Synthesis of some quinoline-2(1*H*)-one and 1, 2, 4 - triazolo [4 , 3 -*a*] quinoline derivatives as potent anticonvulsants

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ABSTRACT - PURPOSE. A new series of substituted quinoline-2(1*H*)-one and 1,2,4-triazolo[4,3-*a*]-quinoline derivatives were designed and synthesized to meet the structural requirements essential for anticonvulsant properties. **METHODS.** 4-substituted-phenyl-3,4-dihydro-2(1*H*)-quinolines, 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3*a*]quinolines and 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo-[4,3-*a*]quinoline-1-(2*H*)-ones derivatives were synthesized using 3-substituted-phenyl-*N*-phenyl-acrylamide as a starting material. Their anticonvulsant activity were evaluated by maximal electroshock (MES) test, subcutaneous pentylenetetrazol (scPTZ) test, and their neurotoxic effects were determined by the rotarod neurotoxicity test. **RESULTS.** The compounds 4-substitued-phenyl-3,4-dihydro-2(1*H*)-quinolines **(2a-f)** had increased anticonvulsant effects compared to the parental compounds. The compounds 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinolines **(3a-f)** had significantly increased anticonvulsant activity compared to **2a-f**. However, the compounds 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinoline-1(2*H*)-ones**(4a-f),** exhibited no anticonvulsant effects even under a high dose of 300 mg/kg. **CONCLUSIONS.** The triazole, but not the triazolone, modified series showed stronger anticonvulsant effects than the parent compounds. Among them, compound **(3f),** 5-(*p*-fluorophenyl)-4,5–dihydro-1,2,4-triazolo[4,3-*a*]quinoline, showed the strongest anticonvulsant effect with ED_{50} of 27.4mg/kg and 22.0mg/kg in the anti-MES and anti-PTZ test, respectively.

INTRODUCTION

Epilepsy, a ubiquitous disease characterized by recurrent seizures, inflicts more than sixty million people worldwide according to epidemiological studies (1). The majority of antiepileptic drugs have been in use since 1985. They do not provide satisfactory seizure control in all patients and typically cause notable adverse side effects (2, 3). Research to find more effective and safer antiepileptic drugs, is, therefore, imperative and challenging in medicinal chemistry.

Quinoline derivatives have been known to possess a variety of biological activities such as antitumor (4), antimalarial (5), antiplatelet (6), antidepressant (7), antiulcer (8) and cardiac stimulant (9).

In our previous research on the positive inotropic activity of quinolines (10), 3,4-dihydro-2 (1*H)*-quinoline (compound **I**) showed a slight positive anticonvulsant activity with an effective dose of 300mg/kg in the anti-MES test. In order to obtain compounds with better anticonvulsant activity, we synthesized 4-substituted-phenyl-3,4-dihydro-2 (1*H*)-quinolines **(2a-f)** using 3,4-dihydro-2 (1*H*)-quinolione as the lead compound. The hypothesis was that the introduction of a substituted-phenyl into the 4-position of compound **I** would increase the lipophilic property of the compounds and increase their permeability to the blood-brain barrier which probably enhances their anticonvulsant activity. Subsequently, 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinoline (**3a-f**) and 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinoline-1(2*H*)-one (**4a-f**) derivatives were synthesized by incorporating a triazole or triazolone ring into N_1-C_2 positions of compounds **2a-f,** hoping to increase their receptor binding and metabolic stability and, as a result, obtaining compounds with increased anticonvulsant activity. Similar designing approaches have also been reported (11-14).

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For instance, when triazole or triazolone was incorporated into 4- and 5-positions of 1-aryl-3,5-dihydro-7,8-dimethoxy-4*H*-2,3-benzodiazepin-4-one, the anticonvulsant activity of the resulting compounds 11*H*-triazolo [4,5-*c*] [2,3] benzodiazepine and 11*H*-triazolo[4,5-*c*][2,3]benzodiazepine-3(2*H*)-ones increased remarkably.

The new compounds were evaluated as anticonvulsant agents in experimental epilepsy models, i.e., maximal electroshock test (MES) and subcutaneous pentylenetetrazol (sc-PTZ) induced seizure in mice. They were also evaluated for neurotoxicity by the rotarod assay performed in mice.

INSTRUMENTS

Melting points were determined in open capillary tubes and were uncorrected. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a 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). Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer. The major chemicals were purchased from Alderich chemical corporation. All other chemicals were of analytical grade.

METHODS AND RESULTS

Chemistry

Compounds **2a-f** were prepared according to a reported procedure (15). Briefly, they were obtained by the cyclization of 3-substituted-phenyl-*N*-phenyl-acrylamide using polyphosphoric acid as a catalyst. Compounds **3a-f** were prepared as reported (16,17) where the compounds **2a-f** were sulfurizated with phosphorous pentasulfide and then cyclized with formhydrazide. Compounds **4a-f** were prepared as reported (15,16) where the compounds **2a-f** were sulfurizated with phosphorous pentasulfide and then cyclized with methyl hydrazinocarboxylate, as depicted in the following scheme.

The structures of the target compounds were confirmed by ^{1}H-NMR, ^{13}H-NMR, MS and elemental analysis techniques.

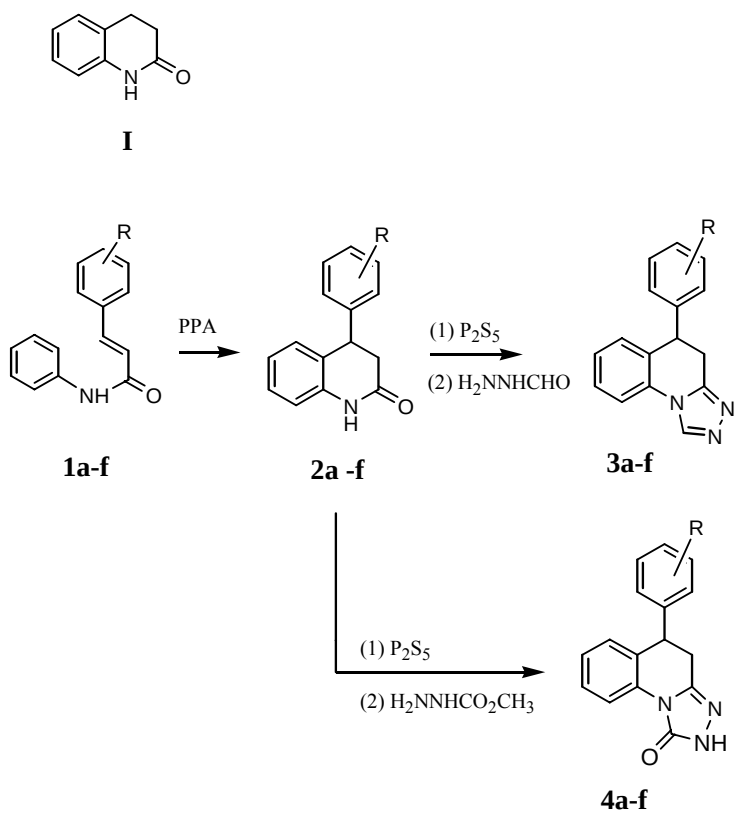
Preparation of compounds 2a-f

A mixture of 3-substituted-phenyl-*N*-phenyl-acrylamide (1.00g) and polyphosphoric acid (20g) was heated to 120℃. After 30 min, the reaction mixture was cooled and hydrolyzed over crushed ice. The reaction product was extracted with three 125 ml portions of dichloromethane. The extracts were combined and dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product that was purified by recrystallization from ethanol. The yield, melting point and spectral data of each compound is given below.

4-Phenyl-3,4-dihydro-2 (1H)-quinolone (**2a**)*:* Yield 70%, mp 180-18℃. ^{1}H-NMR ($CDCl_3$): 2.97 (m, 2H, J = 7.5 Hz, CH_2), 4.35 (t, H, J = 7.5 Hz, CH), 6.94-7.38 (m, 4H, C_6H_4-), 7.01-7.34 (m, 5H, C_6H_5-), 9.41 (s , 1H, NH). ^{13}C-NMR ($CDCl_3$)δ: 38.40， 42.02， 115.76， 123.30， 126.65， 127.20， 127.81， 127.99， 128.33， 128.89，137.12，141.52，170.89. MS : (M + 1) 224. *Anal*. Calcd. for $C_{15}H_{13}NO$: C, 80.69; H, 5.87; N, 6.27. Found: C, 80.41; H, 5.92; N, 6.34.

4-(4-Chlorophenyl)-3,4-dihydro-2 (1H)-quinolone (**2b**): Yield 93.4%, mp 180-182℃. ^{1}H-NMR ($CDCl_3$): 2.99 (m, 2H, J = 6.9 Hz, CH_2), 4.38 (t, H, J = 6.9 Hz, CH), 6.95-7.37 (m, 4H, C_6H_4-), 7.02-7.35 (m, 4H, C_6H_4-), 9.50 (s, 1H, NH). ^{13}C - NMR ($CDCl_3$)δ: 38.34, 41.43, 115.86, 123.50, 126.05, 128.28, 128.98, 129.09, 129.14, 133.06, 137.00, 139.99, 170.53. MS: (M+1) 259. *Anal*. Calcd. for $C_{15}H_{12}ClNO$: C, 69.91; H, 4.69; N, 5.43. Found: C, 69.64; H, 4.49; N, 5.39.

4-(4-Methoxyphenyl)-3,4-dihydro-2 (1H)-quinolone (**2c**): Yield 73.5%, mp 138-140℃. ^{1}H-NMR ($CDCl_3$): 2.87 (m, 2H, J = 7.5 Hz, CH_2), 4.28 (t, H, J = 7.5 Hz, CH), 3.76 (s , 1H, OCH_3), 6.95-7.37 (m , 4H, C_6H_4-), 6.75-7.08 (m, 4H, C_6H_4-), 9.48 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$)δ: 38.55, 41.21, 55.27, 114.28, 115.60, 123.34, 127.13, 127.92, 128.33, 128.80, 133.38, 136.93， 158.71, 170.83. MS: (M+1) 254. *Anal*. Calcd. for $C_{16}H_{15}NO_2$: C, 75.87; H, 5.97; N, 5.53. Found: C, 75.71; H, 5.69; N, 5.37.



R:			
a	H	**d**	p-CH_3-
b	p-Cl	**e**	p-F
c	p-OCH_3	**f**	m-F

Synthesis of compounds 3a-f and 4a-f

4-(4-Methylphenyl)-3,4-dihydro-2 (1H)-quinolone (**2d**): Yield 74.6%, mp 154-156. ^{1}H-NMR ($CDCl_3$): 2.91 (m, 2H, J = 7.2 Hz, CH_2), 4.36 (t, H, J = 7.2 Hz, CH), 2.54 (s,1H, CH_3), 6.96-7.38 (m, 4H, C_6H_4-), 7.01-7.22 (m , 4H, C_6H_4-), 9.36 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$)δ: 21.03, 38.46, 41.62, 115.64, 123.31, 126.96, 127.69, 127.92, 128.34, 129.58, 136.85, 137.01，138.39, 170.92. MS: (M+1) 238. *Anal*. Calcd. for $C_{16}H_{15}NO_2$: C, 80.98; H, 6.37; N, 5.90. Found: C, 81.17; H, 6.16; N, 5.73.

4-(4-Fluorophenyl)-3,4-dihydro-2 (1H)-quinolone (**2e**): Yield 94%, mp 184-186℃. ^{1}H-NMR ($CDCl_3$): 3.01 (m, 2H, J = 6.9 Hz, CH_2), 4.39 (t , H, J = 6.9 Hz, CH), 6.97-7.34 (m, 4H, C_6H_4-), 6.91-7.17 (m, 4H, C_6H_4-), 9.40 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$)δ: 38.54, 41.30, 115.77 (d, $^2J_{C\text{-}F}$ = 21.0 Hz), 115.78, 123.46, 126.42, 128.19, 128.29, 129.30 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 136.97, 137.15, 161.92 (d, $^1J_{C\text{-}F}$ = 244.5 Hz), 170.55. MS: (M+1)228. *Anal*. Calcd. for $C_{15}H_{12}FNO$: C, 74.67; H, 5.01; N, 5.81. Found: C, 74.91; H, 4.91; N, 5.97.

4-(3-Fluorophenyl)-3,4-dihydro-2 (1H)-quinolone (**2f**): Yield 64.5%, mp 177-178℃. ^{1}H-NMR ($CDCl_3$): 3.02 (m, 2H, J = 7.2 Hz, CH_2), 4.39 (t , H , J = 7.2 Hz, CH), 6.95-7.34 (m, 4H, C_6H_4-), 6.75-7.20 (m, 4H, C_6H_4-), 9.39 (s, 1H, NH). ^{13}C-NMR ($CDCl_3$)δ: 38.28, 41.76, 114.88, 114.85 (d, $^2J_{C\text{-}F}$ = 21.0 Hz), 115.73, 116.48, 123.45, 128.92, 129.67, 129.93, 130.42 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 130.60, 137.02, 163.06 (d, $^1J_{C\text{-}F}$ = 244.5 Hz), 169.90. MS: (M+1) 228. *Anal*. Calcd. for $C_{15}H_{12}FNO$: C, 74.67; H, 5.01; N, 5.81. Found: C, 74.78; H, 4.89; N, 5.89.

Preparation of compounds 3a-f.

Acetonitrile (60 ml) and triethylamine (40 ml) were placed in a three-necked round-bottomed flask, to which P_2S_5 (9.99g, 0.06 mol) was added slowly in an ice bath and stirred until dissolved.

Then 4-substituted-phenyl-3,4-dihydro-2(1*H*)-quinolones (0.04 mol) was added while stirring. The mixture was refluxed for 5 h in a nitrogen atmosphere. After removing the solvent under reduced pressure, the residue was dissolved in 120 ml of dichloromethane, washed with water (120 × 3), and dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product, then formhydrazide (2.4g, 0.04 mol), n-butanol (80 ml) and acetic acid (0.5 ml) were added, and the mixture was refluxed for 20 h in a nitrogen atmosphere. Solvents were removed under reduced pressure, and the residue was extracted twice with dichloromethane (60 ml). The dichloromethane layer was washed three times with matured sodium chloride (60 × 3) and dried over anhydrous $MgSO_4$. After removing the solvents, products were purified by silica gel column chromatography (dichloromethane: methanol = 20:1). The yield, melting point and spectral data of each compound is given below.

5-Phenyl-4,5-dihydro-1,2,4- triazolo[4,3-a]quinoline (**3a**): Yield 82.7%, mp 140-142℃. ^{1}H-NMR ($CDCl_3$): 3.45 (m, 2H, J = 8.0 Hz, CH_2), 4.34 (t, H, J = 8.0 Hz, CH), 7.10-7.42 (m, 4H, C_6H_4-), 7.07-7.25 (m, 4H, C_6H_5-), 8.69 (s, 1H, H-1). ^{13}C-NMR ($CDCl_3$)δ: 28.54, 42.44, 116.30, 127.42, 127.71, 127.85, 128.71, 129.08, 129.98, 130.49, 131.88, 137.46, 140.70, 149.65. MS: (M+1) 248. *Anal*. Calcd. for $C_{16}H_{13}N_3$: C, 77.71; H, 5.30; N, 16.99. Found: C, 77.89; H, 5.08; N, 17.28.

5-(4-Chlorophenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinoline (**3b**): Yield 78.7%, mp 176-178℃. ^{1}H-NMR ($CDCl_3$): 3.48 (m, 2H, J = 8.0 Hz, CH_2), 4.37 (t, H, J = 8.0 Hz, CH), 7.11-7.51 (m, 4H, C_6H_4-), 7.08-7.28 (m, 4H, C_6H_4-), 8.70 (s, 1H, H-1). ^{13}C-NMR ($CDCl_3$)δ: 28.47, 41.83, 116.47, 127.40, 128.86, 129.16, 129.25, 129.84, 130.14, 131.81, 133.50, 137.50, 139.18, 149.32. MS: (M+1) 284. *Anal*. Calcd. for $C_{16}H_{12}ClN_3$: C, 68.21; H, 4.29; N, 14.91. Found: C, 68.46; H, 4.64; N, 15.10.

5-(4-Methoxyphenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinoline (**3c**): Yield 63.2%, mp 188-190℃. ^{1}H-NMR ($CDCl_3$): 3.46 (m, 2H, J = 6.9 Hz, CH_2), 4.33 (t, H, J = 6.9 Hz, CH), 3.80 (s, 3H, OCH_3), 7.11-7.49 (m, 4H, C_6H_4-), 6.84-7.28 (m, 4H, C_6H_4-), 8.68 (s,1H, H-1). ^{13}C-NMR ($CDCl_3$)δ: 28.65, 41.66, 55.28, 114.09, 116.27, 127.22, 128.49, 128.86, 129.91, 130.88, 131.82, 132.62, 137.44, 149.76. 158.95. MS: (M+1) 278. *Anal*. Calcd. for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15. Found: C, 73.39; H, 5.71; N, 14.89.

5-(4-Methylphenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinoline (**3d**): Yield 81.6%, mp 158-160℃. ^{1}H-NMR ($CDCl_3$): 3.47 (m , 2H, J = 7.5 Hz, CH_2), 4.34 (t, H, J = 7.5 Hz, CH), 2.34 (s, 3H, CH_3), 7.13-7.49 (m, 4H, C_6H_4-), 7.01-7.28 (m, 4H, C_6H_4-), 8.69 (s, 1H, H-1). ^{13}C-NMR ($CDCl_3$)δ: 21.02, 28.54, 42.03, 116.81, 126.87, 127.71, 128.06, 128.51, 129.27, 129.72, 130.73, 131.82, 137.47, 139.28, 149.76. MS: (M+1) 262. *Anal*. Calcd. for $C_{17}H_{15}N_3$: C, 78.13; H, 5.79; N, 16.08. Found: C, 77.88; H, 5.95; N, 15.86.

5-(4-Fluorophenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinoline (**3e**): Yield 72.8%, mp 168-169℃. ^{1}H-NMR ($CDCl_3$): 3.49 (m, 2H, J = 7.5 Hz, CH_2), 4.38 (t, H, J = 7.5 Hz, CH), 7.10-7.51 (m, 4H, C_6H_4-), 6.92-7.28 (m, 4H, C_6H_4-), 8.69 (s, 1H, H-1). ^{13}C-NMR ($CDCl_3$)δ: 28.64, 41.70, 115.28 (d, $^2J_{C\text{-}F}$ = 21.0 Hz), 115.83, 116.11, 116.44, 127.37, 129.19, 129.38 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 129.85, 130.22, 131.80, 136.44, 162.07 (d, $^1J_{C\text{-}F}$ = 245.3 Hz) . MS: (M+1) 266. *Anal*. Calcd. for $C_{16}H_{12}FN_3$: C, 72.44; H, 4.56; N, 15.84. Found: C, 72.71; H, 4.32; N, 16.12.

5-(3-Fluorophenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinoline (**3f**): Yield 54.5%, mp 158-160℃. ^{1}H-NMR ($CDCl_3$): 3.50 (m, 2H, J = 6.9 Hz, CH_2), 4.39 (t, H , J = 6.9 Hz, CH), 7.12-7.52 (m, 4H, C_6H_4-), 6.81-7.29 (m, 4H, C_6H_4-), 8.70 (s,1H, H-1) . ^{13}C-NMR ($CDCl_3$)δ: 28.45, 42.10, 114.56 (d, $^2J_{C\text{-}F}$ = 18.8 Hz), 114.85 (d, $^2J_{C\text{-}F}$ = 18.8 Hz), 116.50, 123.45, 123.48, 127.40, 128.92, 129.91 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 130.58 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 130.69, 131.41, 131.85, 143.31, 163.05 (d, $^1J_{C\text{-}F}$ = 245.3 Hz) . MS: (M+1) 266. *Anal*. Calcd. for $C_{16}H_{12}FN_3$: C, 72.44; H, 4.56; N, 15.84. Found: C, 72.63; H, 4.32; N, 16.06.

Preparation of compounds 4a-f.

To 4-substituted-phenyl-3,4-dihydro-2(1*H*)-quinolione-thiones (0.04mol),prepared according to the method described by Zappala et al. (13) methyl hydrazinocarboxylate (2.4g, 0.04 mol), n-butanol (80 ml), and acetic acid (0.5ml) was added and the mixture was refluxed for 60 h in a nitrogen atmosphere. Solvents were removed under reduced pressure, and the residue was extracted twice with dichloromethane (60 ml). The dichloromethane layer was washed three times with matured sodium chloride (60 × 3) and

dried over anhydrous $MgSO_4$. After removing the solvents, products were purified by silica gel column chromatography (dichloromethane: methanol = 20:1). The yield, melting point and spectral data of each compound are given below.

5-Phenyl-4,5-dihydro-1,2,4-triazolo[4,3-a]quinolin-1(2H)-one (**4a**): Yield 65.13%. mp 202-204℃. ^{1}H-NMR ($CDCl_3$): 3.20 (m, 2H, J = 6.6 Hz, CH_2), 4.35 (t, H, J = 6.6 Hz, CH), 7.19-8.46 (m, 4H, C_6H_4-), 7.01-7.29 (m, 4H, C_6H_5-), 9.82 (s, 1H, N-H). ^{13}C-NMR ($CDCl_3$)δ: 29.60, 42.38, 114.42, 117.40, 126.09, 127.46, 127.74, 128.49, 128.89, 129.02, 132.71, 140.83, 143.71, 153.13. MS: (M+1) 264. *Anal*. Calcd. for $C_{16}H_{13}N_3O$: C, 72.99; H, 4.98; N, 15.96. Found: C, 72.76; H, 5.26; N, 15.75.

5-(4-Chlorophenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinolin-1(2H)-one **(4b)**: Yield 52.3%, mp 256-258℃. ^{1}H-NMR ($CDCl_3$): 3.18 (m, 2H, J = 6.5 Hz, CH_2), 4.32 (t, H, J = 6.5 Hz, CH), 6.92-8.45 (m, 4H, C_6H_4-), 7.04-7.21 (m, 4H, C_6H_4-) , 9.67 (s, 1H, N-H). ^{13}C-NMR ($CDCl_3$)δ: 29.57, 41.77, 117.51, 126.24, 128.39, 128.75, 128.88, 129.06, 129.20, 130.10, 132.60, 139.28, 143.38 , 152.81. MS: (M+1) 298. *Anal*. Calcd. for $C_{16}H_{12}ClN_3O$: C, 64.54; H, 4.06; N, 14.11. Found: C, 64.46; H, 4.29; N, 13.93.

5-(4-Methoxyphenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinolin-1(2H)-one (**4c**): Yield 57.8%, mp 206-208℃. ^{1}H-NMR ($CDCl_3$): 3.18 (m, 2H, J = 6.9 Hz, CH_2), 4.31 (t, H, J = 6.9 Hz, CH), 3.80 (s, 3H, OCH_3), 6.88-8.45 (m, 4H, C_6H_4-), 6.85-7.15 (m, 4H, C_6H_4-), 9.88 (s, 1H, N-H). ^{13}C-NMR ($CDCl_3$)δ: 29.74, 41.59, 55.28, 114.39, 117.37, 126.08, 128.40, 128.75, 128.91, 129.40, 132.61, 132.75, 143.88, 152.99, 158.83. MS: (M+1) 294. *Anal*. Calcd. for $C_{17}H_{15}N_3O_2$: C, 69.61; H, 5.15; N, 14.33. Found: C, 69.49; H, 5.36; N, 14.12.

5-(4-Methylphenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinolin-1(2H)-one (**4d**): Yield 63.4%, mp 188-190℃. ^{1}H-NMR ($CDCl_3$): 3.20 (m, 2H, J = 6.6 Hz, CH_2), 4.32 (t, H, J = 6.6 Hz, CH), 2.35 (s, 3H, CH_3), 7.00-8.47 (m, 4H, C_6H_4-), 7.02-7.20 (m, 4H, C_6H_4-), 9.99 (s, 1H, N-H). ^{13}C-NMR ($CDCl_3$)δ: 21.01, 29.66, 42.00, 117.36, 126.05, 127.60, 128.40, 128.94, 129.27, 129.68, 132.69, 137.13, 137.79, 143.82, 153.09. MS: (M+1) 278. *Anal*. Calcd. for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15. Found: C, 73.83; H, 5.36; N, 15.32.

5-(4-Fluorophenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinolin-1(2H)-one (**4e**): Yield 51%, mp 255-258℃. ^{1}H-NMR ($CDCl_3$): 3.21 (m, 2H, J = 6.6 Hz, CH_2), 4.36 (t, H, J = 6.6 Hz, CH), 6.92-8.47 (m, 4H, C_6H_4-), 6.88-7.17 (m, 4H, C_6H_4-), 10.10 (s, 1H, N-H). ^{13}C-NMR ($CDCl_3$)δ: 29.73, 41.66, 115.28 (d, $^2J_{C\text{-}F}$ = 21.0 Hz), 115.77, 116.06, 117.50, 126.19, 128.75, 129.32 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 132.61, 136.55, 143.48, 152.98, 162.01 (d, $^1J_{C\text{-}F}$ = 245.3 Hz). MS: (M+1) 282. *Anal*. Calcd. for $C_{16}H_{12}FN_3O$: C, 68.32; H, 4.30; N, 14.94. Found: C, 68.06; H, 4.56; N, 14.76.

5-(3-Fluorophenyl)-4,5-dihydro-1,2,4-triazolo[4,3-a]quinolin-1(2H)-one **(4f)**: Yield 50.4%, mp 224-226℃. ^{1}H-NMR ($CDCl_3$): 3.21 (m, 2H, J = 6.5 Hz, CH_2), 4.37 (t, H, J = 6.5 Hz, CH), 6.97-8.49 (m, 4H, C_6H_4-), 6.81-7.18 (m, 4H, C_6H_4-), 10.26 (s, 1H, N-H). ^{13}C-NMR ($CDCl_3$) δ: 29.44, 42.07, 114.50 (d, $^2J_{C\text{-}F}$ = 21.8 Hz), 114.79 (d, $^2J_{C\text{-}F}$ = 21.8 Hz), 117.52, 123.35, 126.22, 128.23, 128.79, 128.92, 130.52 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 130.63 (d, $^3J_{C\text{-}F}$ = 8.3 Hz), 132.64, 143.42, 152.12, 163.08 (d, $^1J_{C\text{-}F}$ = 246.0 Hz). MS: (M+1) 282. *Anal*. Calcd. for $C_{16}H_{12}FN_3O$: C, 68.32; H, 4.30; N, 14.94. Found: C, 68.46; H, 4.21; N, 14.86.

Anticonvulsant Tests

The MES test, sc-PTZ test, and rotarod test were carried out by the Antiepileptic Drug Development Program (ADD), Epilepsy Branch, National Institutes of Health, Bethesda, MD, USA (18,19). All compounds were tested for anticonvulsant activity with C57B/6 mice in the 18-25g weight range purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University. The tested compounds were dissolved in polyethylene glycol-400.

In Phase I screening (Table 1) each compound was administered at three dose levels (30, 100, and 300 mg/kg i.p., 3 mice for each dose) with anticonvulsant activity and neurotoxicity assessed at 30 min and 4 h intervals after administration. Anticonvulsant efficacy was measured in the MES test and the sc-PTZ test. 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. Abolition of the hind-leg tonic-extensor component of the seizure indicated protection against the spread of MES-induced seizures. The

sc-PTZ test involved subcutaneous injection of a convulsant dose (CD_{97}) of pentylenetetrazol (85mg/kg in mice). Elevation of the pentylenetrazol-induced seizure threshold was indicated by the absence of clonic spasms for at least 5 s duration over a 30 min period following administration of the test compound. Anticonvulsant drug-induced neurologic deficit was detected in mice using the rotorod ataxia test.

The pharmacologic parameters estimated in phase I screening was quantified for compounds **3a-f** in phase II screening (Table 2). Anticonvulsant activity was expressed in terms of the median effective dose (ED_{50}), and neurotoxicity was expressed as the median toxic dose (TD_{50}). For determination of the ED_{50} and TD_{50} values, 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 plot of this data, the respective ED_{50} and TD_{50} 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, Bethesda, MD, USA.

Results of the initial anticonvulsant activity evaluation phase I of the synthesized compounds are presented in Table 1. Based on the result of the preliminary screening, compounds **3a-3f** were selected for phase Ⅱ tests, the results of which are shown in Table 2.

Table 1. Phase I mouse anticonvulsant activity (i.p.)

Compd.	Dosage (mg/kg)	MES[a]		scPTZ[b]		Rotarod[c]	
		0.5h	4h	0.5h	4h	0.5h	4h
1	300	3/3	0/3	3/3	0/3	3/3	1/3
2a	100	2/3	0/3	2/3	0/3	0/3	0/3
2b	100	2/3	0/3	2/3	0/3	0/3	0/3
2c	100	2/3	0/3	2/3	0/3	0/3	0/3
2d	100	1/3	0/3	1/3	0/3	0/3	0/3
2e	100	2/3	0/3	2/3	2/3	0/3	0/3
2f	100	2/3	0/3	2/3	2/3	0/3	0/3
3a	100	3/3	1/3	3/3	1/3	1/3	0/3
3b	100	3/3	1/3	3/3	1/3	2/3	0/3
3c	100	3/3	1/3	3/3	1/3	0/3	0/3
3d	100	2/3	0/3	2/3	0/3	0/3	0/3
3e	30	1/3	1/3	1/3	1/3	0/3	0/3
3f	30	2/3	2/3	2/3	2/3	0/3	0/3
4a	300	0/3	0/3	0/3	0/3	0/3	0/3
4b	300	0/3	0/3	0/3	0/3	0/3	0/3
4c	300	0/3	0/3	0/3	0/3	0/3	0/3
4d	300	0/3	0/3	0/3	0/3	0/3	0/3
4e	300	0/3	0/3	0/3	0/3	0/3	0/3
4f	300	0/3	0/3	0/3	0/3	0/3	0/3

[a] Maximal electroshock test (number of animals protected / number of animals tested).
[b] Subcutaneous pentylenetetrazol test (number of animals protected / number of animals tested).
[c] Rotorod toxicity (number of animals exhibiting toxicity / number of animals tested).

Table 2. Phase II quantitative anticonvulsant data in mice (Test drug administered i.p.)

Compd.	ED_{50}[a]		Rotarod Toxicity	PI[b]	
	MES	scPTZ	TD_{50}[c]	MES	scPTZ
3a	54.8 (46.3-64.8)[e]	42.4 (35.9-50.2)	141.6 (123.70-161.6)	2.58	3.34
3b	45.6 (39.2-53.1)	50.9 (42.2-61.5)	131.2 (109.4-157.5)	2.88	2.58
3c	61.1 (51.3-72.8)	47.3 (39.7-56.4)	136.3 (118.2-157.3)	2.23	2.88
3d	84.9 (75.8-95.0)	84.8 (72.3-99.5)	203.6 (172.2-240.8)	2.40	2.40
3e	32.9 (27.8-38.9)	27.4 (22.7-33.1)	98.2 (85.1-113.3)	2.99	3.58
3f	27.4 (23.4-32.2)	22.0 (18.5-26.2)	91.3 (79.1-105.3)	3.33	4.15
Phenytoin[d]	9.5 (8.1-10.4)	＞300	65.5 (52.5-72.9)	6.9	＜0.22
Carbamazepin[d]	8.8 (5.5-14.1)	＞100	71.6 (45.9-135)	8.1	＜0.22
phenobarbital[d]	21.8 (21.8-25.5)	13.2 (5.8-15.9)	69.0 (62.8-72.9)	3.2	5.2
Valproate[d]	272 (247-338)	149 (123-177)	426 (369-450)	1.6	2.9

[a] Dose measured in mg/kg. [b] $PI = TD_{50} / ED_{50}$

[c] Minimal neurotoxicity was determined by the rotarod test 30 min after the tested compounds were administrated.

[d] Data from Huseyin, U., *et al*. 1998 [18]

[e] The 95% confidence limits

DISCUSSION

The 4-substituted-phenyl-3,4-dihydro-2(1*H*)-quinolines (**2a-f)** showed remarkable anticonvulsant activity. Compound **I** indicated anti-MES effect only under the high dose of 300 mg/kg, whereas compounds **2a-f** showed anti-MES and anti-PTZ effect at the medium dose of 100 mg/kg. We reason that the increase in anticonvulsant activity might be due to their easier transport across biological membranes after the introduction of the substituted-phenyl at the fourth position of compound **I.**

The 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinolines **(3a-f)** were prepared by incorporating a triazole group into **2a-f** at the N_1-C_2 position, which caused stronger anticonvulsant effect. Significantly, compounds **3e** and **3f** showed anticonvulsant effect at the low dose of 30 mg/kg.

Based on the result of the preliminary screening, compounds **3a-f** were selected for phase Ⅱ tests, where their anticonvulsant activity and neurotoxicity in mice were quantified and expressed in terms of median effective dose (ED_{50}) and median neurotoxic dose (TD_{50}). As shown in Table 2, ED_{50} of the anti-MES activity of the six compounds were between 27.4－84.9 mg/kg. The structure-activity relationships were concluded as following Introduction of electron donor groups such as methyl or methoxyl to the phenyl ring reduced anticonvulsant activity, whereas introduction of electron-acceptor groups such as chlorine or fluorine to the phenyl ring increased anticonvulsant activity. For example, the activity of compounds **3c, 3d** (with donor groups introduced) was lower than that of **3a**, while the anti-MES effect of **3b, 3e** and **3f** (with electron acceptor groups introduced) was higher than that of **3a.** The activity was the strongest for the two fluorine introduced compounds where the *m*-F derivative (**3f**) had higher activity than the *p*-F derivative (**3e**)**.** Compound **3f** had an ED_{50} of 27.4 mg/kg in the anti-MES test, which was higher than the control drug valproate, but was lower than phenytoin, carbamazepin and phenobarbital.

Compounds **3a-f** also possessed strong anti-PTZ effect, which was a little higher than their anti-MES effect. The strongest compound was still **3f** with ED_{50} of 22.0 mg/kg, which was stronger than the control drugs phenytoin, carbamazepin and valproate, and only weaker

than phenobarbital. scPTZ has been reported to produce seizures by inhibiting gamma-aminobutyic acid (GABA) neurotransmission (20, 21). 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 (22-24), while enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The standard anticonvulsant drugs used have also been shown to exert their anticonvulsant action by enhancing GABAergic neurotransmission and activity (23). The findings of the present study tend to suggest that the derivatives in this study might have inhibited or attenuated PTZ-induced seizures in mice by enhancing GABAergic neurotransmission.

The protective index (PI) value of compounds **3a-f** was 2.23-3.33 in MES test, among which compound **3f** had the best PI value of 3.33, which was better than control the drugs valproate and phenobarbital, but was lower than phenytoin and carbamazepin.

The 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinolin-1(2*H*)-ones (**4a-f**) were obtained by incorporating a triazolone group into N_1-C_2 positions of compounds **2a-f**, respectively However, none of these compounds showed anticonvulsant effect even at the high dose of 300 mg/kg, which was very much unexpected. It is possible, however, that triazolone incorporation into the substituted quinoline led to dramatic reduction in the lipophilicity of the compounds and made them difficult to pass biological membranes.

CONCLUSION

The 4-substitued-phenyl-3,4-dihydro-2(1*H*)-quinolines (**2a-f),** synthesized by introducing substituted-phenyl to 3,4-dihydro-2(1*H*)-quinoline at the fourth position, had remarkably increased anticonvulsant effects compared to the parent compounds. The 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinolines **(3a-f),** prepared by incorporating a triazole ring into **2a-f** at the N_1-C_2 positions, in turn had significantly increased anticonvulsant activity compared to **2a-f**. However, the compounds prepared by incorporating a triazolone into **2a-f** at the N_1-C_2 positions, namely, 5-substituted-phenyl-l-4,5-dihydro- 1,2,4-triazolo[4,3-*a*] quinoline-1(2*H*)-ones（**4a-f**）**,**exhibited no anticonvulsive effect even under a high dose of 300 mg/kg.

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