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Original article

Design and synthesis of 10-alkoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4] oxazepine derivatives with anticonvulsant activity

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ABSTRACT

A series of novel 10-alkoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine derivatives were synthesized and screened for their anticonvulsant activities by the maximal electroshock (MES) test and their neurotoxicity was evaluated by the rotarod neurotoxicity test (Tox). In the MES test, compound 10-Heptyloxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8f**) was found to possess better anticonvulsant activity and higher safety than marketed drugs carbamazepine and phenytoin with an ED_{50} value of 6.9 mg/kg a PI value of 9.5. To explain the possible mechanism of anticonvulsant activity, compound **8f** was tested in pentylenetetrazole, isoniazid, thiosemicarbazide, 3-mercaptopropionic acid and Bicuculline induced seizures tests. The results suggest that compound **8f** exerts anticonvulsant activity through GABA-mediated mechanism.

1. Introduction

Epilepsy, one of the most frequent neurological afflictions in man characterized by excessive temporary neuronal discharges resulting in uncontrolled convulsion, inflicts more than 2 million Americans and 60 million people worldwide [1,2]. Although the current drugs provide adequate seizure control in many patients, 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. Toxicity, intolerance, and lack of efficacy are the limitations of the current AEDs. Therefore, the continued search for safer and more effective new AEDs is necessary.

In our previous studies [11,12], series of 6-alkyloxyl-3,4-dihydro-2(1*H*)-quinolines and 7-alkoxy-4,5-dihydro-[1,2,4]triazole[4,3-a] quinolines (Fig. 1, **I**) were synthesized and tested for anticonvulsant activity. In these synthesized derivatives, 7-(4-fluorobenzyloxy)-4,5-di-hydro-[1,2,4]triazole[4,3-a]quinoline displayed the best activity, having ED_{50} values of 11.8 mg/kg in the maximal electroshock test (MES).

Intent on exploring effective compounds with lower neurotoxicity, a series of 10-alkoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4] oxazepines (Fig. 1, **II**) were designed and synthesized in this study. Compounds **II**, the ring enlargement analogues of compounds **I** through inserting an oxygen atom, were anticipated to possess a better anticonvulsant activity. Their structures were characterized using IR, 1H NMR, MS and elemental analysis techniques. Their anticonvulsant activity was evaluated using MES test in mice and their neurotoxicity was evaluated with the rotarod test. For explaining the possible mechanism of action, the most active compound (**8k**) was tested in Pentylenetetrazole (PTZ), Isoniazid (ISO), thiosemicarbazide (TSC), 3-Mercaptopropionic acid (3-MP), and Bicuculline (BIC) induced seizure tests.

2. Chemistry

Compounds were prepared as outlined in Scheme 1. The starting material 6-methoxy-2,3-dihydrochromen-4-one (**1**) was prepared by the method reported by Cai [13]. The Beckmann rearrangement reaction is a good method to obtain seven-membered heterocyclic amides, especially the 1, 4-benzoxazepin-5(2*H*)-ones [14,15], This method was used in the next step. Compound (**1**) reacted with hydroxylamine chloride to form 6-methoxy-2,3- dihydrochromen-4-one oxime (**2**) with a high yield, which then, under the Lewis acid condition, using polyphosphoric acid (PPA) in this case, became compound **3**. In this step, the product in the Beckmann

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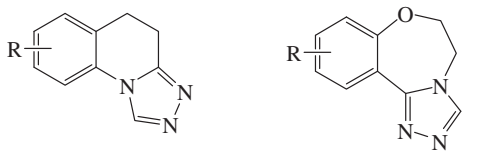


Fig. 1. Structure of compounds I and II.

rearrangement reaction was mostly the expected product, 7-methoxy-3,4-dihydrobenzo[f][1,4]oxazepin-5(2*H*)-one (**3**), rather than the regioisomer. This might be ascribed to the greater difficulty for the aryl group to migrate.

7-Methoxy-3,4-dihydrobenzo[f][1,4]oxazepine-5(2*H*)-thione (**4**) was prepared by the reaction of compound **3** with phosphorous pentasulfide in acetonitrile in the presence of triethylamine. Compounds **4** reacted further with hydrazine hydrate in THF to afford 1-(7-methoxy-3,4-dihydrobenzo[f][1,4]oxazepin-5(2*H*)-ylidene)hydrazine (**5**), which reacted with formic acid to give 10-methoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**6**). Demethylation of compound **6** by treatment with dichloromethane and boron tribromide produced 5, 6-dihydro-triazolo[4,3-d]benzo [f][1,4]oxazepin-10-ol (**7**). Finally, compounds **8a**–**s** were achieved by reacting compound **7** with halogenated hydrocarbon in acetonitrile in the presence of K_2CO_3 (Scheme 1).

3. Pharmacology

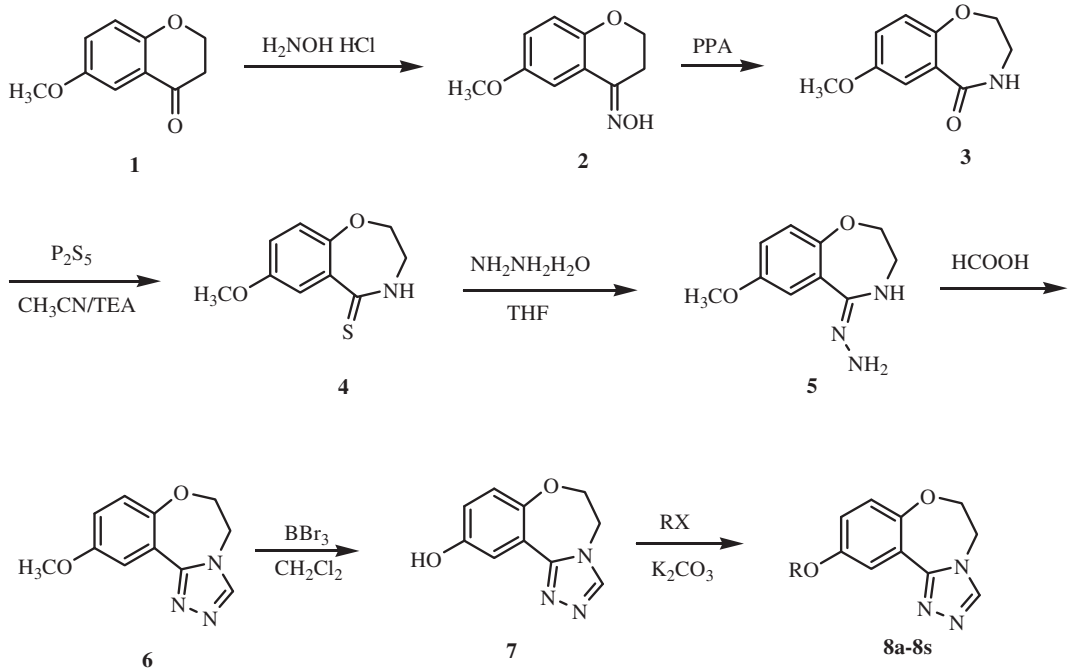
All the titled compounds (**8a**–**s**) were screened for their anticonvulsant activity by using the most adopted seizure models – the Maximal electroshock (MES) test. Neurotoxicity was assessed by rotarod test. The MES test and rotarod test were carried out by the methods described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health following previously described testing procedures (USA) [16,17]. All compounds, which were dissolved in DMSO, were evaluated for anticonvulsant activities with KunMing mice in the 18–22 g weight range purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University.

Most of the titled compounds possessed better activity were quantified their anticonvulsant activity, and the compound **8f** was tested in sc-PTZ, isoniazid, 3-MP, thiosemicarbazide, and Bicuculline induced seizures tests.

4. Results and discussion

As with any other class of drugs, the preclinical discovery and development of a new chemical entity for the treatment of epilepsy rely heavily on the use of predictable animal models. At the present time, there are three in vivo models that are routinely used by most AED discovery programs. They include the maximal electroshock (MES), the subcutaneous pentylenetetrazol (sc-PTZ), and the kindling model. Of these, the MES and sc-PTZ seizure models represent the two animal seizure models most widely used in the search for new AEDs [18,19]. In this study, the MES seizure model was used for preliminary (phase-**I**) screening of compounds **6**, **8a**–**s** and the results were presented in Table 1. As shown in Table 1, all of the compounds were active in the MES test, indicative of their ability to prevent seizure spread, among which five compounds **8c**–**g** showed protection against MES-induced seizure at the dose of 10 mg/kg. At a dose of 30 mg/kg, most compounds showed protection except **6, 8a**, **8m, 8n**, and **8q**–**s**. At a dose of 100 mg/kg all compounds showed protection except **6**. None of the compounds showed protection in the 4 h period.

As a result of preliminary screening, compounds **8b**–**p** 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 phenytoin, are reported in Table 2. Among the tested compounds, 10-Heptyloxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8f**), which gave an ED_{50} value of 6.9 mg/kg and a TD_{50} value of 65.7 mg/kg resulting in a higher protective index (PI) value – that is, TD_{50}/ED_{50} = 9.5 when compared to carbamazepine and phenytoin.



Scheme 1. Synthetic route of target compounds (**8a**–**s**).

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

Compds.	R	Dosage (mg/kg)	MES[a]	
			0.5 h	4 h
6	$-CH_3$	300	2/6	0/6
8a	$-C_2H_5$	100	2/6	0/6
8b	*n*-C_3H_7	30	4/6	0/6
8c	*n*-C_4H_9	10	3/6	0/6
8d	*n*-C_5H_{11}	10	1/6	0/6
8e	*n*-C_6H_{13}	10	4/6	0/6
8f	***n*-C_7H_{15}**	**10**	**6/6**	0/6
8g	*n*-C_8H_{17}	10	2/6	0/6
8h	$-CH_2C_6H_5$	30	6/6	0/6
8i	$-CH_2C_6H_4$ (*o*-F)	30	6/6	0/6
8j	$-CH_2C_6H_4$ (*m*-F)	30	5/6	0/6
8k	$-CH_2C_6H_4$ (*p*-F)	30	4/6	0/6
8l	$-CH_2C_6H_4$ (*o*-Cl)	30	3/6	0/6
8m	$-CH_2C_6H_4$ (*m*-Cl)	100	4/6	0/6
8n	$-CH_2C_6H_4$ (*p*-Cl)	100	5/6	0/6
8o	$-CH_2C_6H_4$(*o*-Br)	30	2/6	0/6
8p	$-CH_2C_6H_4$(*m*-Br)	30	4/6	0/6
8q	$-CH_2C_6H_4$(*p*-Br)	100	2/6	0/6
8r	$-CH_2C_6H_4$(*p*-CH_3)	100	2/6	0/6
8s	$-CH_2C_6H_4$(*p*-OCH_3)	100	1/6	0/6

[a] Maximal electroshock test (number of animals protected/number of animals tested), the number of mice is six.

Therefore, compound **8f** was selected to be the most active and promising compound in this work. With an ED_{50} value of 11.0, 10.0 and 11.0 respectively, compounds **8c**, **8e** and **8g** were nearly equipotent to carbamazepine, but were more toxic than carbamazepine. And the remaining 11 compounds **8b**, **8d**, and **8h**–**p** exhibited comparatively weaker activity than carbamazepine and phenytoin.

Analyzing the activities of the synthesized compounds the following structure–activity relationships (SAR) were obtained.

Besides the derivative **8h** (non-substituted in the ring of benzyl group), some halogen substituted derivatives (**8i**–**q**) were designed and synthesized in this paper. Introduction of halogen on the benzyl ring decreased the anticonvulsant activity (compared with **8h**), although the o-F derivative **8i** exhibited higher safety with *PI* value of 5.1 compared with **8h** (*PI* = 4.6). Comparison of the halogen substituted derivatives indicated that different halogen atoms contributed to the anticonvulsant activity in the order of F > Cl, Br. Comparing the derivatives with different F-substitution positions on the benzyl ring, their activity order was o-F > m-F > p-F. Activity order of the Cl and Br substituted derivatives was o-Cl > p-Cl > m-Cl and m-Br > o-Br > p-Br.

Two electron-donor derivatives were also designed and prepared, containing p-CH_3 and p-OCH_3. The pharmacology test revealed that their activities were obviously lower than the halogen substituted derivatives with an ED_{50} > 100 mg/kg.

The length of the alkyl chain appeared to have a direct impact on anticonvulsant activity of the 10-alkoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine derivatives. From compounds **6**, **8a** to **8f**, as alkyl chain length increased, ED_{50} gradually increased with the compound **8f** (with the n-heptyl substituted group) being the most active. The trend reversed, however, when the alkyl chain had more than seven carbon atoms. Obviously, the activity curve of the alkyl chain substituted derivatives is bell-shaped with a maximum activity peak. Compound **8f**, with the maximum activity peak, reflected the optimal partition coefficient associated with the easiest crossing of the biological membranes. Compound **8f**, with ED_{50} value of 6.9 mg/kg and PI value of 9.5, was the most potent of all the tested and superior to the carbamazepine and phenytoin.

To further investigate the effects of the anticonvulsant activity in several different models and speculate about the possible mechanism of anticonvulsant action, compounds **8f** was tested against convulsions induced by chemical substances, including PTZ, ISO, 3-MP, TSC, and BIC. Compounds **8f** was administered into mice i.p. at a dose of 30 mg/kg, which was higher than four times its ED50 value and far below its TD50 value. The reference drug carbamazepine was also administered i.p. at a dose of 30 mg/kg.

In the sc-PTZ model, compounds **8f** partially inhibited the clonic seizures induced by sc-PTZ, while the reference drug carbamazepine did not inhibit the clonic seizures induced by sc-PTZ. Both of them inhibited the tonic seizures and reduced lethality compared with the control group (Table 3). In the isoniazid model, carbamazepine inhibited the clonic seizures, tonic seizures and death induced by isoniazid at the rates of 40%, 100% and 100%, respectively; and compound **8f** showed complete inhibition of the clonic seizures, tonic seizures and death induced by isoniazid (Table 3). PTZ and ISO have been reported to produce seizures by inhibiting γ-aminobutyric acid (GABA) neurotransmission [20,21]. GABA is the

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

Compds.	R	ED_{50} (mg/kg) (MES)	TD_{50} (mg/kg) (Rotarod)	PI[a]
6	$-CH_3$	>100	–[b]	–
8a	$-C_2H_5$	>100	–	–
8b	*n*-C_3H_7	19.0 (17.1–21.2)[c]	54.8 (49.3–61.0)	2.9
8c	*n*-C_4H_9	11.0 (9.8–12.2)	45.6 (41.0–50.7)	4.2
8d	*n*-C_5H_{11}	15.8 (14.2–17.6)	60.0 (48.5–74.2)	3.8
8e	*n*-C_6H_{13}	10.0 (8.1–12.4)	60.0 (48.5–74.2)	6.0
8f	***n*-C_7H_{15}**	**6.9** (**5.6**–**8.5**)	**65.7** (**59.1**–**73.1**)	**9.5**
8g	*n*-C_8H_{17}	11.0 (9.9–12.2)	63.4 (57.6–69.7)	5.8
8h	$-CH_2C_6H_5$	16.4 (14.7–18.2)	76.0 (69.1–83.6)	4.6
8i	$-CH_2C_6H_4$ (*o*-F)	18.0 (16.2–20.0)	91.3 (83.0–100.4)	5.1
8j	$-CH_2C_6H_4$ (*m*-F)	23.7 (21.3–26.4)	76.0 (69.1–83.6)	3.2
8k	$-CH_2C_6H_4$ (*p*-F)	27.4 (24.6–30.5)	63.4 (57.6–69.7)	2.3
8l	$-CH_2C_6H_4$ (*o*-Cl)	38.3 (34.4–42.6)	91.3 (82.1–101.6)	2.4
8m	$-CH_2C_6H_4$ (*m*-Cl)	82.2 (73.9–91.4)	126.8 (114.0–141.1)	1.5
8n	$-CH_2C_6H_4$ (*p*-Cl)	60.0 (53.8–66.9)	109.6 (98.5–121.9)	1.8
8o	$-CH_2C_6H_4$(*o*-Br)	41.7 (33.7–51.6)	109.6 (98.5–121.9)	2.6
8p	$-CH_2C_6H_4$(*m*-Br)	25.0 (20.2–30.9)	91.3 (83.0–100.4)	3.6
8q	$-CH_2C_6H_4$(*p*-Br)	>100	–	–
8r	$-CH_2C_6H_4$(*p*-CH_3)	>100	–	–
8s	$-CH_2C_6H_4$(*p*-OCH_3)	>100	–	–
Carbamazepine	–	11.8 (9.7–14.1)	76.1(69.1–83.7)	6.4
Phenytoin	–	9.5 (8.1–10.4)	65.5 (52.5–72.9)	6.9

[a] PI = Protective (TD_{50}/ED_{50}).
[b] Not test.
[c] The 95% confidence limits.

Table 3
Effects of compound **8f** on chemically-induced seizures in mice.

Chemical substances	Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
Pentylenetetrazol	DMSO	–	0.5	100	40	40
	Carbamazepine	30	0.5	100	0	0
	8f	30	0.5	80	0	0
Isoniazid	DMSO	–	1	100	100	60
	Carbamazepine	30	1	60	0	0
	8f	30	1	0	0	0
3-Mercaptopropionic acid	DMSO	–	0.5	100	100	100
	Carbamazepine	30	0.5	100	0	0
	8f	30	0.5	100	0	20
Thiosemicarbazide	DMSO	–	2.5	100	100	80
	Carbamazepine	30	2.5	100	0	0
	8f	30	2.5	100	30	30
Bicuculline	DMSO	–	0.5	100	100	100
	Carbamazepine	30	0.5	100	0	20
	8f	30	0.5	100	0	0

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 [22], while enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study suggest that the newly synthesized compound **8f** might have inhibited or attenuated pentylenetetrazole-induced seizures and isoniazid-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, compounds **8f** showed the anticonvulsant effect similar to that of carbamazepine in inhibiting the clonic and tonic seizures, and showed partial inhibition of the death induced by 3-MP with the inhibition rates of 0%, 100% and 80%, respectively (Table 3). In the TSC-induced seizure model, the anticonvulsant effect is similar to that of the 3-MP induced seizure model. Compared with the control group, carbamazepine showed inhibition at the rates of 0%, 100% and 100%, respectively of the clonic seizures, tonic seizures and death. Compound **8f** showed inhibition at the rates of 0%, 70% and 70%, respectively (Table 3). 3-MP and TSC were competitive inhibitors of GABA synthesis enzyme glutamate decarboxylase (GAD), and they inhibit the synthesis of GABA resulting in decrease of GABA level in the brain [23]. Compound **8f** showed moderate antagonism to 3-MP induced seizures and thiosemicarbazide-induced seizures, suggesting that it might activate GAD or inhibit aminotransferase (GABA-T) in the brain.

In the BIC induced seizure model, both carbamazepine and **8f** inhibited the tonic seizures and death, and did not inhibit clonic seizures. Carbamazepine showed inhibition at the rate of 0%, 100% and 80% of the clonic seizures, tonic seizures and death, respectively. And **8f** showed inhibition at the rates of 0%, 100% and 80%, respectively (Table 3). BIC is a competitive antagonist of $GABA_A$ receptor. BIC produces convulsions through its antagonism of $GABA_A$ receptor [24]. Compound **8f** can inhibit the seizures induced by BIC, which suggested that it exerts anticonvulsant activity at least partially through $GABA_A$-mediated mechanisms.

5. Conclusion

In conclusion, the results of this study demonstrated that 10-alkoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine derivatives have potent anticonvulsant activity. Especially, compound **8f** showed better anticonvulsant activity and higher safety than marketed drugs carbamazepine and phenytoin. In addition, compound **8f** demonstrated antagonistic activity against seizures induced by PTZ, isoniazid, 3-MP, thiosemicarbazide, and Bicuculline. These experiments suggested that compound **8f** exert anticonvulsant activity through GABA-mediated mechanisms, such as effecting GABAergic neurotransmission, activating GAD, inhibiting GABA-T or agonizing $GABA_A$ receptor in the brain.

6. Experimental protocols

6.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on 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). Elemental analyses were performed on a 204Q CHN (Perkin Elmer, USA). The major chemicals were purchased from Aldrich Chemical Corporation.

6.1.1. Synthesis of 6-methoxy-2,3-dihydrochromen-4-one oxime (**2**)

6-Methoxy-2,3-dihydrochromen-4-one (7.8 g, 44 mmol), pyridine (8 mL) and hydroxylamine chloride (9.17 g, 132 mmol) were placed into a round-bottomed flask containing 150 mL of methanol. The reaction mixture was stirred for 4 h at 70 °C. After removing most of the solvent, the mixture was added to 100 ml of ice-water and filtered to a white solid.

M.p. 119–121 °C, yield = 91.5%. ^{1}H NMR (DMSO-d_6, 300 MHz), δ2.79 (t, 2H, J = 6.1 Hz, N–CH_2), 3.73 (s, 3H, O–CH_3), 4.11 (t, 2H, J = 6.1 HZ, O–CH_2), 6.82–7.26 (m, 3H, Ar-H), 11.26 (s, 1H, N–OH). IR (KBr) cm^{-1}: 3345 (O–H), 1616 (C=N). MS m/z 194 (M + 1).

6.1.2. Synthesis of 7-methoxy-3,4-dihydrobenzo[f][1,4]oxazepin-5 (2H)-one (**3**)

Compound **2** (2.6 g, 13 mmol) and polyphosphoric acid (45 g) were placed into a three-necked round-bottomed flask. The mixture was heated and stirred with a blade stirrer at 90 °C for 4 h. A mass of water was added to the mixture after the temperature of the mixture was equal to room temperature, and then the aqueous layer was extracted by CH_2Cl_2 3 times. The combined organic layer was dried overnight with anhydrous magnesium sulfate and evaporated under reduced pressure to get a light yellow solid.

M.p. 90–92 °C, yield = 61.5%. ^{1}H NMR (CDCl3, 300 MHz), δ3.24 (q, 2H, J = 5.2 Hz, N–CH_2), 3.73 (s, 3H, O–CH_3), 4.17 (t, 2H, J = 5.0 Hz, O–CH_2), 6.96 (d, 1H, J = 8.8 Hz, Ar-H), 7.03 (dd, 1H, J_1 = 2.9 Hz, J_2 = 8.8 Hz, Ar-H), 7.16 (d, 1H, J_1 = 2.9 Hz, Ar-H). 8.34

(s, 1H, –NH–). IR (KBr) cm^{-1}: 3204 (NH), 1674 (C=O). MS (*m*/*z*): 194 (M + 1).

*6.1.3. Synthesis of 7-methoxy-3,4-dihydrobenzo[f][1,4]oxazepine-5 (2H)-thione (**4**)*

To a stirring mixture of acetonitrile and triethylamine in a three-necked round-bottomed flask in an ice bath, P_2S_5 (1.2 eq), divided into multiple portions, was added one portion at a time after the previous portion had completely dissolved. Then, 7-methoxy-3,4-dihydrobenzo[f][1,4]oxazepin-5(2*H*)-one compound 3 was added and the solution was refluxed for 6 h under nitrogen. After removing the solvent under reduced pressure, the residue was dissolved in dichloromethane (30 mL), washed with water (3 × 30 mL) and dried over anhydrous MgSO4. Evaporation of the solvents gave a crude product, which was purified by silica gel column chromatography with dichloromethane to give a light yellow solid (**4**).

M.p. 114–116 °C, yield = 62.5%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ3.51 (d, 2H, *J* = 5.8 Hz, N–CH_2), 3.85 (s, 3H, O–CH_3), 4.39 (t, 2H, *J* = 5.8 HZ, O–CH_2), 6.98 (dd, 1H, J_1 = 2.9 Hz, J_2 = 8.9 Hz, Ar-H), 7.02 (d, 1H, *J* = 8.9 Hz, Ar-H), 7.51 (d, 1H, *J* = 2.9 Hz, Ar-H), 9.53 (s, 1H, N–H). IR (KBr) cm^{-1}: 3145 (N–H), 1256 (C–N). MS *m*/*z* 210 (M + 1). *Anal*. Calcd. for $C_{10}H_{11}NO_2S$: C, 57.39; H, 5.30; N, 6.69; S, 15.32. Found: C, 57.48; H, 5.46; N, 6.49; S, 15.19.

*6.1.4. Synthesis of 1-(7-methoxy-3,4-dihydrobenzo[f][1,4]oxazepin-5 (2H)-ylidene) hydrazine (**5**)*

7-Methoxy-3,4-dihydrobenzo[f][1,4]oxazepine-5(2H)-thione (**4**) (0.79 g, 3.8 mmol) was dissolved in tetrahydrofuran in a round-bottomed flask and 0.95 g of anhydrous hydrazine (19 mmol) was added. After the mixture was refluxed for 8 h, the solvent and excess hydrazine were removed under reduced pressure to yield a yellow solid, which was used in the next step without refinement.

M.p. 149–150 °C, yield = 87.6%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ3.31 (t, 2H, *J* = 5.6 Hz, N–CH_2), 3.80 (s, 3H, O–CH_3), 4.14 (t, 2H, *J* = 5.6 HZ, O–CH_2), 6.88 (dd, 1H, J_1 = 3.0 Hz, J_2 = 8.7 Hz, Ar-H), 6.95 (d, 1H, *J* = 8.7 Hz, Ar-H), 7.12 (d, 1H, *J* = 3.0 Hz, Ar-H). IR (KBr) cm^{-1}: 3323 (N–H), 1641 (C=N). MS *m*/*z* 208 (M + 1). *Anal*. Calcd. for $C_{10}H_{13}N_3O_2$: C, 57.96; H, 6.32; N, 20.28. Found: C, 58.12; H, 6.39; N, 20.09.

*6.1.5. Synthesis of 10-methoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**6**)*

A mixture of compound **5** (0.79 g, 3.8 mmol) and formic acid (20 mL) was heated at 120 °C for 4 h. After removing the excess formic acid under reduced pressure, the residue was dissolved with CH_2Cl_2 and washed with water. The CH_2Cl_2 layer was dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product, which was purified by silica gel column chromatography (dichloromethane: methanol = 60:1) to a white solid **6**.

M.p. 152–153 °C, yield = 83.2%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ3.86 (s, 3H, O–CH_3), 4.44 (s, 4H, N–CH_2CH_2–O), 6.91 (dd, 1H, J_1 = 9.0 Hz, J_2 = 2.9 Hz, Ar-H), 6.98 (d, 1H, *J* = 9.0 Hz, Ar-H), 8.07 (d, 1H, *J* = 2.9 Hz, Ar-H), 8.20 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1615 (C=N), 1271 (C–N), 1132 (N–N). MS *m*/*z* 218 (M + 1). *Anal*. Calcd. for $C_{11}H_{11}N_3O_2$: C, 60.82; H, 5.10; N, 19.34. Found: C, 60.94; H, 5.24; N, 19.27.

*6.1.6. Synthesis of 5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepin-10-ol (**7**)*

10-Methoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**6**) (2.00 g, 9.22 mmol) was dissolved in dichloromethane (60 mL). BBr_3 (46.1 mmol) was added dropwise to the solution and the mixture was stirred at room temperature. After 4 h the mixture was added slowly 20 mL ice cold water and allowed to stir for half a hour. The resulting white precipitate was obtained by filtration.

M.p. 247–248 °C, yield = 90.4%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.53 (t, 2H, *J* = 3.6 Hz, N–CH_2), 4.71 (t, 2H, *J* = 3.6 Hz, O–CH_2), 6.99–7.01 (m, 2H, Ar-H), 7.63 (s, 1H, Ar-H), 8.04 (s, 1H, O–H), 9.76 (s, 1H, N=CH). IR (KBr) cm^{-1}: 3340 (O–H), 1628 (C=N), 1348 (C–N), 1121 (N–N). MS *m*/*z* 204 (M + 1). *Anal*. Calcd. for $C_{10}H_9N_3O_2$: C, 59.11; H, 4.46; N, 20.68. Found: C, 59.18; H, 4.54; N, 20.69.

*6.1.7. General procedure for the synthesis of 10-alkoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine derivatives (**8a–s**)*

K_2CO_3 (1.24 g, 9 mmol) and 5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepin-10-ol (**7**) (3 mmol) were dissolved in acetonitrile (50 ml) and refluxed for 30 min, then alkyl bromide or benzyl chloride derivatives (3.3 mmol) were added into the mixture accompanied with some of benzyltriethylamine chloride (TEBA). The reaction mixture was heated at reflux temperature for 4–24 h then poured into 100 ml of water. Aqueous layer was extracted with dichloromethane (30 ml × 3). The combined layer of dichloromethane was dried by anhydrous $MgSO_4$. The evaporation of the solvent gave a crude product, which was purified by silica gel column chromatography with CH_2Cl_2–CH_3OH (40:1) to a white solid. The yield, melting point and spectral data of each compound were given below.

*6.1.7.1. 10-Ethoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8a**).* M.p. 134–135 °C, yield = 74.5%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ1.42 (t, 3H, *J* = 6.9 Hz, CH_3), 4.10 (q, 2H, *J* = 6.9 Hz, O–CH_2), 4.43 (s, 4H, N–CH_2CH_2–O), 6.92 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.6 Hz, Ar-H), 6.98 (d, 1H, *J* = 8.9 Hz, Ar-H), 8.08 (d, 1H, *J* = 2.6 Hz, Ar-H), 8.21 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1619 (C=N), 1276 (C–N), 1138 (N–N). MS *m*/*z* 232 (M + 1). *Anal*. Calcd. for $C_{12}H_{13}N_3O_2$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.47; H, 5.81; N, 18.01.

*6.1.7.2. 10-Propoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8b**).* M.p. 99–100 °C, yield = 79.1%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ1.04 (t, 3H, *J* = 7.4 Hz, CH_3), 1.75–1.87 (m, 2H, CH_2), 3.98 (t, 2H, *J* = 6.6 Hz, O–CH_2), 4.43 (s, 4H, N–CH_2CH_2–O), 6.92 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.8 Hz, Ar-H), 6.97 (d, 1H, *J* = 8.9 Hz, Ar-H), 8.07 (d, 1H, *J* = 2.8 Hz, Ar-H), 8.19 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1616 (C=N), 1271 (C–N), 1132 (N–N). MS *m*/*z* 246 (M + 1). *Anal*. Calcd. for $C_{13}H_{15}N_3O_2$: C, 63.66; H, 6.16; N, 17.13. Found: C, 63.82; H, 6.25; N, 17.07.

*6.1.7.3. 10-Butoxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8c**).* M.p. 109–110 °C, yield = 82.8%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ0.98 (t, 3H, *J* = 7.3 Hz, CH_3), 1.44–1.54 (m, 2H, CH_2), 1.71–1.81 (m, 2H, CH_2), 4.02 (t, 2H, *J* = 6.5 Hz, O–CH_2), 4.43 (s, 4H, N–CH_2CH_2–O), 6.92 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.8 Hz, Ar-H), 6.98 (d, 1H, *J* = 8.9 Hz, Ar-H), 8.07 (d, 1H, *J* = 2.8 Hz, Ar-H), 8.21 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1610 (C=N), 1266 (C–N), 1127 (N–N). MS *m*/*z* 260 (M + 1). *Anal*. Calcd. for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.20. Found: C, 64.99; H, 6.68; N, 16.30.

*6.1.7.4. 10-Pentyloxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8d**).* M.p. 85–86 °C, yield = 83.3%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ0.93 (t, 3H, *J* = 6.9 Hz, CH_3), 1.38–1.46 (m, 4H, $(-CH_2-)_2$), 1.73–1.82 (m, 2H, CH_2), 4.02 (t, 2H, *J* = 6.5 Hz, O–CH_2), 4.43 (s, 4H, N–CH_2CH_2–O), 6.92 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.7 Hz, Ar-H), 6.98 (d, 1H, *J* = 8.9 Hz, Ar-H), 8.07 (d, 1H, *J* = 2.7 Hz, Ar-H), 8.20 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1626 (C=N), 1281 (C–N), 1123 (N–N). MS *m*/*z* 274 (M + 1). *Anal*. Calcd. for $C_{15}H_{19}N_3O_2$: C, 65.91; H, 7.01; N, 15.37. Found: C, 66.13; H, 7.16; N, 15.19.

*6.1.7.5. 10-Hexyloxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8e**).* M.p. 105–106 °C, yield = 80.7%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ0.91 (t, 3H, *J* = 6.9 Hz, CH_3), 1.33–1.46 (m, 6H, $(-CH_2-)_3$), 1.73–1.81 (m, 2H, CH_2), 4.02 (t, 2H, *J* = 6.6 Hz, O–CH_2), 4.43 (s, 4H, N–CH_2CH_2–O), 6.92 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.8 Hz,

Ar-H), 6.98 (d, 1H, J = 8.9 Hz, Ar-H), 8.08 (d, 1H, J = 2.8 Hz, Ar-H), 8.21 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1631 (C=N), 1284 (C–N), 1128 (N–N). MS m/z 288 (M + 1). *Anal*. Calcd. for $C_{16}H_{21}N_3O_2$: C, 66.88; H, 7.37; N, 14.62. Found: C, 66.98; H, 7.54; N, 14.41.

*6.1.7.6. 10-Heptyloxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8f**).* M.p. 100–101 °C, yield = 71.1%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ0.91 (t, 3H, J = 6.8 Hz, CH_3), 1.32–1.47 (m, 8H, $(-CH_2-)_4$), 1.75–1.82 (m, 2H, CH_2), 4.03 (t, 2H, J = 6.6 Hz, O–CH_2), 4.44 (s, 4H, N–CH_2CH_2–O), 6.93 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.8 Hz, Ar-H), 6.99 (d, 1H, J = 8.9 Hz, Ar-H), 8.09 (d, 1H, J = 2.8 Hz, Ar-H), 8.22 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1633 (C=N), 1287 (C–N), 1119 (N–N). MS m/z 302 (M + 1). *Anal*. Calcd. for $C_{17}H_{23}N_3O_2$: C, 67.75; H, 7.69; N, 13.94. Found: C, 67.91; H, 7.77; N, 13.78.

*6.1.7.7. 10-Octyloxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8g**).* M.p. 106–107 °C, yield = 73.6%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ0.88 (t, 3H, J = 6.6 Hz, CH_3), 1.28–1.44 (m, 10H, $(-CH_2-)_5$), 1.72–1.79 (m, 2H, CH_2), 3.99 (t, 2H, J = 6.5 Hz, O–CH_2), 4.42 (s, 4H, N–CH_2CH_2–O), 6.91 (dd, 1H, J_1 = 8.9 Hz, J_2 = 2.3 Hz, Ar-H), 6.95 (d, 1H, J = 8.9 Hz, Ar-H), 8.09 (d, 1H, J = 2.3 Hz, Ar-H), 8.21 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1639 (C=N), 1291 (C–N), 1135 (N–N). MS m/z 316 (M + 1). *Anal*. Calcd. for $C_{18}H_{25}N_3O_2$: C, 68.54; H, 7.99; N, 13.32. Found: C, 68.72; H, 7.87; N, 13.42.

*6.1.7.8. 10-Benzyloxy-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8h**).* M.p. 149–151 °C, yield = 88.9%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.39 (s, 4H, N–CH_2CH_2–O), 5.09 (s, 2H, O–CH_2), 6.94–6.97 (m, 2H, Ar-H), 7.31–7.46 (m, 5H, Ar-H), 8.17 (s, 1H, Ar-H), 8.17 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1601 (C=N), 1252 (C–N), 1135 (N–N). MS m/z 294 (M + 1). *Anal*. Calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.77; H, 5.28; N, 14.13.

*6.1.7.9. 10-(2-Fluorobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8i**).* M.p. 140–142 °C, yield = 57.4%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.44 (s, 4H, N–CH_2CH_2–O), 5.18 (s, 2H, O–CH_2), 6.98–7.01 (m, 2H, Ar-H), 7.06–7.56 (m, 4H, Ar-H), 8.21 (s, 1H, Ar-H), 8.24 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1605 (C=N), 1266 (C–N), 1141 (N–N). MS m/z 312 (M + 1). *Anal*. Calcd. for $C_{17}H_{14}FN_3O_2$: C, 65.59; H, 4.53; N, 13.50. Found: C, 65.71; H, 4.64; N, 13.29.

*6.1.7.10. 10-(3-Fluorobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8j**).* M.p. 128–130 °C, yield = 72.5%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.42 (s, 4H, N–CH_2CH_2–O), 5.10 (s, 2H, O–CH_2), 6.97–7.00 (m, 2H, Ar-H), 7.00–7.38 (m, 4H, Ar-H), 8.16 (s, 1H, Ar-H), 8.20 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1609 (C=N), 1271 (C–N), 1137 (N–N). MS m/z 312 (M + 1). *Anal*. Calcd. for $C_{17}H_{14}FN_3O_2$: C, 65.59; H, 4.53; N, 13.50. Found: C, 65.65; H, 4.61; N, 13.33.

*6.1.7.11. 10-(4-Fluorobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8k**).* M.p. 178–179 °C, yield = 72.4%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.43 (s, 4H, N–CH_2CH_2–O), 5.07 (s, 2H, O–CH_2), 6.97–6.99 (m, 2H, Ar-H), 7.07 (dd, 2H, J_1 = 8.6 Hz, J_2 = 8.5 Hz, Ar-H), 7.43 (dd, 2H, J_1 = 8.6 Hz, J_2 = 5.6 Hz, Ar-H), 8.18 (s, 1H, Ar-H), 8.21 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1607 (C=N), 1260 (C–N), 1155 (N–N). MS m/z 312 (M + 1). *Anal*. Calcd. for $C_{17}H_{14}FN_3O_2$: C, 65.59; H, 4.53; N, 13.50. Found: C, 65.70; H, 4.67; N, 13.38.

*6.1.7.12. 10-(2-Chlorobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8l**).* M.p. 131–132 °C, yield = 62.2%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.43 (s, 4H, N–CH_2CH_2–O), 5.19 (s, 2H, O–CH_2), 6.97–7.00 (m, 2H, Ar-H), 7.28–7.68 (m, 4H, Ar-H), 8.22 (s, 1H, Ar-H), 8.22 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1611 (C=N), 1271 (C–N), 1142 (N–N). MS m/z 328 (M+1). *Anal*. Calcd. for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.44; H, 4.25; N, 12.89.

*6.1.7.13. 10-(3-Chlorobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8m**).* M.p. 138–140 °C, yield = 61.2%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.44 (s, 4H, N–CH_2CH_2–O), 5.10 (s, 2H, O–CH_2), 6.99–7.02 (m, 2H, Ar-H), 7.31–7.46 (m, 4H, Ar-H), 8.19 (s, 1H, Ar-H), 8.21 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1618 (C=N), 1277 (C–N), 1135 (N–N). MS m/z 328 (M + 1). *Anal*. Calcd. for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.49; H, 4.20; N, 12.92.

*6.1.7.14. 10-(4-Chlorobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8n**).* M.p. 183–184 °C, yield = 66.5%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.44 (s, 4H, N–CH_2CH_2–O), 5.10 (s, 2H, O–CH_2), 6.97–7.01 (m, 2H, Ar-H), 7.36 (d, 2H, J = 8.6 Hz, Ar-H), 7.40 (d, 2H, J = 8.6 Hz, Ar-H), 8.20 (s, 1H, Ar-H), 8.21 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1623 (C=N), 1270 (C–N), 1165 (N–N). MS m/z 328 (M + 1). *Anal*. Calcd. for $C_{17}H_{14}ClN_3O_2$: C, 62.30; H, 4.31; N, 12.82. Found: C, 62.41; H, 4.46; N, 12.86.

*6.1.7.15. 10-(2-Bromobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8o**).* M.p. 142–144 °C, yield = 68.9%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.44 (s, 4H, N–CH_2CH_2–O), 5.17 (s, 2H, O–CH_2), 6.98–7.02 (m, 2H, Ar-H), 7.17–7.60 (m, 4H, Ar-H), 8.21 (s, 1H, Ar-H), 8.24 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1622 (C=N), 1264 (C–N), 1161 (N–N). MS m/z 372 (M + 1). *Anal*. Calcd. for $C_{17}H_{14}BrN_3O_2$: C, 54.86; H, 3.79; N, 11.29. Found: C, 54.97; H, 3.85; N, 11.16.

*6.1.7.16. 10-(3-Bromobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8p**).* M.p. 141–142 °C, yield = 73.3%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.43 (s, 4H, N–CH_2CH_2–O), 5.08 (s, 2H, O–CH_2), 6.98–7.02 (m, 2H, Ar-H), 7.23–7.62 (m, 4H, Ar-H), 8.17 (s, 1H, Ar-H), 8.20 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1616 (C=N), 1266 (C–N), 1147 (N–N). MS m/z 372 (M + 1). *Anal*. Calcd. for $C_{17}H_{14}BrN_3O_2$: C, 54.86; H, 3.79; N, 11.29. Found: C, 55.03; H, 3.90; N, 11.21.

*6.1.7.17. 10-(4-Bromobenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8q**).* M.p. 187–188 °C, yield = 75.5%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ4.43 (s, 4H, N–CH_2CH_2–O), 5.06 (s, 2H, O-CH_2), 6.97–7.01 (m, 2H, Ar-H), 7.33 (d, 2H, J = 8.2 Hz, Ar-H), 7.51 (d, 2H, J = 8.2 Hz, Ar-H), 8.17 (s, 1H, Ar-H), 8.19 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1609 (C=N), 1264 (C–N), 1149 (N–N). MS m/z 372 (M + 1). *Anal*. Calcd. for C17H14BrN3O2: C, 54.86; H, 3.79; N, 11.29. Found: C, 54.93; H, 3.72; N, 11.19.

*6.1.7.18. 10-(4-Methylbenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8r**).* M.p. 164–166 °C, yield = 44.5%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ2.36 (s, 3H, Ar-CH_3), 4.42 (s, 4H, N–CH_2CH_2–O), 5.08 (s, 2H, O-CH_2), 6.96–7.01 (m, 2H, Ar-H), 7.20 (d, 2H, J = 7.8 Hz, Ar-H), 7.34 (d, 2H, J = 7.8 Hz, Ar-H), 8.20 (s, 1H, Ar-H), 8.20 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1607 (C=N), 1263 (C–N), 1152 (N–N). MS m/z 308 (M + 1). *Anal*. Calcd. for $C_{18}H_{17}N_3O_2$: C, 70.34; H, 5.58; N, 13.67. Found: C, 70.47; H, 5.66; N, 13.49.

*6.1.7.19. 10-(4-Methoxybenzyloxy)-5, 6-dihydro-triazolo[4,3-d]benzo[f][1,4]oxazepine (**8s**).* M.p. 140–141 °C, yield = 58.9%. ^{1}H NMR ($CDCl_3$, 300 MHz), δ3.82 (s, 3H, O–CH_3), 4.43 (s, 4H, N–CH_2CH_2–O), 5.05 (s, 2H, O–CH_2), 6.97–7.01 (m, 2H, Ar-H), 6.92 (d, 2H, J = 8.3 Hz, Ar-H), 7.39 (d, 2H, J = 8.3 Hz, Ar-H), 8.21 (s, 1H, Ar-H), 8.21 (s, 1H, N=CH). IR (KBr) cm^{-1}: 1614 (C=N), 1273 (C–N), 1139 (N–N). MS m/z 224 (M + 1). *Anal*. Calcd. for $C_{18}H_{17}N_3O_3$: C, 66.86; H, 5.30; N, 13.00. Found: C, 66.98; H, 5.41; N, 12.86.

6.2. Pharmacology

6.2.1. Anticonvulsant effects in the maximal electroshock seizure (MES) test [25,26]

The MES test was carried out by the methods described in the ADD of the National Institutes of Health (USA) [16,17]. Seizures

were elicited with a 60 Hz alternating current of 50 mA intensity in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. At 30 min 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, 100, and 300 mg/kg for evaluating the preliminary anticonvulsant activity. For determination of the median effective dose (ED50) 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.

6.2.2. Neurotoxicity screening (NT) [16,17]

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.

6.2.3. sc-PTZ-induced seizures [16,17]

At 30 min after the administration of the test compound, 85 mg/kg PTZ dissolved in saline administered sc. The animals (10 mice in one group) placed in individual cages and observed for 30 min. The number of clonic and tonic seizures as well as the number of deaths were noted.

6.2.4. Isoniazid-induced seizures test [27]

At 30 min after the administration of the test compound, the animals (10 mice in one group) were given in i.p. at dose of ISO (250 mg/kg), a dose at which 100% of the animals showed convulsive reactions. The mice were placed in individual cages and observed for 1 h. The number of clonic and tonic seizures as well as the number of deaths were noted.

6.2.5. 3-MP induced seizures test [28]

At 30 min after the administration of the test compound, 60 mg/kg of 3-MP in saline solution was injected sc to mice (10 mice in one group). The mice were placed in individual cages and observed for 0.5 h. The number of clonic and tonic seizures as well as the number of deaths were noted.

6.2.6. Thiosemicarbazide-induced seizures test [29]

At 30 min after the administration of the test compound, the animals (10 mice in one 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 recorded.

6.2.7. Bicuculline-induced seizures test [27]

At 30 min after the administration of compounds, the animals (10 mice in one group) were given a subcutaneous dose of 2.7 mg/kg for Bicuculline (within 15–45 min after preparation due to instability). Individual mice were then placed in isolation cages and observed for at least 30 min for the presence or absence of clonic seizures, and tonic seizures, and lethality was also recorded.

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