz, 8-H), 7.43 (d, 2H, $J = 9.0$ Hz, 2' and 6'-H), 7.25 (d, 2H, $J = 9.0$ Hz, 3' or 5'-H), 5.50 (s, 2H, CH_2), 2.45 (s, 3H, CH_3). IR (KBr) cm^{-1} : 1507 (C=N). MS (APCI) m/z 291 (M+1). *Anal.* Calc. for $C_{17}H_{14}N_4O$: C, 70.33; H, 4.86; N, 19.30. Found: C, 70.31; H, 4.96; N, 19.32.

6-(Cyclohexyloxy)-[1,2,4]triazolo[3,4-a]phthalazine (17)

Yield: 67%, m.p. 139–142°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.95 (s, 1H, 3-H), 8.43 (d, 1H, $J = 9.0$ Hz, 10-H), 8.18 (d, 1H, $J = 9.0$ Hz, 7-H), 7.90 (t, 1H, $J = 9.0$ Hz, 9-H), 7.73 (t, 1H, $J = 9.0$ Hz, 8-H), 5.24–5.16 (m, 1H, CH), 2.36–2.09 (m, 2H, CH_2), 1.88–1.25 (m, 8H, $(\text{CH}_2)_4$). IR (KBr) cm^{-1} : 1509 (C=N). MS (APCI) m/z 269 (M+1). *Anal.* Calc. for

$C_{15}H_{16}N_4O$: C, 67.15; H, 6.01; N, 20.88. Found: C, 67.21; H, 6.19; N, 20.77.

6-(1-Phenylethoxy)-[1,2,4]triazolo[3,4-a]phthalazine (18)

Yield: 71%, m.p. 68–70°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.56 (s, 1H, 3-H), 8.19 (d, 1H, $J = 8.4$ Hz, 10-H), 7.91 (d, 1H, $J = 8.4$ Hz, 7-H), 7.50–7.44 (m, 2H, 8 and 9-H), 7.42 (d, 2H, $J = 7.8$ Hz, 2' and 6'-H), 7.13 (t, 2H, $J = 7.8$ Hz, 3' and 5'-H), 7.08 (d, $J = 7.8$ Hz, 4'-H), 6.01 (q, 1H, $J = 6.6$ Hz, -OCH), 1.62 (d, 3H, $J = 6.6$ Hz, - CH_3). IR (KBr) cm^{-1} : 1513 (C=N). MS (APCI) m/z 269 (M+1). *Anal.* Calc. for $C_{17}H_{14}N_4O$: C, 70.33; H, 4.86; N, 19.30. Found: C, 70.37; H, 4.91; N, 19.29.

6-(1-(4-Fluorophenyl)ethoxy)-[1,2,4]triazolo[3,4-a]phthalazine (19)

Yield: 71%, m.p. 68–70°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.78 (s, 1H, 3-H), 8.52 (d, 1H, $J = 8.4$ Hz, 10-H), 8.22 (d, 1H, $J = 8.4$ Hz, 7-H), 7.87 (t, 1H, $J = 8.6$ Hz, 9-H), 7.77 (t, 1H, $J = 8.6$ Hz, 8-H), 7.53–7.49 (m, 2H, 3' and 5'-H), 7.07–7.09 (t, 2H, $J = 7.8$ Hz, 2' and 6'-H), 6.24 (q, 1H, $J = 6.5$ Hz, -OCH), 1.62 (d, 3H, $J = 6.5$ Hz, - CH_3). IR (KBr) cm^{-1} : 1509 (C=N). MS (APCI) m/z 309 (M+1). *Anal.* Calc. for $C_{17}H_{13}FN_4O$: C, 66.23; H, 4.25; N, 18.17. Found: C, 66.12; H, 4.34; N, 18.21.

6-(1-(4-Chlorophenyl)ethoxy)-[1,2,4]triazolo[3,4-a]phthalazine (20)

Yield: 78%, m.p. 130–132°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.77 (s, 1H, 3-H), 8.52 (d, 1H, $J = 8.4$ Hz, 10-H), 8.21 (d, 1H, $J = 8.4$ Hz, 7-H), 7.85 (t, 1H, $J = 8.6$ Hz, 9-H), 7.78 (t, 1H, $J = 8.6$ Hz, 8-H), 7.45 (d, 2H, $J = 7.8$ Hz, 3' and 5'-H), 7.34 (d, 2H, $J = 7.8$ Hz, 2' and 6'-H), 6.24 (q, 1H, $J = 6.6$ Hz, -OCH), 1.62 (d, 3H, $J = 6.6$ Hz, - CH_3). IR (KBr) cm^{-1} : 1521 (C=N). MS (APCI) m/z 325 (M+1). *Anal.* Calc. for $C_{17}H_{13}ClN_4O$: C, 62.87; H, 4.03; N, 17.25. Found: C, 62.79; H, 4.12; N, 17.33.

6-(1-(4-Methoxyphenyl)ethoxy)-[1,2,4]triazolo[3,4-a]phthalazine (21)

Yield: 86%, m.p. 84–86°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.78 (s, 1H, 3-H), 8.54 (d, 1H, $J = 8.6$ Hz, 10-H), 8.24 (d, 1H, $J = 8.4$ Hz, 7-H), 7.90 (t, 1H, $J = 8.6$ Hz, 9-H), 7.75 (t, 1H, $J = 8.4$ Hz, 8-H), 7.45 (d, 2H, $J = 7.8$ Hz, 3' and 5'-H), 6.90 (d, 2H, $J = 7.8$ Hz, 2' and 6'-H), 6.22 (q, 1H, $J = 6.6$ Hz, -OCH), 3.80 (s, 3H, - OCH_3), 1.79 (d, 3H, $J = 6.6$ Hz, - CH_3). IR (KBr) cm^{-1} : 1512 (C=N). MS (APCI) m/z 321 (M+1). *Anal.* Calc. for $C_{18}H_{16}N_4O_2$: C, 67.49; H, 5.03; N, 17.49. Found: C, 67.54; H, 5.23; N, 17.33.

6-(1-4-Tolylethoxy)-[1,2,4]triazolo[3,4-a]phthalazine (22)

Yield: 81%, m.p. 68–70°C. $^1\text{H-NMR}$ (CDCl_3): δ 8.76 (s, 1H, 3-H), 8.52 (d, 1H, $J = 8.6$ Hz, 10-H), 8.23 (d, 1H, $J = 8.4$ Hz, 7-H), 7.83 (t, 1H, $J = 8.6$ Hz, 9-H), 7.76 (t, 1H, $J = 8.4$ Hz, 8-H), 7.40 (d, 2H, $J = 7.8$ Hz, 3' and 5'-H), 7.18 (d, 2H, $J = 7.8$ Hz, 2' and 6'-H), 6.22 (q, 1H, $J = 6.6$ Hz, -OCH), 2.33 (s, 3H, - CH_3), 1.77 (d, 3H, $J = 6.6$ Hz, - CH_3). IR (KBr) cm^{-1} : 1509 (C=N). MS (APCI) m/z 305 (M+1). *Anal.* Calc.

for $C_{18}H_{16}N_4O$: C, 71.04; H, 5.30; N, 18.41. Found: C, 71.11; H, 5.26; N, 18.45.

6-(Benzhydryloxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (23)

Yield: 84%, m.p. 188–190°C. 1H -NMR ($CDCl_3$): δ 8.76 (s, 1H, 3-H), 8.58 (d, 1H, $J = 7.2$ Hz, 10-H), 8.36 (d, 1H, $J = 7.2$ Hz, 7-H), 7.90 (t, 1H, $J = 7.2$ Hz, 9-H), 7.80 (t, 1H, $J = 7.2$ Hz, 8-H), 7.51 (d, 4H, $J = 8.4$ Hz, (2' and 6'-H)₂), 7.41–7.30 (m, 6H, (3', 4' and 5'-H)₂), 7.24 (s, 1H, -OCH). IR (KBr) cm^{-1} : 1516 (C=N). MS (APCI) m/z 353 (M+1). *Anal.* Calc. for $C_{22}H_{16}N_4O$: C, 74.98; H, 4.58; N, 15.90. Found: C, 75.01; H, 4.62; N, 15.78.

6-(Bis(4-fluorophenyl)methoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (24)

Yield: 82%, m.p. 180–182°C. 1H -NMR ($CDCl_3$): δ 8.77 (s, 1H, 3-H), 8.60 (d, 1H, $J = 8.0$, 10-H), 8.32 (d, 1H, $J = 7.8$ Hz, 7-H), 7.94 (t, 1H, $J = 8.0$ Hz, 9-H), 7.82 (t, 1H, $J = 7.8$ Hz, 8-H), 7.48–7.44 (m, 4H, (3' and 5'-H)₂), 7.22 (s, 1H, -OCH), 7.06 (t, 4H, $J = 8.2$ Hz, (2' and 6'-H)₂). IR (KBr) cm^{-1} : 1524 (C=N). MS (APCI) m/z 389 (M+1). *Anal.* Calc. for $C_{22}H_{14}N_4O$: C, 68.04; H, 3.63; N, 14.43. Found: C, 68.23; H, 3.51; N, 14.39.

6-((4-Chlorophenyl)(phenyl)methoxy)-[1,2,4]triazolo[3,4-*a*]phthalazine (25)

Yield: 69%, m.p. 182–185°C. 1H -NMR ($CDCl_3$): δ 8.76 (s, 1H, 3-H), 8.54 (d, 1H, $J = 7.8$ Hz, 10-H), 8.32 (d, 1H, $J = 7.6$ Hz, 7-H), 7.87 (t, 1H, $J = 7.8$ Hz, 9-H), 7.80 (t, 1H, $J = 7.6$ Hz, 8-H), 7.52–7.33 (m, 9H, Ar-H), 7.20 (s, 1H, -OCH). IR (KBr) cm^{-1} : 1532 (C=N). MS (APCI) m/z 387 (M+1). *Anal.* Calc. for $C_{22}H_{15}ClN_4O$: C, 68.31; H, 3.91; N, 14.48. Found: C, 68.23; H, 3.78; N, 14.61.

Synthesis of 6-chlorotetrazolo[5,1-*a*]phthalazine (39)

Compound **37** (3 g, 15.4 mmol) was dissolved in 50 ml of 30% H_2SO_4 , then a solution of $NaNO_2$ (1.06 g, 15.4 mmol) in 15 ml H_2O was added dropwise to the mixture under ice-bath, be sure that the reaction temperature was below 5°C. Then the mixture was stirred at room temperature for 2 h (TLC monitoring). The mixture was extracted twice with dichloromethane (60 ml). The dichloromethane layer was washed three times with saturated aqueous NaCl (60 × 3) and dried over anhydrous $MgSO_4$. After removing the solvents, 2.15 g light yellow product was gained, yield 68%.

The general procedure for the synthesis of compounds 26–34

Compound **38** (0.65 g, 3.2 mmol), K_2CO_3 (0.44 g, 3.2 mmol), benzyltriethylammonium chloride (TEBA) (0.23g, 1 mmol) and corresponding alcohol (0.70 mmol) were suspended in acetonitrile (50 mL), and reacted with stirring and refluxing for about 3 h. After the acetonitrile was removed under reduced pressure, the solid residue was purified by silica gel chromatography with ethyl acetate: petroleum ether (2:3) to obtain compounds **26–34**. The yield, melting point and spectral data of each compound were given below.

6-(Benzyloxy)tetrazolo[5,1-*a*]phthalazine (26)

Yield: 89%, m.p. 165–167°C; 1H -NMR ($CDCl_3$, 300 MHz) δ : 8.61 (d, 1H, $J = 8.5$ Hz, 9-H), 8.33 (d, 1H, $J = 8.2$ Hz, 6-H), 8.01 (t, 1H, $J = 4.2$ Hz, 8-H), 7.94 (t, 1H, $J = 4.0$ Hz, 7-H), 7.58 (d, 2H, $J = 7.9$ Hz, 3' and 5'-H), 7.28–7.48 (m, 3H, 2', 4' and 6'-H), 5.68 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1509 (C=N). MS (APCI) m/z : 278 (M+1); *Anal.* Calcd for $C_{15}H_{11}N_5O$: C, 64.97; H, 4.00; N, 25.26. Found: C, 64.78; H, 4.13; N, 25.12.

6-(4-Fluorobenzyloxy)tetrazolo[5,1-*a*]phthalazine (27)

Yield: 86%, m.p. 164–166°C; 1H -NMR ($CDCl_3$, 300 MHz) δ : 8.64 (d, 1H, $J = 8.0$ Hz, 9-H), 8.33 (d, 1H, $J = 8.0$ Hz, 6-H), 8.05 (t, 1H, $J = 4.2$ Hz, 8-H), 7.96 (t, 1H, $J = 4.0$ Hz, 7-H), 7.57 (d, 2H, $J = 7.9$ Hz, 3' and 5'-H), 7.13 (d, 2H, $J = 7.9$ Hz, 2' and 6'-H), 5.66 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1523 (C=N). MS (APCI) m/z : 296 (M+1); *Anal.* Calcd for $C_{15}H_{10}FN_5O$: C, 61.02; H, 3.41; N, 23.72. Found: C, 60.87; H, 3.53; N, 23.68.

6-(2-Chlorobenzyloxy)tetrazolo[5,1-*a*]phthalazine (28)

Yield: 89%, m.p. 162–164°C; 1H -NMR ($CDCl_3$, 300 MHz) δ : 8.61 (d, 1H, $J = 8.0$ Hz, 9-H), 8.34 (d, 1H, $J = 8.0$ Hz, 6-H), 8.03 (t, 1H, $J = 4.2$ Hz, 8-H), 7.97 (t, 1H, $J = 7.6$ Hz, 7-H), 7.69–7.34 (m, 4H, 3', 4', 5', and 6'-H), 5.77 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1527 (C=N). MS (APCI) m/z : 312 (M+1); *Anal.* Calcd for $C_{15}H_{10}ClN_5O$: C, 57.79; H, 3.23; N, 22.47. Found: C, 57.81; H, 3.39; N, 22.23.

6-(4-Chlorobenzyloxy)tetrazolo[5,1-*a*]phthalazine (29)

Yield: 89%, m.p. 190–192°C; 1H -NMR ($CDCl_3$, 300 MHz) δ : 8.65 (d, 1H, $J = 8.0$ Hz, 9-H), 8.33 (d, 1H, $J = 8.0$ Hz, 6-H), 8.06 (t, 1H, $J = 4.2$ Hz, 8-H), 7.94 (t, 1H, $J = 7.6$ Hz, 7-H), 7.57 (d, 2H, $J = 7.9$ Hz, 3' and 5'-H), 7.42 (d, 2H, $J = 7.9$ Hz, 2' and 6'-H), 5.66 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1517 (C=N). MS (APCI) m/z : 312 (M+1); *Anal.* Calcd for $C_{15}H_{10}ClN_5O$: C, 57.79; H, 3.23; N, 22.47. Found: C, 57.58; H, 3.42; N, 22.39.

6-(3-Bromobenzyloxy)tetrazolo[5,1-*a*]phthalazine (30)

Yield: 81%, m.p. 180–183°C; 1H -NMR ($CDCl_3$, 300 MHz) δ : 8.67 (d, 1H, $J = 8.20$ Hz, 9-H), 8.35 (d, 1H, $J = 8.20$ Hz, 6-H), 8.07 (t, 1H, $J = 4.2$ Hz, 8-H), 7.97 (t, 1H, $J = 7.6$ Hz, 7-H), 7.73–7.33 (m, 4H, Ar-H), 5.66 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1519. MS (APCI) m/z : 356 (M+1); *Anal.* Calcd for $C_{15}H_{10}BrN_5O$: C, 50.58; H, 2.83; N, 19.66. Found: C, 50.62; H, 2.91; N, 19.57.

6-(3-(Trifluoromethyl)benzyloxy)-tetrazolo[5,1-*a*]phthalazine (31)

Yield: 80%, m.p. 155–157°C; 1H -NMR ($CDCl_3$, 300 MHz) δ : 8.87 (d, 1H, $J = 9.00$ Hz, 10-H), 8.35 (d, 1H, $J = 9.00$ Hz, 7-H), 8.05 (t, 1H, $J = 9.0$ Hz, 9-H), 7.98 (s, 1H, 2'-H), 7.79 (t, 1H, $J = 9.0$ Hz, 8-H), 7.76 (d, 1H, $J = 9.0$ Hz, 4'-H), 7.68 (d, 1H, $J = 9.0$ Hz, 6'-H), 7.58 (d, 1H, $J = 9.0$ Hz, 5'-H), 5.59 (s, 2H, CH_2). IR (KBr) cm^{-1} : 1527. MS (APCI) m/z : 346 (M+1); *Anal.* Calcd for $C_{16}H_{10}F_3N_5O$: C, 55.66; H, 2.92; N, 20.28. Found: C, 55.87; H, 2.91; N, 20.34.

6-(4-(Methyl)benzyloxy)-tetrazolo[5,1-*a*]phthalazine (32)

Yield: 85%, m.p. 153–155°C; ¹H-NMR (CDCl₃, 300 MHz) δ: 8.61 (d, 1H, *J* = 7.90 Hz, 9-H), 8.31 (d, 1H, *J* = 8.20 Hz, 6-H), 8.02 (t, 1H, *J* = 8.2 Hz, 8-H), 7.90 (t, 1H, *J* = 8.2 Hz, 7-H), 7.46 (d, 2H, *J* = 8.0 Hz, 3' and 5'-H), 7.25 (d, 1H, *J* = 8.0 Hz, 2' and 6'-H), 5.63 (s, 2H, CH₂), 2.39 (s, 3H, CH₃). IR (KBr) cm⁻¹: 1523. MS (APCI) *m/z*: 292 (M+1); *Anal.* Calcd for C₁₆H₁₃N₅O: C, 65.97; H, 4.50; N, 24.04. Found: C, 65.78; H, 4.65; N, 24.09.

6-(1-(4-Fluorophenyl)ethoxy)tetrazolo[5,1-*a*]phthalazine (33)

Yield: 82%, m.p. 113–115°C; ¹H-NMR (CDCl₃, 300 MHz) δ: 8.55 (d, 1H, *J* = 7.90 Hz, 9-H), 8.37 (d, 1H, *J* = 7.90 Hz, 6-H), 8.01–7.95 (m, 2H, Ar-H), 7.61–7.56 (m, 2H, Ar-H), 7.10–7.04 (m, 2H, Ar-H), 6.45 (q, 2H, *J* = 6.5 Hz CH₂), 1.89 (d, 3H, *J* = 6.5 Hz, CH₃). IR (KBr) cm⁻¹: 1509. MS (APCI) *m/z*: 310 (M+1); *Anal.* Calcd for C₁₆H₁₂FN₅O: C, 62.13; H, 3.91; N, 22.64. Found: C, 62.21; H, 4.02; N, 22.56.

6-(Bis(4-fluorophenyl)methoxy)tetrazolo[5,1-*a*]phthalazine (34)

Yield: 89%, m.p. 204–206°C; ¹H-NMR (CDCl₃, 300 MHz) δ: 8.65 (d, 1H, *J* = 7.90 Hz, 9-H), 8.47 (d, 1H, *J* = 7.90 Hz, 6-H), 8.07 (t, 1H, *J* = 7.20 Hz, 8-H), 7.99 (t, 1H, *J* = 7.20 Hz, 7-H), 7.54–7.49 (m, 4H, Ar-H), 7.44 (s, 1H, CH), 7.12–7.06 (m, 4H, Ar-H). IR (KBr) cm⁻¹: 1544. MS (APCI) *m/z*: 390 (M+1); *Anal.* Calcd for C₂₁H₁₃F₂N₅O: C, 64.78; H, 3.37; N, 17.99. Found: C, 64.86; H, 3.45; N, 18.12.

Biological assays***Anticonvulsant effects in the maximal electroshock seizure (MES) test***

The MES test and rotarod test were carried out according to procedures described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA) (15, 16). Seizures were elicited with a 60 Hz alternating current of 50 mA intensity in KunMing 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 tonic maximal extension of the hind leg. At 0.5 h after the administration of the compounds, the activities were evaluated in MES test. In phase-I screening, each compound was administered at the dose levels of 30 and 100 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. Groups of 10 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 the plot of this data, the respective ED₅₀ and TD₅₀ values, 95% confidence intervals, slope of the regression line, and the standard error of the slope

were calculated by means of a computer program written at National Institute of Neurological Disorders and Stroke^{13,14}.

Neurotoxicity (NT) screening

The neurotoxicity of the compounds was measured in Kunming mice by the rotarod test for three times. The mice were trained to stay on an accelerating rotarod of diameter 3.2 cm 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^{15,16}.

Sc-PTZ-induced seizures

At 0.5 h after the administration of the test compound, 85 mg/kg PTZ dissolved in saline was administered sc. The animals (10 KunMing mice in each group) were placed in individual cages and observed for 0.5 h. The numbers of clonic seizure (range from exaggerated twitches of the limbs to violent shaking or vibrating of the stiffened extremities) and tonic seizure (the extremities pull towards the body or rigidly push away from it, usually maximal extension of the hind leg) as well as the number of death were noted^{15,16}.

Isoniazid-induced seizures test

At 0.5 h after the administration of the test compound, the animals (10 KunMing mice in each group) were given ISO by ip at a dose of 250 mg/kg at which 100% of the animals showed convulsive reactions. The mice were placed in individual cages and observed for 1 h. The numbers of clonic and tonic seizures as well as the number of death were noted¹⁷.

3-MP induced seizures test

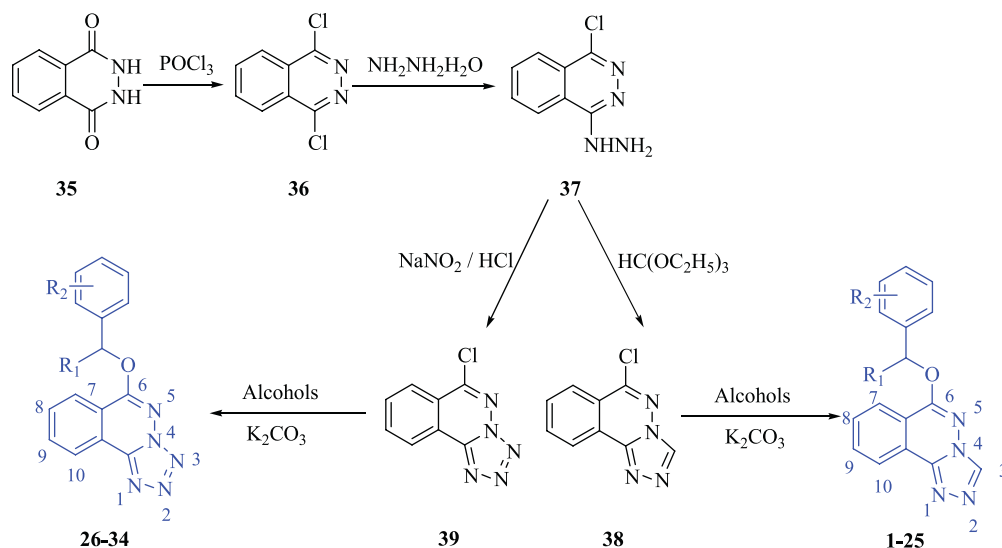
At 0.5 h after the administration of the test compound, 60 mg/kg of 3-MP in saline solution was injected by sc to mice (10 KunMing mice in each group). The mice were placed in individual cages and observed for 0.5 h. The numbers of clonic and tonic seizures as well as the number of death were noted¹⁸.

Thiosemicarbazide-induced seizures test

At 0.5 h after the administration of the test compound, the animals (10 KunMing mice in each group) were given an i.p. dose of TSC (50 mg/kg). The mice were placed in individual cages and observed for 2.5 h. The number of clonic seizures, tonic seizures and the lethality were recorded¹⁹.

Results and discussion**Chemistry**

All the target compounds **1–34** were synthesized according to Scheme 1. Compound **36**, a key substrate for the whole reaction, was prepared by an established procedure²⁰. The starting material 2,3-dihydrophthalazine-1,4-dione



Scheme 1. Synthesis route of Compounds 1–34.

(35) reacted with the refluxing phosphorus oxychloride to yield compound 36. Compound 36 reacted further with hydrazine hydrate in tetrahydrofuran to afford 1-hydrazine-4-chlorophthalazine (37). Cyclization of compound 37 with triethyl orthoformate yielded compound 38. The target compounds 1–25 were gained from compound 38 by reacting with corresponding alcohol in acetonitrile in the presence of K_2CO_3 . And via the diazotization of compound 37 with $NaNO_2$ at 5°C in 30% H_2SO_4 , 6-chloro-tetrazolo[5,1-*a*] phthalazine (39) was obtained²¹. The target compounds 26–34 were gained from compound 39 by reacting with corresponding alcohol in acetonitrile in the presence of K_2CO_3 .

Biological evaluation

Phase I evaluation of anticonvulsant activity

As we know, there are two models *in vivo*, the maximal electroshock seizure (MES), the subcutaneous Pentylene-tetrazole (sc-PTZ) models represent the two animal seizure models most widely used in the search for new AEDs^{22,23}. In the present study, we used the MES seizure model for screening the anticonvulsant activity of target compounds. Compounds 1–25 were prepared to evaluate the influence of the triazole ring and 26–34 were prepared to evaluate the influence of the tetrazole ring on anticonvulsant activity. Their preliminary anticonvulsant activities were obtained and listed in Table 1. After i.p. injection in mice using doses of 30 and 100 mg/kg, the following observations can be made. At the dose of 100 mg/kg, compounds 4, 6, 8, 9, 13, 14, 15, 16, 18, 20–22, 24, 25 and 34 showed complete protection. Compounds 4, 13, 14, 15, 19 and 24 exhibited complete protection at 30 mg/kg.

Phase II evaluation of anticonvulsant activity

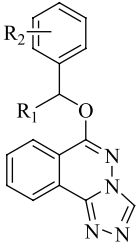
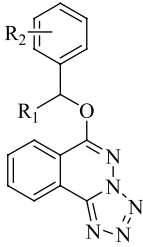

On the basis of the considerable anticonvulsant activity suggested in phase I testing, compounds 4, 13, 14, 15,

19 and 24 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 test for selected compounds, along with the data on the current antiepileptic drug, were shown in Table 2. Among the tested compounds, 6-(3-(Trifluoromethyl)benzyloxy)-[1,2,4]triazolo[3,4-*a*] phthalazine (14) was the most active and promising compound in this work. It possessed strong anti-MES activity with ED_{50} of 9.3 mg/kg, which was much better than currently used antiepileptic drug Carbamazepine. The neurotoxicity caused by it was minimal and was markedly lower than the drug compared. It showed a protective index of 91.7, which was many folds higher than the current antiepileptic drug having the PI value of 6.9. Furthermore, compounds 4, 13, 15 and 24 also showed higher protective index than Carbamazepine.

Structure activity relationships discussions

Analysing the activities of the synthesized compounds, the following structure activity relationships (SAR) were obtained. We can divide all the compounds into two groups, in the first group compounds (1–25) with triazole ring compounds: among compounds 1–16, R1 was H and R2 was substituted part, effects of different substitutions at benzyl were observed. The bioevaluation led to an understanding of the importance of the position of the substituted group at the phenyl. For example, the activity order of the F position on the phenyl ring was $p-F > m-F > o-F$; the activity order of the Cl position on the phenyl ring was $m-Cl > o-Cl > p-Cl$; the activity order of the $-OCH_3$ position was $o-OCH_3 > m-OCH_3 > p-OCH_3$; and the activity order of the $-CF_3$ position was $m-CF_3 > p-CF_3 > o-CF_3$. Among compounds 18–22: R1 was CH_3 and R2 was substituted part, effects of different substitutions at benzyl were observed. Among the five para-position compounds (18–22), the similar

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

<div style="display: flex; justify-content: space-around; align-items: center;"> <div style="text-align: center;">  <p>1-25</p> </div> <div style="text-align: center;"> <p>and</p>  <p>26-34</p> </div> </div>									
No.	R ₁	R ₂	100	30	No.	R ₁	R ₂	100	30
1	H	H	0/3	0/3	18	CH ₃	H	3/3	1/3
2	H	<i>o</i> -F	0/3	1/3	19	CH ₃	<i>p</i> -F	— ^a	3/3
3	H	<i>m</i> -F	2/3	1/3	20	CH ₃	<i>p</i> -Cl	3/3	1/5
4	H	<i>p</i> -F	3/3	3/3	21	CH ₃	<i>p</i> -OCH ₃	3/3	1/3
5	H	<i>o</i> -Cl	1/3	0/3	22	CH ₃	<i>p</i> -CH ₃	3/3	1/3
6	H	<i>m</i> -Cl	3/3	2/3	23	C ₆ H ₅	H	1/6	—
7	H	<i>p</i> -Cl	0/3	—	24	C ₆ H ₄ (<i>p</i> -F)	<i>p</i> -F	3/3	3/3
8	H	<i>o</i> -Br	3/3	1/3	25	C ₆ H ₄ (<i>p</i> -Cl)	<i>p</i> -Cl	3/3	1/3
9	H	<i>m</i> -Br	3/3	0/3	26	H	H	0/3	—
10	H	<i>o</i> -OCH ₃	2/3	0/3	27	H	<i>p</i> -F	0/3	—
11	H	<i>m</i> -OCH ₃	1/3	0/3	28	H	<i>o</i> -Cl	0/3	—
12	H	<i>p</i> -OCH ₃	0/3	—	29	H	<i>p</i> -Cl	0/3	—
13	H	<i>o</i> -CF ₃	3/3	3/3	30	H	<i>m</i> -Br	0/3	—
14	H	<i>m</i> -CF ₃	3/3	3/3	31	H	<i>m</i> -CF ₃	0/3	—
15	H	<i>p</i> -CF ₃	3/3	3/3	32	H	<i>p</i> -CH ₃	0/3	—
16	H	<i>p</i> -CH ₃	3/3	0/3	33	CH ₃	<i>p</i> -F	0/3	—
17 ^b		—	2/3	0/3	34	C ₆ H ₄ (<i>p</i> -F)	<i>p</i> -F	3/3	0/3

^aNot tested.

^bFor compound 17, the cyclohexane group was on the O atom.

Table 2. Quantitative anticonvulsant data in mice (i.p.).

Compound	ED ₅₀ (mg/kg) (MES)	TD ₅₀ (mg/kg)	PI (TD ₅₀ /ED ₅₀)
4	12.5 (9.5–18.4)	560.3 (354–726)	44.8
13	16.1 (10.2–24.5)	448.7 (256–667)	27.9
14	9.3 (6.7–14.2)	852.8 (645–1032)	91.7
15	15.4 (10.2–23.8)	663.2 (502–872)	43.1
19	19.8 (14.4–30.2)	67.5 (45.5–86.5)	3.4
24	14.6 (10.1–21.3)	267.4 (205–312)	18.3
CBZ ^a	11.8 (7.2–15.6)	76.1 (54.4–99.5)	6.4

^aCBZ: carbamazepine

anticonvulsant activities were observed. Among compound **23–25**: R₁ was a substituted phenyl and R₂ were H, *p*-F and *p*-Cl respectively, the activity order of three compounds was *p*-F > *p*-Cl > H. We also can see that the other nine compounds (**26–34**) exhibited lower anticonvulsant activity with tetrazole ring structure,

Chemicals induced seizures

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). To further investigate the effects of the anticonvulsant activity in several different

Table 3. Effects of compound 13 on chemical-induced seizures in mice.

Chemical sub- stances	Compound	Doses (mg/ kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
Sc-PTZ	DMSO	—	0.5	100	60	60
	CBZ	30	0.5	100	0	0
	14	30	0.5	20	0	0
ISO	DMSO	—	1	100	100	60
	CBZ	30	1	50	0	0
	14	30	1	50	0	0
3-MP	DMSO	—	0.5	100	100	100
	CBZ	30	0.5	100	0	0
	14	30	0.5	40	0	0
TSC	DMSO	—	2.5	100	100	100
	CBZ	30	2.5	100	0	0
	14	30	2.5	40	0	20

models and speculate about the possible mechanism of anticonvulsant action, compound **14** was tested against convulsions induced by chemical substances, including PTZ, ISO, 3-MP, and TSC. Compound **14** was administered to mice at 30 mg/kg i.p., which was higher than its ED₅₀ value and far below its TD₅₀ 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 100%, respectively. While compound **14** inhibited the clonic seizures, tonic seizures and lethality at the rates of 80%, 100% and 100% induced by sc-PTZ (Table 3), respectively, which revealed that compound **14** possessed excellent activity against sc-PTZ. Compound **14**, exhibiting high anticonvulsant activity in the MES and sc-PTZ models which are most widely used in the search for new AEDs, suggested that it really possesses a good anticonvulsant profile. In the ISO model, both Carbamazepine and compound **14** inhibited the clonic seizures, tonic seizures and death induced by ISO at the rates of 50%, 100% and 100%, respectively (Table 3). PTZ and ISO have been reported to produce seizures by inhibiting γ -aminobutyric acid (GABA) neurotransmission^{24,25}. 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 seizures²⁶, while enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study suggest that the newly synthesized compound **14** might inhibit or attenuate PTZ-induced seizures and ISO-induced seizures in mice by enhancing GABAergic neurotransmission.

In the 3-MP induced seizure model, carbamazepine inhibited the clonic seizures, tonic seizures and death at the rates of 0%, 100% and 100%, respectively. In comparison, compound **14** showed the anticonvulsant effect higher than Carbamazepine in inhibiting the clonic seizures induced by 3-MP with the inhibition rate of 60% (Table 3). In the TSC-induced seizure model, the anticonvulsant

effect was similar to that of the 3-MP induced seizure model. Compared with the control group, Carbamazepine showed inhibition of clonic and tonic seizures and death at the rates of 0%, 100% and 100%, respectively. Compound **14** showed inhibition of clonic seizure, tonic seizures and death at the rates of 60%, 100% and 80% induced by TSC (Table 3), respectively. 3-MP and TSC are competitive inhibitors of the GABA synthesis enzyme glutamate decarboxylase (GAD), and they inhibit the synthesis of GABA resulting in decrease of GABA levels in the brain²⁷. Compound **14** showed moderate antagonism to both 3-MP induced seizures and TSC-induced seizures.

Conclusion

In the present study we described the syntheses and anticonvulsant activity evaluation of 6-substituted-[1,2,4]triazolo[3,4-*a*](tetrazolo[5,1-*a*])phthalazine (**1-34**). Most of the synthesized compounds exhibited potent anticonvulsant activities in the maximal electroshock test (MES). The most promising compound **14** showed significant anticonvulsant activity in MES test with ED₅₀ value of 9.3 mg/kg. It displayed a wide margin of safety with protective index much higher than the standard drug Carbamazepine. And the potency of compound **14** against seizures induced by Pentylentetrazole, Isoniazid, Thiosemicarbazide and 3-Mercaptopropionic acid in the chemical-induced seizure tests, suggested that compound **14** displayed wide spectrum activity in several models.

Declaration of interest

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