Full Paper

Synthesis and Anticonvulsant Activity Evaluation of 5-Phenyl-[1,2,4]triazolo[4,3-*c*]quinazolin-3-amines

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In the present study we describe the syntheses and anticonvulsant activity evaluation of 5-phenyl-[1,2,4]triazolo[4,3-*c*]quinazolin-3-amine derivatives. Their anticonvulsant activity and neurotoxicity were evaluated by the maximal electroshock seizure test (MES) and the rotarod test, respectively. The majority of the compounds prepared were effective in the MES screens at a dose level of 100 mg/kg. Of these compounds, the most promising was compound **8h**, which showed an ED_{50} value of 27.4 mg/kg and a protective index (PI) value of 5.8. These values were superior to those provided by valproate (ED_{50} and PI values of 272 and 1.6, respectively) in the MES test in mice. As well as its anti-MES efficacy, the potencies of compound **8h** against seizures induced by pentylenetetrazole and thiosemicarbazide were also established, with the results suggesting that the GABAergic system-mediated mechanisms might be involved in its anticonvulsant activity.

Keywords: Anticonvulsant / Maximal electroshock / Quinazoline / Synthesis / Triazolo

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Introduction

Epilepsy, one of the most frequent neurological afflictions in men characterized by excessive temporary neuronal discharges resulting in uncontrolled convulsion, inflicts more than 60 million people worldwide [1, 2]. Despite the development of several new anticonvulsants, the treatment of epilepsy remains still inadequate. It is roughly estimated that up to 28–30% of patients are poorly treated with the available antiepileptic drugs (AEDs) [3, 4]. Moreover, many AEDs have serious side effects [5–10], and lifelong medication may be required. Therefore, there is a continuing demand for new anticonvulsant agents with more selectivity and lower toxicity.

One of the most frequently encountered heterocycles in medicinal chemistry is 4(3*H*)-quinazolinones, which have diverse pharmacological activities like antitumor [11, 12], antiinflammatory [13], antimicrobial activities [14, 15], and CNS activities such as CNS depressant [16, 17] and anticonvulsant [18, 19]. Since the appearance of methaqualone (a well known sedative-hypnotic containing the quinazolin-4(3*H*)-one nucleus) in the early 1970s, more and more researches are focused on the program that aims to develop an anticonvulsant in 4(3*H*)-quinazolinones [20–25]. In fact, these studies confirm that the quinazolin-4(3*H*)-one nucleus possesses a pharmacophoric character for anticonvulsant activities. Moreover, the 1,2,4-triazole nucleus and its derivatives have received considerable attention owing to their effective biological importance. And their biological importance associated with anticonvulsant activity has been established in our previous work [26–32].

Based on the above mentioned facts and in continuation of an ongoing program aiming at finding new lead structures with potential anticonvulsant activities, we have incorporated five nitrogenous heterocyclic molecules (1,2,4-triazole, tetrazole, 1,2,4-triazol-3-amine, 1,2,4-triazol-3(4*H*)-one, and 3-methyl-1,2,4-triazole) into the 2-phenyl-4(3*H*)-quinazolinone nucleus to get several new lead structures **6**–**10**. Preliminary anticonvulsant screening demonstrated that compound **8** possessed positive activity at the dose of 100 mg/kg, while compounds **6**, **7**, **9**, and **10** did not show the protection at the same dose. As a consequence, a series of 5-phenyl-[1,2,4]-triazolo[4,3-*c*]quinazolin-3-amine derivatives was designed

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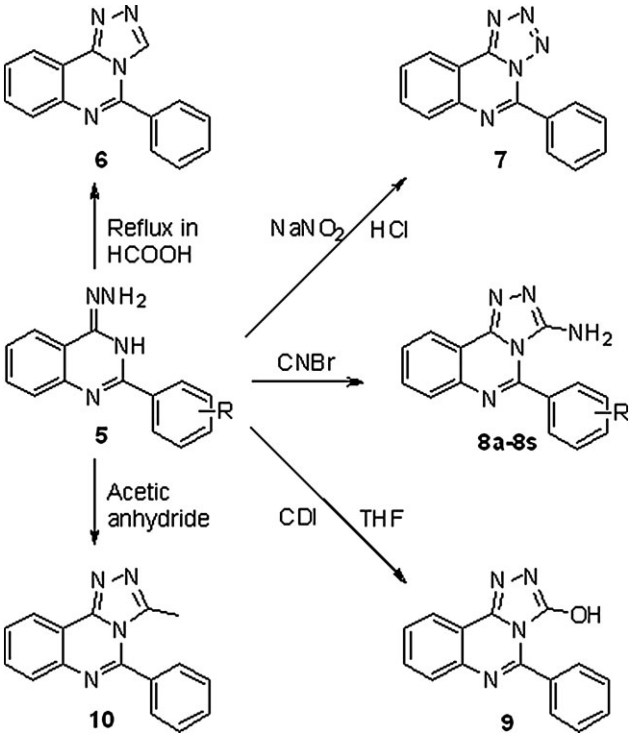
with substituted moieties possessing different electronic environment on the benzene in the hope of developing potent and safe new anticonvulsant compounds. The structures of all target compounds were characterized. Anticonvulsant activity and neurotoxicity were evaluated by the maximal electroshock seizure (MES) test and the rotarod test, respectively. The most active compound **8h** was subjected to some chemical-induced seizure models, including pentylenetetrazole (PTZ) and thiosemicarbazide (TSC) induced seizure tests.

Results and discussion

Chemistry

All the compounds were prepared as outlined in Schemes 1 and 2. Cyclization of 2-aminobenzamide with appropriate benzaldehydes gave substituted 2-phenyl-2,3-dihydroquinazolin-4(1*H*)-ones (**2**), which were oxidized by $KMnO_4$ to obtain compounds **3**. The compounds **3** underwent sulfurization in the presence of Lawesson's reagent to form 2-phenylquinazoline-4(3*H*)-thiones (**4**). Replacing the sulfur atom in compounds **4** with the hydrazine group by treatment with hydrazine hydrate produced the key intermediates **5**. Finally, intermediates **5** reacted with formic acid, $NaNO_2$/HCl, cyanogene bromide, carbonyl diimidazole, and acetic anhydride to obtain compounds **6**, **7**, **8**, **9**, and **10**, respectively. Their chemical structures were characterized using IR, ^{1}H-NMR, MS, and elemental analysis techniques. The detailed physical and analytical data are listed in the Experimental part.



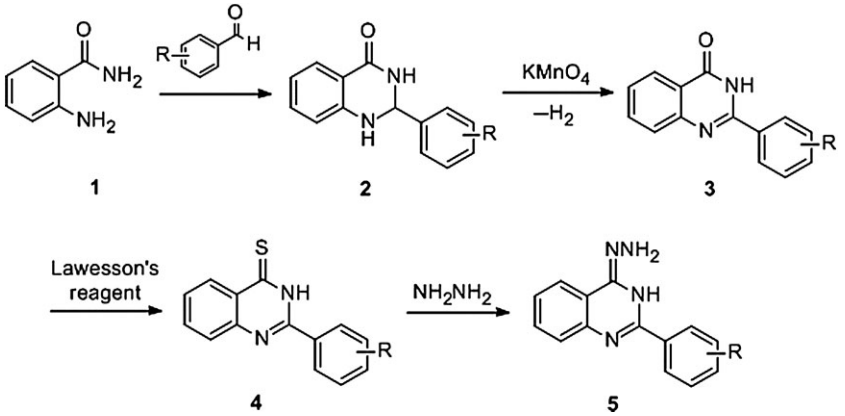
Scheme 2. Synthetic route of target compounds.

Pharmacology

Phase I evaluation of anticonvulsant activity

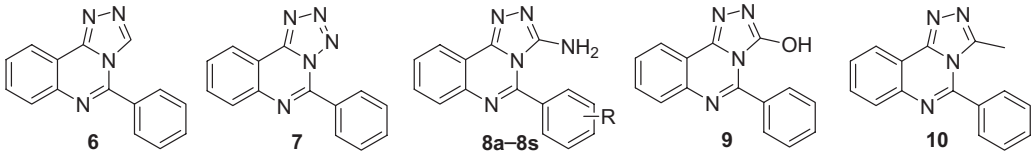
The pharmacological tests of all the target compounds were performed according to the standard protocol given by the epilepsy branch of the National Institute of Neurological Disorders and Stroke (NINDS) and adopted by the Antiepileptic Drug Development (ADD) program [33]. Their anticonvulsant activity was assessed by the most adopted animal model electroshock (MES) test. All the synthesized compounds were administered intraperitoneally into mice using doses of 30 and 100 mg/kg, and the observations were taken at two different time intervals (0.5 and 4.0 h). The results were presented in Table 1.

In the preliminary anticonvulsant screening, several compounds showed a certain degree of protection in the MES screen which was indicative of the anticonvulsant ability of these compounds. Nine compounds were active at a dose of 100 mg/kg after 0.5 h. These include compounds **8a–8e**, **8h**,



Scheme 1. Synthetic route of the key intermediate compound **5**.

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



Compounds	R	MES[a] (mg/kg) 0.5 h		MES (mg/kg) 4 h	
		100	30	100	30
8a	-H	2/3[b]	0/3	0/3	–
8b	*o*-F	2/3	0/3	0/3	–
8c	*m*-F	3/3	1/3	0/3	–
8d	*p*-F	1/3	0/3	0/3	–
8e	*o*-Cl	2/3	0/3	0/3	–
8f	*m*-Cl	0/3	–	0/3	–
8g	*p*-Cl	0/3	–	0/3	–
8h	*o*-Br	3/3	2/3	0/3	–
8i	*m*-Br	1/3	0/3	0/3	–
8j	*p*-Br	0/3	–	0/3	–
8k	*o*-CF_3	1/3	0/3	0/3	–
8l	*m*-CF_3	0/3	–	0/3	–
8m	*p*-CF_3	1/3	0/3	0/3	–
8n	*m*-NO_2	0/3	–	0/3	–
8o	*p*-CH_3	0/3	–	0/3	–
8p	*o*-OCH_3	0/3	–	0/3	–
8q	*m*-OCH_3	0/3	–	0/3	–
8r	*p*-OCH_3	0/3	–	0/3	–
6	-H	0/3	–	0/3	–
7	-H	0/3	–	0/3	–
9	-H	0/3	–	0/3	–
10	-H	0/3	–	0/3	–

[a] Maximal electroshock: Doses of 30, 100, and 300 mg/kg were administrated intraperitoneally in mice; the animals were examined 0.5 h after administration.
[b] The figures n_1/n_2: the animals protected/the animals tested.
The dash (–): The compound was not tested in mice at the dose of 30 mg/kg.

8i, **8k**, and **8m**. Among these, compounds **8c** and **8h** showed protection against seizure at the dose 30 mg/kg after 0.5 h. No compound showed activity at the dose of 100 mg/kg at 4 h interval. In the series of **8a–8r**, we can see that electron withdrawing groups (**8b–8n**) were found to be uncertain on the anticonvulsant activity, while electron releasing groups (**8o–8r**) obviously decreased the anticonvulsant activity when compared to compound **8a**. Introduction of the same halogen in different positions seems to have a different effect on the activity. For the three F-substituted derivatives (**8b–8d**), the potency order was *m*-F > *o*-F > *p*-F. Among the three Cl-substituted derivatives (**8e–8g**), the potency order was *o*-Cl > *m*-Cl = *p*-Cl. For the three Br-substituted derivatives (**8h–8j**), the potency order was *o*-Br > *m*-Br > *p*-Br.

Phase II evaluation of anticonvulsant activity

Because of the considerable anticonvulsant activity of compounds **8c** and **8h** presented in the preliminary anticonvulsant screening, they 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 standard drug carbamazepine and valproate, are reported in Table 2. 5-(3-Fluorophenyl)-[1,2,4]triazolo[4,3-*c*]-quinazolin-3-amine (**8c**) and 5-(2-bromophenyl)-[1,2,4]triazolo-[4,3-*c*]quinazolin-3-amine (**8h**) gave an ED_{50} of 44.8 and 27.4 mg/kg, and a protective index (PI) of 4.4 and 5.8, respectively. Both of them showed weaker anticonvulsant activity compared to the currently used AED carbamazepine but better than valproate. Moreover, both compounds showed a safer profile than valproate, and a comparable PI when compared with carbamazepine.

Speculation upon mechanism

To speculate about the possible mechanism of anticonvulsant action of the structures, compound **8h** was tested against

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

Compounds	ED_{50} mg/kg (MES)	TD_{50} mg/kg (rotarod)	PI (TD_{50}/ED_{50})
8c	44.8 (26.24–76.48)	182.6 (137.16–242.96)	4.1
8h	27.4 (19.89–37.70)	157.8 (114.61–217.19)	5.8
Carbamazepine	9.8 (8.9–10.8)	44.0 (40.2–48.1)	4.5
Valproate	272 (247–338)	426 (369–450)	1.6

convulsions induced by chemical substances, including PTZ and TSC. Compound **8h** was administered to mice at 50 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 50 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 **8h** inhibited the clonic seizures and tonic seizures induced by PTZ at the rates of 20 and 90%, respectively, and also showed complete inhibition of the death compared to the control (Table 3). These results revealed that compound **8h** possessed potent activity against sc-PTZ. PTZ have been reported to produce seizures by inhibiting γ-aminobutyric acid (GABA) neurotransmission [34, 35]. 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 [36], while enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study suggest that the newly synthesized compound **8h** might inhibit or attenuate PTZ-induced seizures in mice by enhancing GABAergic effects.

In the TSC-induced seizure model, the anticonvulsant effect is similar to that of the sc-PTZ induced seizure model. Compared with the control group, carbamazepine showed inhibition of clonic and tonic seizures and death at rates of 0, 100, and 100%, respectively. Compound **8h** showed inhibition of clonic seizures at a rate of 30%, and absolute protection against the tonic seizures and death induced by TSC. TSC are competitive inhibitors of the GABA synthesis enzyme glutamate decarboxylase (GAD), and they inhibit the synthesis of GABA, resulting in decreasing GABA levels in the brain [37]. Compound **8h** showed antagonism to TSC-induced seizures, suggesting that the GABAergic system-mediated mechanisms might be involved in its anticonvulsant activity.

In summary, the current study has demonstrated that a series of 5-phenyl-[1,2,4]triazolo[4,3-*c*]quinazolin-3-amine derivatives were effective in the MES screens. The most promising was compound **8h**, which showed an ED_{50} value of 27.4 mg/kg and a PI value of 5.8. These values were superior to those provided by valproate (ED_{50} and PI values of 272 and 1.6, respectively) in the MES test in mice. As well as its anti-MES efficacy, the potencies of compound **8h** against seizures induced by PTZ and TSC were also established, with the results suggesting that the GABAergic system-mediated mechanisms might be involved in its anticonvulsant activity.

Experimental

Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a IRPrestige-21. ^{1}H-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 ionization technique used for recording mass spectra was atmospheric pressure chemical ionization (APCI). Elemental analyses were performed on a 204Q CHN (Perkin Elmer, USA). The chemicals were purchased from Aldrich Chemical Corporation.

*General procedure for the preparation of compounds **2** [38]*

Compound **1** (1 g, 7.3 mmol) and appropriate benzaldehyde (9.6 mmol) were dissolved in dichloromethane (25 mL) and refluxed for 2–3 d. After completion of the reaction, excess solvent was removed under reduced pressure. Then water was added to the residue to get white crystal, which was further filtered and washed with excess of water. The white crystal was

Table 3. Effects of compound **8h** on chemical-induced seizures in mice (i.p.).

Chemical substances	Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
Pentylenetetrazol	DMSO	–	0.5	100	60	60
	Carbamazepine	50	0.5	100	0	0
	8h	50	0.5	80	10	0
Thiosemicarbazide	DMSO	–	0.5	100	100	100
	Carbamazepine	50	0.5	100	0	0
	8h	50	0.5	70	0	0

directly used in the next step without purification, whose yield was above 98%.

*General procedure for the preparation of compounds **3** [39]*

A mixture of **2** (6.7 mmol) and potassium permanganate (0.53 g, 3.3 mmol) in dimethyl formamide (20 mL) was refluxed for 2–3 h. The resulting suspension was filtered to remove the insoluble solid and excess solvent of the filtrate was removed under reduced pressure. Then water was added to the residue to get a white precipitate with a yield range of 85–90%, which was directly used in the next step without purification.

*General procedure for the preparation of compounds **4** [40]*

A mixture of **3** (8.5 mmol) and Lawesson's reagent (2.1 g, 5 mmol) in toluene (20 mL) was refluxed for 2–3 h. After removing the solvent under reduced pressure, the crude product was purified by silica gel column chromatography (V CH_3OH/CH_2Cl_2 = 1:100) with a yield range of 30–56%.

*General procedure for the preparation of compounds **5** [41]*

To a solution of compounds **4** (6.3 mmol) in methanol, 80% hydrazine hydrate (3.15 g, 50 mmol) was added and the mixture was refluxed for 12 h. After completion of the reaction, the major solvent was removed under reduced pressure. Waiting for a moment, a precipitate formed, which was filtered and washed by a small amount of methanol to obtain a pure product with a yield range of 70–76%.

*Procedure for the preparation of 5-phenyl-[1,2,4]triazolo[4,3-c]quinazoline (**6**)*

A mixture of compound **5** (R = H) (0.45 g, 1.9 mmol) and formic acid (30 mL) was heated at 120°C for 6 h. After removing the excess formic acid under reduced pressure, the residue was purified by silica gel column chromatography (V CH_3OH/CH_2Cl_2 = 1:60) to get a white solid **6**. Yield: 81%, m.p. 180–182°C. ^{1}H-NMR ($CDCl_3$): δ 7.59–7.63 (m, 3H, Ar–H), 7.74 (t, 1H, J = 7.5 Hz, Ar–H), 7.84–7.90 (m, 1H, Ar–H), 8.47 (s, 1H, triazole-H), δ 8.50–8.59 (m, 4H, Ar–H). IR (KBr) cm^{-1}: 1527, 1604 (C=N). MS *m/z* 247 (M+H). Anal. calcd. for $C_{15}H_{10}N_4$: C, 73.16; H, 4.09; N, 22.75. Found: C, 73.32; H, 4.27; N, 22.48.

*Procedure for the preparation of 5-phenyltetrazolo-[1,5-c]quinazoline (**7**)*

To a solution of compound **5** (R = H) (0.5 g, 2.1 mmol) in 9 mL 30% HCl, 10% $NaNO_2$ (0.15 g $NaNO_2$ dissolved in 1.5 mL H_2O) solution was added dropwise below 5°C. The mixture was stirred for 12 h below 5°C. The precipitate formed was filtered to get the crude product, which was purified by silica gel column chromatography (V CH_3OH/CH_2Cl_2 = 1:80) to get a white solid **7**. Yield: 73%, m.p. 162–163°C. ^{1}H-NMR ($CDCl_3$): δ 7.63–7.71 (m, 3H, Ar–H), 7.80–7.86 (m, 1H, Ar–H), 7.95–8.00 (m, 1H, Ar–H), 8.21(d, 1H, J = 8.2 Hz, Ar–H), 8.64–8.73 (m, 3H, Ar–H). IR (KBr) cm^{-1}: 1510, 1610 (C=N). MS *m/z* 248 (M+H). Anal. calcd. for $C_{14}H_9N_5$: C, 68.01; H, 3.67; N, 28.32. Found: C, 67.86; H, 3.75; N, 28.53.

*Procedure for the preparation of compound **8***

In a round-bottom flask, compound **5** (1.9 mmol) was dissolved in dioxane (15 mL), then the solution was treated with Na_2CO_3 solution (0.21 g Na_2CO_3 dissolved in 6 mL H_2O). Cyanogene bromide (0.24 g, 2.1 mmol) in dioxane (6 mL) was added dropwise to the mixture under ice-bath and the reaction temperature was kept below 10°C. After stirring for 12 h, the solvent was removed under reduced pressure. The residue was recrystallized from ethanol to get a pure product **8**.

*5-Phenyl-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8a**)*

Yield: 73%, m.p. 256–257°C. ^{1}H-NMR (DMSO-d_6): δ 5.20 (s, 2H, –NH_2), 7.59–7.84 (m, 8H, Ar–H), 8.31 (d, 1H, J = 7.4 Hz, Ar–H). IR (KBr) cm^{-1}: 1510, 1610 (C=N), 3086, 3114 (N–H). MS *m/z* 262 (M+H). Anal. calcd. for $C_{15}H_{11}N_5$: C, 68.95; H, 4.24; N, 26.80. Found: C, 68.95; H, 4.24; N, 26.80.

*5-(2-Fluorophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8b**)*

Yield: 76%, m.p. 250–252°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.14 (s, 2H, NH_2), 7.30–7.42 (m, 2H, Ar–H), 7.64–7.75 (m, 4H, Ar–H), 7.82 (d, 1H, J = 7.5 Hz, Ar–H), 8.35 (d, 1H, J = 7.8 Hz, Ar–H). IR (KBr) cm^{-1}: 1534, 1670 (C=N), 3034, 3432 (N–H). MS *m/z* 280 (M+H). Anal. calcd. for $C_{15}H_{10}FN_5$: C, 64.51; H, 3.61; N, 25.08. Found: C, 64.74; H, 3.89; N, 24.85.

*5-(3-Fluorophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8c**)*

Yield: 71%, m.p. 270–271°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.18 (s, 2H, NH_2), 7.37–7.42 (m, 1H, Ar–H), 7.52–7.73 (m, 5H, Ar–H), 7.82–7.87 (m, 1H, Ar–H), 8.42 (d, 1H, J = 6.9 Hz, Ar–H). IR (KBr) cm^{-1}: 1515, 1646 (C=N), 3111, 3419 (N–H). MS *m/z* 280 (M+H). Anal. calcd. for $C_{15}H_{10}FN_5$: C, 64.51; H, 3.61; N, 25.08. Found: C, 64.70; H, 3.84; N, 24.88.

*5-(4-Fluorophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8d**)*

Yield: 78%, m.p. 257–258°C. ^{1}H-NMR (DMSO-d_6): δ 5.30 (s, 2H, NH_2), 7.41 (t, 2H, J = 8.1 Hz, Ar–H), 7.64–7.75 (m, 2H, Ar–H), 7.80–7.84 (m, 3H, Ar–H), 8.32 (d, 1H, J = 7.9 Hz, Ar–H). IR (KBr) cm^{-1}: 1508, 1643 (C=N), 3105, 3417 (N–H). MS *m/z* 280 (M+H). Anal. calcd. for $C_{15}H_{10}FN_5$: C, 64.51; H, 3.61; N, 25.08. Found: C, 64.66; H, 3.78; N, 24.89.

*5-(2-Chlorophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8e**)*

Yield: 80%, m.p. 246–248°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.00 (s, 2H, NH_2), 7.52–7.80 (m, 7H, Ar–H), 8.32 (d, 1H, J = 8.0 Hz, Ar–H). IR (KBr) cm^{-1}: 1532, 1662 (C=N), 3025, 3426 (N-H). MS *m/z* 296 (M+H). Anal. calcd. for $C_{15}H_{10}ClN_5$: C, 60.92; H, 3.41; N, 23.68. Found: C, 61.14; H, 3.50; N, 23.82.

*5-(3-Chlorophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8f**)*

Yield: 70%, m.p. 244–246°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.25 (s, 2H, NH_2), 7.58–7.78 (m, 7H, Ar–H), 8.31 (d, 1H, J = 7.4 Hz, Ar–H). IR (KBr) cm^{-1}: 1511, 1610 (C=N), 3082, 3113 (N–H). MS *m/z* 296 (M+H). Anal. calcd. for $C_{15}H_{10}ClN_5$: C, 60.92; H, 3.41; N, 23.68. Found: C, 61.11; H, 3.54; N, 23.79.

*5-(4-Chlorophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8g**)*

Yield: 78%, m.p. 266–268°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.17 (s, 2H, NH_2), 7.52 (d, 2H, J = 8.4 Hz, Ar–H), 7.56–7.65 (m, 2H,

Ar–H), 7.69 (d, 2H, J = 8.4 Hz, Ar–H), 7.74–7.77 (m, 1H, Ar–H), 8.33 (d, 1H, J = 7.3 Hz, Ar–H). IR (KBr) cm^{-1}: 1492, 1643 (C=N), 3093, 3410 (N–H). MS *m/z* 296 (M+H). Anal. calcd. for $C_{15}H_{10}ClN_5$: C, 60.92; H, 3.41; N, 23.68. Found: C, 61.11; H, 3.56; N, 23.87.

*5-(2-Bromophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8h**)*

Yield: 79%, m.p. 216–218°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 4.75 (s, 2H, NH_2), 7.50 (t, 1H, J = 7.5 Hz, Ar–H), 7.63–7.90 (m, 6H, Ar–H), 8.49 (d, 1H, J = 7.6 Hz, Ar–H). IR (KBr) cm^{-1}: 1529, 1668 (C=N), 3029, 3426 (N–H). MS *m/z* 340 (M+H). Anal. calcd. for $C_{15}H_{10}BrN_5$: C, 52.96; H, 2.96; N, 20.59. Found: C, 53.08; H, 3.11; N, 20.38.

*5-(3-Bromophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8i**)*

Yield: 76%, m.p. 232–234°C. ^{1}H-NMR (DMSO-d_6): δ 5.23 (s, 2H, NH_2), 7.45–7.89 (m, 7H, Ar–H), 8.32 (d, 1H, J = 8.0 Hz, Ar–H). IR (KBr) cm^{-1}: 1531, 1669 (C=N), 3039, 3433 (N–H). MS *m/z* 340 (M+H). Anal. calcd. for $C_{15}H_{10}BrN_5$: C, 52.96; H, 2.96; N, 20.59. Found: C, 53.14; H, 3.08; N, 20.41.

*5-(4-Bromophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8j**)*

Yield: 75%, m.p. 282–284°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.21 (s, 2H, NH_2), 7.64–7.88 (m, 7H, Ar–H), 8.40 (d, 1H, J = 7.3 Hz, Ar–H). IR (KBr) cm^{-1}: 1513, 1642 (C=N), 3113, 3418 (N–H). MS *m/z* 340 (M+H). Anal. calcd. for $C_{15}H_{10}BrN_5$: C, 52.96; H, 2.96; N, 20.59. Found: C, 53.17; H, 3.12; N, 20.40.

*5-(2-(Trifluoromethyl)phenyl)-[1,2,4]triazolo-[4,3-c]quinazolin-3-amine (**8k**)*

Yield: 80%, m.p. 172–174°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.26 (s, 2H, NH_2), 7.46–7.91 (m, 7H, Ar–H), 8.32 (d, 1H, J = 7.6 Hz, Ar–H). IR (KBr) cm^{-1}: 1512, 1647 (C=N), 3111, 3419 (N–H). MS *m/z* 330 (M+H). Anal. calcd. for $C_{16}H_{10}F_3N_5$: C, 58.36; H, 3.06; N, 21.27. Found: C, 58.59; H, 3.18; N, 21.04.

*5-(3-(Trifluoromethyl)phenyl)-[1,2,4]triazolo-[4,3-c]quinazolin-3-amine (**8l**)*

Yield: 73%, m.p. 174–176°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.34 (s, 2H, NH_2), 7.68–8.05 (m, 7H, Ar–H), 8.44 (d, 1H, J = 7.9 Hz, Ar–H). IR (KBr) cm^{-1}: 1530, 1668 (C=N), 3029, 3427 (N–H). MS *m/z* 330 (M+H). Anal. calcd. for $C_{16}H_{10}F_3N_5$: C, 58.36; H, 3.06; N, 21.27. Found: C, 58.62; H, 3.14; N, 21.07.

*5-(4-(Trifluoromethyl)phenyl)-[1,2,4]triazolo-[4,3-c]quinazolin-3-amine (**8m**)*

Yield: 78%, m.p. 263–266°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 6.34 (s, 2H, NH_2), 7.65–7.85 (m, 4H, Ar–H), 7.98 (d, 1H, J = 8.0 Hz, Ar–H), 8.25 (d, 1H, J = 8.0 Hz, Ar–H), 8.78–8.82 (m, 2H, Ar–H). IR (KBr) cm^{-1}: 1539, 1669 (C=N), 3029, 3426 (N–H). MS *m/z* 330 (M+H). Anal. calcd. for $C_{16}H_{10}F_3N_5$: C, 58.36; H, 3.06; N, 21.27. Found: C, 58.51; H, 3.21; N, 21.10.

*5-(3-Nitrophenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8n**)*

Yield: 71%, m.p. 238–239°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 5.37 (s, 2H, NH_2), 7.67–7.86 (m, 4H, Ar–H), 8.17 (s, 1H, J = 7.6 Hz, Ar–H), 8.39–8.65 (m, 3H, Ar–H). IR (KBr) cm^{-1}: 1534, 1667 (C=N), 3028, 3423 (N–H). MS *m/z* 307 (M+H). Anal. calcd. for $C_{15}H_{10}N_6O_2$: C, 58.82; H, 3.29; N, 27.44. Found: C, 58.60; H, 3.33; N, 27.61.

*5-p-Tolyl-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8o**)*

Yield: 75%, m.p. 250–251°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 2.41 (s, 3H, –CH_3), 5.07 (s, 2H, NH_2), 7.33 (d, 1H, J = 8.2 Hz, Ar–H), 7.52 (d, 2H, J = 9.0 Hz, Ar–H), 7.56–7.76 (m, 3H, Ar–H), 8.31 (d, 1H, J = 9.0 Hz, Ar–H). IR (KBr) cm^{-1}: 1534, 1671 (C=N), 3037, 3432 (N–H). MS *m/z* 276 (M+H). Anal. calcd. for $C_{16}H_{13}N_5$: C, 69.80; H, 4.76; N, 25.44. Found: C, 69.99; H, 4.64; N, 25.32.

*5-(2-Methoxyphenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8p**)*

Yield: 72%, m.p. 230–231°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 3.79 (s, 3H, OCH_3), 4.62 (s, 2H, NH_2), 7.08–7.26 (m, 2H, Ar–H), 7.55–7.72 (m, 4H, Ar–H), 7.90 (d, 1H, J = 9.0 Hz, Ar–H), 8.50 (d, 1H, J = 8.2 Hz, Ar–H). IR (KBr) cm^{-1}: 1517, 1619 (C=N), 3083, 3112 (N–H). MS *m/z* 292 (M+H). Anal. calcd. for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.50; N, 24.04. Found: C, 66.18; H, 4.59; N, 23.87.

*5-(3-Methoxyphenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8q**)*

Yield: 73%, m.p. 238–239°C. ^{1}H-NMR (DMSO-d_6, $CDCl_3$): δ 3.82 (s, 3H, OCH_3), 5.16 (s, 2H, NH_2), 7.13–7.23 (m, 3H, Ar–H), 7.43–7.69 (m, 3H, Ar–H), 7.76 (d, 1H, J = 9.0 Hz, Ar–H), 8.31 (d, 1H, J = 8.3 Hz, Ar–H). IR (KBr) cm^{-1}: 1539, 1670 (C=N), 3039, 3433 (N–H). MS *m/z* 292 (M+H). Anal. calcd. for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.50; N, 24.04. Found: C, 66.14; H, 4.63; N, 23.82.

*5-(4-Methoxyphenyl)-[1,2,4]triazolo[4,3-c]quinazolin-3-amine (**8r**)*

Yield: 79%, m.p. 244–245°C. ^{1}H-NMR (DMSO-d_6,$CDCl_3$): δ 3.83 (s, 3H, OCH_3), 5.02 (s, 2H, NH_2), 7.01 (d, 2H, J = 8.6 Hz, Ar–H), 7.54–7.58 (m, 4H, Ar–H), 7.75 (d, 1H, J = 9.0 Hz, Ar–H), 8.33 (d, 1H, J = 8.6 Hz, Ar–H). IR (KBr) cm^{-1}: 1512, 1613 (C=N), 3081, 3113 (N–H). MS *m/z* 292 (M+H). Anal. calcd. for $C_{16}H_{13}N_5O$: C, 65.97; H, 4.50; N, 24.04. Found: C, 66.09; H, 4.41; N, 23.91.

*Procedure for the preparation of 5-phenyl-[1,2,4]triazolo[4,3-c]quinazolin-3-ol (**9**)*

To a tetrahydrofuran solution (8 mL) of compound **5** (R = H) (0.5 g, 2.1 mmol), carbonyl diimidazole (0.4 g, 2.5 mmol) was added. The reaction mixture was heated at reflux for 8 h. After removing the solvent under reduced pressure, the crude product was purified by silica gel column chromatography (V CH_3OH/CH_2Cl_2 = 1:20) to obtain a white solid. Yield: 44%, m.p. 248–249°C. ^{1}H-NMR ($CDCl_3$): δ 7.49–7.56 (m, 4H, Ar–H), 7.78–7.87 (m, 2H, Ar–H), 8.28–8.36 (m, 3H, Ar–H), 11.99 (s, 1H, –OH). IR (KBr) cm^{-1}: 1532, 1614 (C=N). MS *m/z* 263 (M+H). Anal. calcd. for $C_{15}H_{10}N_4O$: C, 68.69; H, 3.84; N, 21.36. Found: C, 68.46; H, 3.95; N, 21.58.

*Procedure for the preparation of 3-methyl-5-phenyl-[1,2,4]triazolo[4,3-c]quinazoline (**10**)*

A mixture of compound **5** (R = H) (0.5 g, 2.1 mmol) and acetic anhydride (5 mL) was heated at reflux for 4 h. After the completion of the reaction, the mixture was poured into 15 mL of water, and then extracted with CH_2Cl_2. The CH_2Cl_2 layer was

dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product that was purified by silica gel column chromatography (V CH_3OH/CH_2Cl_2 = 1:60) to get a white solid. Yield: 64%, m.p. 120–122°C. ^{1}H-NMR ($CDCl_3$): δ 2.72 (s, 3H, $-CH_3$), 7.59–7.63 (m, 3H, Ar–H), 7.70 (t, 1H, J = 7.5 Hz, Ar–H), 7.84 (t, 1H, J = 7.5 Hz, Ar–H), 8.49–8.53 (m, 4H, Ar–H). IR (KBr) cm^{-1}: 1522, 1608 (C=N). MS *m/z* 261 (M+H). Anal. calcd. for $C_{16}H_{12}N_4$: C, 73.83; H, 4.65; N, 21.52. Found: C, 73.98; H, 4.42; N, 21.40.

Pharmacology

All the target compounds were screened for their anticonvulsant activity using the most adopted seizure model – the MES test. Neurotoxicity was assessed by rotarod test. All the compounds, dissolved in DMSO-d_6, were evaluated for anticonvulsant activities with KunMing mice in the 18–22 g weight range, which were purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University. Compound **8h** was chosen to test in PTZ- and TSC-induced seizure models for the speculation about the possible mechanism of anticonvulsant action.

Maximal electroshock seizure (MES) test [42, 43]

In the MES test, 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 and 4.0 h of drug administration, the activities were evaluated in the MES test. In phase I screening, each compound was administered at the dose levels of 30, 100, and 300 mg/kg for evaluating the preliminary anticonvulsant activity. For determination of the median effective dose (ED_{50}) and the median toxic dose (TD_{50}), the phase II screening was prepared. Groups of five 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) [42, 43]

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 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.

Chemical induced seizure tests

In chemically induced seizures (see Table 3), mice were given doses of convulsant drugs that could induce seizures in at least 97% of the animals. The doses used were: PTZ, 85 mg/kg; TSC, 50 mg/kg. The test compounds and carbamazepine were administered i.p. in a volume of 50 mg/kg to groups of ten mice 30 min before injection of PTZ (s.c.) and TSC (i.p.). The mice were placed in individual cages and observed for 30 min; the numbers of clonic seizures and tonic seizures, and the lethality were recorded [44, 45].

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