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Design, synthesis of 8-alkoxy-5,6-dihydro-[1,2,4]triazino[4,3-*a*]quinolin-1-ones with anticonvulsant activity

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ABSTRACT

A new series of 8-alkoxy-5,6-dihydro-[1,2,4]triazino[4,3-*a*]quinolin-1-one derivatives were synthesized. Their anticonvulsant activities were evaluated by the maximal electroshock (MES) test, and their neurotoxicities were evaluated by the rotarod neurotoxicity test. The results showed that 8-heptyloxy-5,6-dihydro-[1,2,4]triazino[4,3-*a*]quinolin-1-one **5t** was the most potent with median effective dose (ED_{50}) value of 11.4 mg/kg, median toxicity dose (TD_{50}) of 114.1 mg/kg, providing a protective index ($PI = TD_{50}/ED_{50}$) value of 10.0, which is much greater than the PI of the prototype drug carbamazepine (PI = 6.4). To explain the possible mechanism of anticonvulsant activity, the compound **5t** was tested in chemically induced seizures.

1. Introduction

Epilepsy, a ubiquitous disease characterized by recurrent seizures, inflicts more than 60 million people worldwide according to epidemiological studies [1]. 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 conditions [5]. Research to find more effective and safer antiepileptic drugs is, therefore, imperative and challenging in medicinal chemistry.

In our previous work [6], a series of derivatives of 6-alkoxy-3,4-dihydro-2(1*H*)-quinoline were first found to have anticonvulsant activities, among which 6-benzyloxy-3,4-dihydro-2(1*H*)-quinoline (compound **I**) showed the strongest activity with an ED_{50} value of 29.6 mg/kg in the MES test Fig. 1. Introduction of triazole ring to the first and second positions of this compound **I** caused a remarkable increase in the anticonvulsant activity, as seen in 7-benzyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline (compound **II**), which showed ED_{50} values of 17.3 mg/kg and 24 mg/kg in the MES and the sc PTZ tests, respectively [7].

Aimed at exploring effective compounds with better anticonvulsant activity and lower neurotoxicity, compounds **5a**–**5v**, **6a** and **6s** were designed and synthesized, which substituted by triazone the triazole in compound **II**. The hypothesis was that triazone compound may have higher affinity to the receptor due to the carbonyl group, and thus may increase the anticonvulsant activity. As shown in the preliminary work [8], the introduction of alkoxy to the 7th position of 1,2,4-triazole quinoline remarkably increased their anticonvulsant activity. So, we designed and synthesized a series of 8-alkoxy-5,6-dihydro-[1,2,4]triazino[4,3-*a*]quinolin-1-one derivatives, to discuss the influence of the 8-alkoxy to anticonvulsant activity; the structure–activity relationship was also discussed in this paper. Their structures were characterized using IR, ^{1}H NMR, MS, and elemental analysis techniques. In additional, their anticonvulsant activity was evaluated using MES test and reported for the first time. Their neurotoxicity was evaluated using the rotarod test in mice. For explaining the possible mechanism of action, the compound **5t** was tested in pentylenetetrazole (PTZ), isoniazid, thiosemicarbazide, 3-mercaptopropionic acid (3-MP) and strychnine induced test. The anti-MES activity and the neurotoxicity of the

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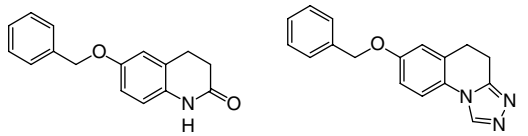


compound **I** compound **II**

Fig. 1. Previous active quinolines reported by Quan et al. **I**: 6-(benzyloxy)-3,4-dihydroquinolin-2(1*H*)-one; **II**: 7-benzyloxyl-4,5-dihydro-[1,2,4]triazolo[4,3-*a*]quinoline.

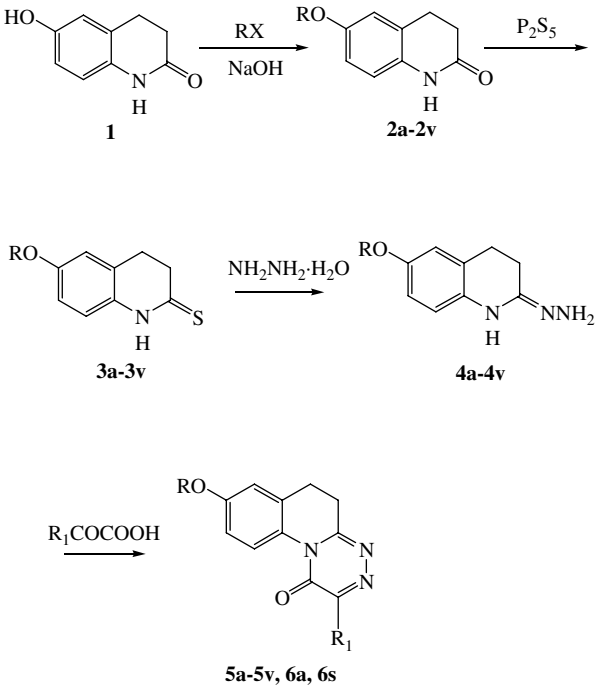
marketed agent carbamazepine were evaluated in our laboratory under the same conditions for the purpose of comparison.

2. Results and discussions

2.1. Chemistry

Compounds were prepared according to Scheme 1. The starting material 6-hydroxy-3,4-dihydro-2(1*H*)-quinolone reacted with an appropriate amount of alkyl halide in a solution of sodium hydroxide in DMF and yielded 6-alkoxy-3,4-dihydroquinolin-2(1*H*)-one **2a**–**2v**. 6-Alkoxy-3,4-dihydroquinolin-2(1*H*)-thiones **3a**–**3d** and **3j**–**3v** were prepared by the reaction of compounds **2a**–**2d** and **2j**–**2v** with phosphorous pentasulfide in acetonitrile in the presence of triethylamine. Since compounds **2e**–**2i**, with Cl atom substituted at 8-alkoxy group, did not dissolve in acetonitrile, toluene was used as solvent and compounds **2e**–**2i** and phosphorous pentasulfide reacted in toluene with stirring and refluxing, which produced derivatives **3e**–**3i** with a moderate yield.

Compounds **3a**–**3v** reacted further with hydrazine hydrate in THF to afford 1-(6-alkoxy-3,4-dihydroquinolin-2(1*H*)-ylidene)hydrazines **4a**–**4v**. Briefly, to a solution of hydrazine hydrate in THF, a solution of compounds **3a**–**3v** in THF was added dropwise at room temperature, and the mixture was stirred at 60 °C for 1 h. Then, half of the solvent was removed under reduced pressure, and the mixture was poured into petroleum ether. The precipitate was filtered and washed with petroleum ether, and then kept below 0 °C. The compounds obtained were pure enough for the next step. The structures of compounds **4a**–**4v** may change gradually at room temperature in about 10 h, and the molecular weight indicated the disubstitution of hydrazine with compounds **3a**–**3v**, as shown in Scheme 2. But this change could be avoided by keeping compounds **4a**–**4v** below 0 °C.



Scheme 1. The synthesis route of target compounds **5a**–**5v**, **6a** and **6s**.

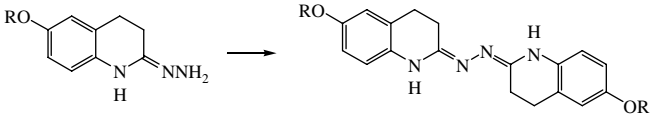
Compounds **4a**–**4v** reacted further with glyoxylic acid monohydrate in ethanol at room temperature, then evaporated the solvent under reduced pressure, the residue continued to react in refluxing toluene for about 2 h to obtain target compounds **5a**–**5v**. Compounds **4a** and **4s** reacted further with pyruvic acid in ethanol at room temperature, then evaporated the solvent under reduced pressure, the residue continued to react in refluxing acetic acid for about 2 h to obtain target compounds 8-(benzyloxy)-2-methyl-5,6-dihydro-[1,2,4]triazino[4,3-*a*]quinolin-1-one **6a** and 8-hexyloxy-2-methyl-5,6-dihydro-[1,2,4]triazino[4,3-*a*]quinolin-1-one **6s**, respectively [9].

2.2. Pharmacology

The results of pharmacology test of all synthesized compounds and reference drug are shown in Table 1. As shown in Table 1, most of the compounds showed remarkable anticonvulsant activity. But 1-methyl derivatives **6a** and **6s** did not exhibit anticonvulsant activity at dosages up to 100 mg/kg. The result illustrated that 1-methyl may block the combination of 1,2,4-triazinone ring and receptor.

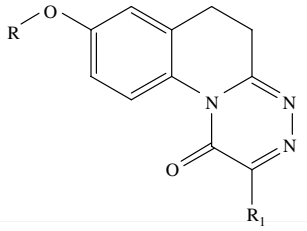
Analyzing the activities of 14 phenyl-substituted derivatives **5a**–**5n**, the following structure–activity relationship was obtained. Compared the activities of these halogen atom substituted derivatives; it was found that the Cl atom gave more contribution to the anticonvulsant activity than the Br, F atom on the whole level. And the position of halogen atom on the phenyl ring greatly influenced the anticonvulsant activity, the activity order is $o > m > p$. The *o*-Cl derivative **5g** exhibited the most potent activity with ED_{50} value of 13.7 mg/kg and TD_{50} value of 105.6 mg/kg, providing a PI value of 7.7. Although the *o*-Br derivative **5j** exhibited weaker activity than **5g,** with ED_{50} value of 13.7 mg/kg, it possessed lower neurotoxicity and a safer PI value was gained. The derivatives **5h** and **5i**, containing double Cl atom, did not exhibited better anticonvulsant activity than derivatives with single Cl atom. The compounds **5h** and **5i**, which were 2,4-Cl_2 and 2,6-Cl_2 substituted-benzyloxyl at the eighth position, respectively, had corresponding ED_{50} values in the MES test of 26.4 mg/kg and 20.5 mg/kg. Compared the influence of electric-donor group to anticonvulsant activity, their contribution order is *p*-CH_3 > 3,4-$(OCH_3)_2$– > *p*-OCH_3. Three electric-donor derivatives **5l**–**5n** showed weaker anticonvulsant activity ranging from 27.4 mg/kg to 56.8 mg/kg than non-substituted derivative **5a** with ED_{50} value of 22.8 mg/kg.

The length of the alkyl chain appeared to have a direct impact on anticonvulsant activity of the 8-alkoxy-5,6-dihydro-[1,2,4]triazino[4,3-*a*]quinolin-1-one derivatives. From compounds **5m**–**5v**, as alkyl chain length increased, ED_{50} gradually increased with the compound **5t** (with the *n*-heptyl substituted group) being the most active. The trend reversed, however, when the alkyl chain had more than seven carbon atoms. Compound **5t**, with ED_{50} value of 11.4 mg/kg, was comparable to reference agent carbamazepine in anti-MES activity, but it possessed weaker neurotoxicity with TD_{50} value of 114.1 mg/kg, so a safer PI value of 10.00 was



Scheme 2. Structural change of compounds **4a**–**4v**.

Table 1
Quantitative anticonvulsant activity in MES test in mice (test drug administered ip)



Comp.	R	R_1	ED_{50} (mg/kg)	TD_{50}[c] (mg/kg)	PI
5a	$-C_6H_5$	H	22.8 (15.3–28.4)[a]	–[b]	–
5b	$-C_6H_4$(*p*-F)	H	38.0 (27.9–51.8)	–	–
5c	$-C_6H_4$(*m*-F)	H	31.7 (23.3–43.2)	–	–
5d	$-C_6H_4$(*o*-F)	H	23.7 (17.1–32.9)	–	–
5e	$-C_6H_4$(*p*-Cl)	H	32.9 (24.1–44.8)	–	–
5f	$-C_6H_4$(*m*-Cl)	H	22.8 (16.7–31.1)	–	–
5g	$-C_6H_4$(*o*-Cl)	H	13.7 (10.1–18.7)	105.6 (79.3–140.5)	7.7
5h	$-C_6H_3$(2,4-Cl_2)	H	26.4 (19.6–34.7)	–	–
5i	$-C_6H_3$(2,6-Cl_2)	H	20.5 (16.5–25.4)	–	–
5j	$-C_6H_4$(*o*-Br)	H	16.4 (12.0–22.3)	132.0 (99.2–175.7)	8.0
5k	$-C_6H_4$(*p*-Br)	H	64.2 (47.1–87.5)	–	–
5l	$-C_6H_4$(*p*-OCH_3)	H	56.8 (40.9–78.8)	–	–
5m	$-C_6H_4$(*p*-CH_3)	H	27.4 (20.1–37.4)	–	–
5n	$-C_6H_3$(3,4-$(OCH_3)_2$)	H	45.6 (33.5–62.1)	–	–
5o	*n*-C_2H_5	H	39.4 (28.9–53.6)	–	–
5p	*n*-C_3H_7	H	32.9 (24.1–44.8)	–	–
5q	*n*-C_4H_9	H	22.7 (16.4–31.5)	–	–
5r	*n*-C_5H_{11}	H	21.9 (16.1–29.8)	–	–
5s	*n*-C_6H_{13}	H	19.7 (14.5–26.8)	82.2 (60.3–112.0)	4.2
5t	*n*-C_7H_{15}	H	11.4 (8.4–15.5)	114.1 (83.7–155.5)	10.0
5u	*n*-C_8H_{17}	H	12.7 (9.3–17.3)	98.6 (72.4–134.4)	7.76
5v	*n*-$C_{12}H_{25}$	H	54.8 (40.2–74.7)	–	
6a	$-C_6H_5$	$-CH_3$	>100	–	–
6s	*n*-C_6H_{13}	$-CH_3$	>100	–	–
Carbamazepine	–	–	11.8 (8.5–16.4)	76.1(55.8–103.7)	6.4

[a] The 95% confidence limits.
[b] Not tested.
[c] Taking consideration of their anti-MES activity, only compounds with an ED_{50} value lower than 20 mg/kg in the MES test have been selected to neurotoxicity test.

obtained. Compound **5t**, could be considered as a potentially useful and safe therapeutic. And the anticonvulsant activity decreased obviously when alkyl chain number lengthened to 12, even showed effective anticonvulsant activity at dosages up to 54.8 mg/kg.

As a result of preliminary test, compound **5t** exhibited the strongest anti-MES activity and the lowest neurotoxicity, it was then selected for further investigations against seizures induced by pentylenetetrazole, 3-MP, thiosemicarbazide, isoniazid and strychnine to prove its anticonvulsant activity and speculate about the possible mechanism of anticonvulsant action. As shown in Table 2, compound **5t** was effective against the seizures induce by pentylenetetrazole, isoniazid, 3-mercaptopropionic acid and thiosemicarbazide, and the potency of compound **5t** was stronger than carbamazepine in these chemically induced seizures.

Pentylenetetrazole and isoniazid have been reported to produce seizures by inhibiting gamma-aminobutyric acid (GABA) neurotransmission [10]. GABA is the main inhibitory neurotransmitter substance in the brain, and is widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures, while enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study tend to suggest that the derivatives in this study might have inhibited or attenuated pentylenetetrazole-induced and isoniazid-induced seizures in mice by enhancing GABAergic neurotransmission.

3-Mercaptopropionic acid and thiosemicarbazide were seen as the competitive inhibitors of GABA synthesis; enzyme glutamate decarboxylase (GAD) inhibited the synthesis of GABA to decrease the GABA level in the brain [11]. The moderate antagonism of 3-mercaptopropionic acid-induced and thiosemicarbazide-induced seizures suggests that this series of compounds might activate GAD or inhibit (GABA)-α-oxoglutarate aminotransferase (GABA-T) in the brain.

As shown in Table 2, compound **5t** failed to protect animals from seizure induced by strychnine. It is known that strychnine directly antagonizes the inhibitory spinal reflexes of glycine [12], so the result suggested that compounds could not influence glycine system.

In conclusion, in the present study, we found that 8-heptyloxy-5,6-dihydro-[1,2,4]triazino[4,3-*a*]quinolin-1-one (**5t**) possessed comparable anticonvulsant activity to marketed drug carbamazepine, with ED_{50} value of 11.4 mg/kg, but a safer PI value of 10.0 was

Table 2
Anticonvulsant activity of compound **5t** in chemically induced seizures tests

Comp.	sc PTZ	ip Isoniazid	ip Thiosemicarbazide	sc 3-MP	ip Strychnine
5t	11.3 (8.7–14.5)	19.7 (14.5–26.8)	15.8 (11.6–21.5)	11.8 (8.5–16.4)	–[a]
Carbamazepine	11.4 (8.4–15.5)	22.8 (16.7–31.1)	19.7 (14.5–26.8)	13.7 (10.0–18.7)	–

[a] Compound failed to control the seizure induced by strychnine.

obtained. Especially, compound **5t** produced significant antagonism activity against seizures induced by pentylenetetrazole, 3-mercaptopropionic acid, thiosemicarbazide and isoniazid, suggested that the compound **5t** might have effects on GABAergic neurotransmission and activate glutamate decarboxylase (GAD) or inhibit (GABA)-α-oxoglutarate aminotransferase (GABA-T) in the brain.

3. Experimental procedures

3.1. Chemistry

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on an FTIR1730. ^{1}H 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 an HP1100LC (Agilent Technologies, USA). Elemental analyses were performed on a 204Q CHN (Perkin–Elmer, USA). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer. The major chemicals were purchased from Aldrich Chemical Corporation. All other chemicals were of analytical grade.

3.1.1. 6-Alkoxy-3,4-dihydro-2(1H)-quinolones (**2a**–**2v**)

The starting compound **1** (10 mmol) and appropriate alkyl halide (10 mmol) were added to a solution of sodium hydroxide in DMF with stirring and refluxing for 3 h. The reaction mixture was cooled and then poured into ice water. The white precipitate was collected through filtration and dried in a vacuum to produce the crude products **2a**–**2v** with a moderate yield and sufficient purity for the next stage.

3.1.2. 6-Alkoxy-3,4-dihydro-1H-quinoline-2-thiones (**3a**–**3v**)

To a stirring mixture of acetonitrile and triethylamine in a three-necked round-bottomed flask in an ice bath, P_2S_5 (1.2 equiv), divided into multiple portions, was added one portion at a time after the previous portion had completely dissolved. Then, compounds **2a**–**2d** and **2j**–**2v** were added and the solution was refluxed for 3 h under nitrogen. After cooling, the light yellow precipitate was collected through filtration and dried in a vacuum to produce the crude products **3a**–**3d** and **3j**–**3v**; compounds **2e**–**2i** and phosphorous pentasulfide reacted in toluene with stirring and refluxing for about 2 h. The solvent was evaporated under reduced pressure; the residue was washed with water to produce a light yellow solid **3e**–**3i**.

3.1.3. 6-Alkoxy-3,4-dihydro-2-hydrazine-1H-quinolines (**4a**–**4v**)

A solution of compounds **3a**–**3v** (5 mmol) in 30 mL THF was added dropwise to a solution of hydrazine hydrate (25 mmol) in 20 mL THF at room temperature, then the mixture was stirred and heated at 60 °C for 1 h. After the reaction, half of the solvent was evaporated under reduced pressure, then the solution was poured to petroleum ether, the yellow precipitate was filtered and then kept at 0 °C for the next step.

3.2. General procedure for the synthesis of 6-alkoxy-[1,2,4]triazolo[3,4-a]phthalazine (**5a**–**5v**, **6a** and **6s**)

A solution of compounds **4a**–**4v** (4 mmol) and glyoxylic acid monohydrate (4 mmol) in 30 mL of ethanol was stirred for 2 h at room temperature. The mixture was evaporated under reduced pressure. The acid intermediate was then reacted in refluxing toluene for 2–3 h. The solution was evaporated to dryness, and the oily residue was filtered on a silica gel chromatographic column (ethyl acetate:methanol, 15:1) to give a white or light yellow solid. Compounds **4a** and **4s** (4 mmol) reacted with pyruvic acid in ethanol at room temperature, then evaporated the solvent under reduced pressure, the acid intermediate continued to react in refluxing acetic acid for about 2 h to obtain targets **6a** and **6s**, respectively.

3.2.1. 8-Benzyloxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5a**)

M.p. 167–169 °C, yield = 58.6%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.02–3.25 (m, 4H, H-5, H-6), 5.10 (s, 2H, –OCH_2–), 6.94–7.43 (m, 8H, Ar-H), 8.44 (s, 1H, H-2). IR (KBr) cm^{-1}: 1702 (C═O). MS *m*/*z* 304 (M + 1). Anal. Calcd. for $C_{18}H_{15}N_3O_2$: C 70.81, H 4.95, N 13.76. Found: C 70.92, H 4.97, N 13.73.

3.2.2. 8-(4-Fluorobenzyloxy)-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5b**)

M.p. 126–128 °C, yield = 63.7%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.01–3.23 (m, 4H, H-5, H-6), 5.06 (s, 2H, –OCH_2–), 6.93–7.44 (m, 7H, Ar-H), 8.57 (s, 1H, H-2). IR (KBr) cm^{-1}: 1698 (C═O). MS *m*/*z* 324 (M + 1). Anal. Calcd. for $C_{18}H_{14}FN_3O_2$: C 66.87, H 4.36, N 13.00. Found: C 66.90, H 4.37, N 13.02.

3.2.3. 8-(3-Fluorobenzyloxy)-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5c**)

M.p. 121–123 °C, yield = 60.3%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.01–3.24 (m, 4H, H-5, H-6), 5.10 (s, 2H, –OCH_2–), 6.92–7.39 (m, 7H, Ar-H), 8.58 (s, 1H, H-2). IR (KBr) cm^{-1}: 1704 (C═O). MS *m*/*z* 324 (M + 1). Anal. Calcd. for $C_{18}H_{14}FN_3O_2$: C 66.87, H 4.36, N 13.00. Found: C 66.91, H 4.37, N 13.01.

3.2.4. 8-(2-Fluorobenzyloxy)-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5d**)

M.p. 200–202 °C, yield = 65.9%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.02–3.24 (m, 4H, H-5, H-6), 5.17 (s, 2H, –OCH_2–), 6.96–7.53 (m, 7H, Ar-H), 8.59 (s, 1H, H-2). IR (KBr) cm^{-1}: 1701 (C═O). MS *m*/*z* 324 (M + 1). Anal. Calcd. for $C_{18}H_{14}FN_3O_2$: C 66.87, H 4.36, N 13.00. Found: C 66.91, H 4.37, N 13.02.

3.2.5. 8-(4-Chlorobenzyloxy)-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5e**)

M.p. 179–181 °C, yield = 59.6%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.01–3.25 (m, 4H, H-5, H-6), 5.07 (s, 2H, –OCH_2–), 6.92–7.38 (m, 7H, Ar-H), 8.60 (s, 1H, H-2). IR (KBr) cm^{-1}: 1693 (C═O). MS *m*/*z* 340 (M + 1), 342 (M + 3). Anal. Calcd. for $C_{18}H_{14}ClN_3O_2$: C 63.63, H 4.15, N 12.37. Found: C 63.71, H 4.16, N 12.32.

3.2.6. 8-(3-Chlorobenzyloxy)-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5f**)

M.p. 114–116 °C, yield = 56.3%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.01–3.24 (m, 4H, H-5, H-6), 5.08 (s, 2H, –OCH_2–), 6.92–7.45 (m, 7H, Ar-H), 8.58 (s, 1H, H-2). IR (KBr) cm^{-1}: 1696 (C═O). MS *m*/*z* 340 (M + 1), 342 (M + 3). Anal. Calcd. for $C_{18}H_{14}ClN_3O_2$: C 63.63, H 4.15, N 12.37. Found: C 63.72, H 4.16, N 12.31.

3.2.7. 8-(2-Chlorobenzyloxy)-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5g**)

M.p. 185–187 °C, yield = 60.6%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.03–3.25 (m, 4H, H-5, H-6), 5.20 (s, 2H, –OCH_2–), 6.96–7.56 (m, 7H, Ar-H), 8.61 (s, 1H, H-2). IR (KBr) cm^{-1}: 1693 (C═O). MS *m*/*z* 340 (M + 1), 342 (M + 3). Anal. Calcd. for $C_{18}H_{14}ClN_3O_2$: C 63.63, H 4.15, N 12.37. Found: C 63.72, H 4.16, N 12.32.

3.2.8. 8-(2,4-Dichlorobenzyloxy)-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5h**)

M.p. 170–172 °C, yield = 62.5%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.02–3.24 (m, 4H, H-5, H-6), 5.15 (s, 2H, –OCH_2–), 6.94–7.50 (m, 6H, Ar-H), 8.58 (s, 1H, H-2). IR (KBr) cm^{-1}: 1689 (C═O). MS *m*/*z* 374 (M + 1), 376 (M + 3). Anal. Calcd. for $C_{18}H_{13}Cl_2N_3O_2$: C 57.77, H 3.50, N 11.23. Found: C 57.82, H 2.51, N 11.16.

*3.2.9. 8-(2,6-Dichlorobenzyloxy)-5,6-dihydro-[1,2,4]triazino [4,3-a]quinolin-1-one (**5i**)*

M.p. 206–208 °C, yield = 64.3%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.03–3.25 (m, 4H, H-5, H-6), 5.31 (s, 2H, $-OCH_2-$), 7.01–7.41 (m, 6H, Ar-H), 8.59 (s, 1H, H-2). IR (KBr) cm^{-1}: 1687 (C═O). MS *m*/*z* 374 (M + 1), 376 (M + 3). Anal. Calcd. for $C_{18}H_{13}Cl_2N_3O_2$: C 57.77, H 3.50, N 11.23. Found: C 57.81, H 2.51, N 11.13.

*3.2.10. 8-(2-Brominebenzyloxy)-5,6-dihydro-[1,2,4]triazino [4,3-a]quinolin-1-one (**5j**)*

M.p. 158–160 °C, yield = 50.8%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.02–3.24 (m, 4H, H-5, H-6), 5.17 (s, 2H, $-OCH_2-$), 6.95–7.64 (m, 7H, Ar-H), 8.57 (s, 1H, H-2). IR (KBr) cm^{-1}: 1690 (C═O). MS *m*/*z* 384 (M + 1). Anal. Calcd. for $C_{18}H_{14}BrN_3O_2$: C 56.27, H 3.67, N 10.94. Found: C 56.33, H 3.68, N 10.91.

*3.2.11. 8-(4-Brominebenzyloxy)-5,6-dihydro-[1,2,4]triazino [4,3-a]quinolin-1-one (**5k**)*

M.p. 207–209 °C, yield = 54.4%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.01–3.24 (m, 4H, H-5, H-6), 5.05 (s, 2H, $-OCH_2-$), 6.91–7.55 (m, 7H, Ar-H), 8.60 (s, 1H, H-2). IR (KBr) cm^{-1}: 1695 (C═O). MS *m*/*z* 384 (M + 1). Anal. Calcd. for $C_{18}H_{14}BrN_3O_2$: C 56.27, H 3.67, N 10.94. Found: C 56.34, H 3.68, N 10.90.

*3.2.12. 8-(4-Methoxybenzyloxy)-5,6-dihydro-[1,2,4]triazino [4,3-a]quinolin-1-one (**5l**)*

M.p. 163–165 °C, yield = 58.8%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.01–3.24 (m, 4H, H-5, H-6), 3.84 (s, 3H, $-OCH_3$), 5.02 (s, 2H, $-OCH_2-$), 6.93–7.38 (m, 7H, Ar-H), 8.59 (s, 1H, H-2). IR (KBr) cm^{-1}: 1697 (C═O). MS *m*/*z* 336 (M + 1). Anal. Calcd. for $C_{19}H_{17}N_3O_3$: C 68.05, H 5.11, N 12.53. Found: C 58.14, H 5.12, N 12.49.

*3.2.13. 8-(4-Methylbenzyloxy)-5,6-dihydro-[1,2,4]triazino [4,3-a]quinolin-1-one (**5m**)*

M.p. 143–145 °C, yield = 53.9%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.38 (s, 3H, $-CH_3$) 2.88–3.22 (m, 4H, H-5, H-6), 3.84 (s, 3H, $-CH_3$), 5.30 (s, 2H, $-OCH_2-$), 6.92–7.34 (m, 7H, Ar-H), 8.56 (s, 1H, H-2). IR (KBr) cm^{-1}: 1694 (C═O). MS *m*/*z* 320 (M + 1). Anal. Calcd. for $C_{19}H_{17}N_3O_2$: C 71.47, H 5.37, N 13.16. Found: C 71.52, H 5.39, N 13.09.

*3.2.14. 8-(3,4-Dimethoxybenzyloxy)-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5n**)*

M.p. 168–170 °C, yield = 51.7%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 3.01–3.23 (m, 4H, H-5, H-6), 3.91 (s, 6H, $-OCH_3$), 5.02 (s, 2H, $-OCH_2-$), 6.88–7.34 (m, 6H, Ar-H), 8.58 (s, 1H, H-2). IR (KBr) cm^{-1}: 1689 (C═O). MS *m*/*z* 366 (M + 1). Anal. Calcd. for $C_{20}H_{19}N_3O_4$: C 65.74, H 5.24, N 11.50. Found: C 65.81, H 5.25, N 11.42.

*3.2.15. 8-Ethoxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5o**)*

M.p. 165–167 °C, yield = 65.5%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.43 (t, 3H, *J* = 7.0 Hz, $-CH_3$), 2.99–3.21 (m, 4H, H-5, H-6), 4.02–4.08 (q, 2H, *J* = 7.0 Hz, $-OCH_2-$), 6.84–7.32 (m, 3H, Ar-H), 8.56 (s, 1H, H-2). IR (KBr) cm^{-1}: 1698 (C═O). MS *m*/*z* 244 (M + 1). Anal. Calcd. for $C_{13}H_{13}N_3O_2$: C 64.19, H 5.39, N 17.27. Found: C 64.23, H 5.38, N 17.21.

*3.2.16. 8-Propyloxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5p**)*

M.p. 123–125 °C, yield = 59.3%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.03 (t, 3H, *J* = 7.0 Hz, $-CH_3$), 1.78–1.87 (m, 2H, $-CH_2-$), 3.00–3.22 (m, 4H, H-5, H-6), 3.94 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 6.85–7.32 (m, 3H, Ar-H), 8.57 (s, 1H, H-2). IR (KBr) cm^{-1}: 1695 (C═O). MS *m*/*z* 258 (M + 1). Anal. Calcd. for $C_{14}H_{15}N_3O_2$: C 65.35, H 5.88, N 16.33. Found: C 65.40, H 5.87, N 16.29.

*3.2.17. 8-Butyloxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5q**)*

M.p. 100–102 °C, yield = 51.1%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.99 (t, 3H, *J* = 7.4 Hz, $-CH_3$), 1.45–1.84 (m, 4H, $-CH_2-CH_2-$), 3.00–3.22 (m, 4H, H-5, H-6), 3.99 (t, 2H, *J* = 9.5 Hz, $-OCH_2-$), 6.85–7.32 (m, 3H, Ar-H), 8.57 (s, 1H, H-2). IR (KBr) cm^{-1}: 1696 (C═O). MS *m*/*z* 272 (M + 1). Anal. Calcd. for $C_{15}H_{17}N_3O_2$: C 66.40, H 6.32, N 15.49. Found: C 66.45, H 6.34, N 15.43.

*3.2.18. 8-Pentyloxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5r**)*

M.p. 115–117 °C, yield = 56.8%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.94 (t, 3H, *J* = 7.1 Hz, $-CH_3$), 1.34–1.85 (m, 6H, $-(CH_2)_3-$), 2.99–3.22 (m, 4H, H-5, H-6), 3.97 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 6.84–7.32 (m, 3H, Ar-H), 8.56 (s, 1H, H-2). IR (KBr) cm^{-1}: 1689 (C═O). MS *m*/*z* 286 (M + 1). Anal. Calcd. for $C_{16}H_{19}N_3O_2$: C 67.35, H 6.71, N 14.73. Found: C 67.45, H 6.74, N 14.62.

*3.2.19. 8-Hexyloxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5s**)*

M.p. 98–100 °C, yield = 51.8%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.92 (t, 3H, *J* = 7.0 Hz, $-CH_3$), 1.35–1.83 (m, 8H, $-(CH_2)_4-$), 3.01–3.24 (m, 2H, H-5, H-6), 3.98 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 6.86–7.33 (m, 3H, Ar-H), 8.60 (s, 1H, H-2). IR (KBr) cm^{-1}: 1694 (C═O). MS *m*/*z* 300 (M + 1). Anal. Calcd. for $C_{17}H_{21}N_3O_2$: C 68.20, H 7.07, N 14.04. Found: C 68.29, H 7.10, N 13.94.

*3.2.20. 8-Heptyloxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5t**)*

M.p. 94–96 °C, yield = 63.2%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88–1.88 (m, 13H, $-(CH_2)_5CH_3$), 3.00–3.23 (m, 2H, H-5, H-6), 3.98 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 6.86–7.33 (m, 3H, Ar-H), 8.58 (s, 1H, H-2). IR (KBr) cm^{-1}: 1688 (C═O). MS *m*/*z* 314 (M + 1). Anal. Calcd. for $C_{18}H_{23}N_3O_2$: C 68.98, H 7.40, N 13.41. Found: C 67.04, H 7.38, N 13.36.

*3.2.21. 8-Octyloxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5u**)*

M.p. 63–65 °C, yield = 53.7%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.88–1.83 (m, 15H, $-(CH_2)_6CH_3$), 3.00–3.23 (m, 2H, H-5, H-6), 3.98 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 6.85–7.33 (m, 3H, Ar-H), 8.59 (s, 1H, H-2). IR (KBr) cm^{-1}: 1692 (C═O). MS *m*/*z* 328 (M + 1). Anal. Calcd. for $C_{19}H_{25}N_3O_2$: C 69.70, H 7.70, N 12.83. Found: C 69.79, H 7.72, N 12.76.

*3.2.22. 8-Dodecyloxy-5,6-dihydro-[1,2,4]triazino[4,3-a]quinolin-1-one (**5v**)*

M.p. 62–64 °C, yield = 63.1%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.87–1.82 (m, 23H, $-(CH_2)_{10}CH_3$), 3.00–3.22 (m, 2H, H-5, H-6), 3.97 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 6.86–7.32 (m, 3H, Ar-H), 8.57 (s, 1H, H-2). IR (KBr) cm^{-1}: 1690 (C═O). MS *m*/*z* 384 (M + 1). Anal. Calcd. for $C_{23}H_{33}N_3O_2$: C 72.03, H 8.67, N 10.96. Found: C 72.14, H 8.69, N 10.84.

*3.2.23. 2-Methyl-8-benzyloxy-5,6-dihydro-[1,2,4]triazino [4,3-a]quinolin-1-one (**6a**)*

M.p. 156–158 °C, yield = 58.6%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 2.55 (s, 3H, $-CH_3$), 3.01–3.25 (m, 4H, H-5, H-6), 5.08 (s, 2H, $-OCH_2-$), 6.94–7.44 (m, 8H, Ar-H). IR (KBr) cm^{-1}: 1794 (C═O). MS *m*/*z* 318 (M + 1). Anal. Calcd. for $C_{19}H_{17}N_3O_2$: C 71.46, H 5.37, N 13.16. Found: C 71.53, H 5.40, N 13.06.

*3.2.24. 2-Methyl-8-hexyloxy-5,6-dihydro-[1,2,4]triazino [4,3-a]quinolin-1-one (**6s**)*

M.p. 71–73 °C, yield = 58.9%. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, 3H, *J* = 7.0 Hz, $-CH_3$), 1.31–1.80 (m, 8H, $-(CH_2)_4-$), 2.57 (s, 3H, $-CH_3$), 3.01–3.24 (m, 2H, H-5, H-6), 3.99 (t, 2H, *J* = 6.5 Hz, $-OCH_2-$), 6.896–

7.34 (m, 3H, Ar-H). IR (KBr) cm^{-1}: 1693 (C=O). MS *m*/*z* 314 (M + 1). Anal. Calcd. for $C_{18}H_{23}N_3O_2$: C 68.98, H 7.40, N 13.41. Found: C 69.04, H 7.42, N 13.33.

3.3. Pharmacology

The MES test and rotarod test were carried out by the standard described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health following previously described testing procedures (USA) [13,14]. All compounds, which were dissolved in polyethyleneglycol-400, were evaluated for anticonvulsant activities with C57B/6 mice in the 18–25 g weight range. Groups of 10 mice were given a range of intraperitoneal doses of the test drug until at least three points were established in the range of 10–90% seizure protection or minimal observed neurotoxicity. From the plots of these data, the respective ED_{50} and TD_{50} values, 95% confidence intervals, slopes of the regression line and the standard error of the slopes were calculated by means of a computer program written by the National Institute of Neurological Disorders and Stroke.

3.3.1. 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 abolition of the hind leg and tonic maximal extension component of the seizure. 30 min after the administration of the compounds, the activities were evaluated in MES test.

3.3.2. PTZ-induced seizure [13,14]

30 min after the administration of the synthesized compounds at various doses, the animals were given a subcutaneous dose of pentylenetetrazole (85 mg/kg), a dose at which 100% of the naïve animals showed convulsive reactions. The dose which prevented 50% of the treated animals from tonic convulsions (ED_{50}) was then calculated.

3.3.3. Isoniazid-induced seizure [15]

30 min after the administration of the compounds at various doses, the animals were given an ip dose of isoniazid (250 mg/kg), a dose at which 100% of the naïve animals showed convulsive reactions. The mice were placed in individual cages and observed for 1 h. The dose which prevented 50% of the treated animals from tonic convulsions (ED_{50}) was calculated.

3.3.4. Thiosemicarbazide-induced seizure [16]

30 min after the administration of the compounds at various doses, the animals were given an ip dose of thiosemicarbazide (50 mg/kg), a dose at which 100% of the animals showed convulsive reaction. This dose was then administered to animals 1 h after different treatments. The doses were calculated which prevented 50% of the treated animals from tonic convulsions (ED_{50}).

3.3.5. 3-MP-induced seizures [17]

30 min after the administration of the compounds at various doses, 40 mg/kg 3-MP in saline solution was injected sc. This dose was then administered to animals 1 h after different treatments. The doses were calculated which prevented 50% of the treated animals from tonic convulsions (ED_{50}).

3.3.6. Strychnine-induced seizures [18]

30 min after the administration of the compounds, the animals were given a subcutaneous dose of strychnine chlorhydrate in saline (1.2 mg/mL, 1 mL/kg), a dose at which 100% of the naïve animals showed convulsive reactions. The mice were placed in individual cages and observed for 30 min.

3.3.7. Rotarod test [19]

30 min after the administration of the compounds, the animals were tested on a 1-in. diameter knurled plastic rod rotating at 6 rpm for 1 min. Neurotoxicity was indicated by the inability of an animal to maintain equilibrium in each of three trials.

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