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The Synthesis and Anticonvulsant Activity of 1-Substituted-7-Methoxy-1,2,4-Triazolo [4, 3-*a*]Quinoline

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A new series of 1-substituted-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-*a*]quinolines were synthesized using ethyl-3-oxo-3-phenylpropanoate and 4-methoxybenzenamine as the starting material. Their anticonvulsant activity was evaluated by the maximal electroshock (MES) test and 7-methoxy-5-phenyl-1,2,4-triazolo[4,3-*a*]quinoline **(4a)** was identified as the most potent compound, with an ED_{50} value of 9.2 mg kg^{-1}, which is comparable to the reference drug phenytoin (ED_{50} = 9.9 mg kg^{-1}). To explore the possible mechanism of its anticonvulsant activity, compound **4a** was tested with the rotarod neurotoxicity test, pentylenetetrazole (sc-PTZ) test, and isoniazid test. Compound **4a** had a higher protective index (PI = TD_{50}/ED_{50}) value (16.6) than phenytoin (PI = 7.0), and it antagonized pentylenetetrazole- and isoniazid-induced seizures with an ED_{50} of 21.1 mg kg^{-1} and 83.3 mg kg^{-1}, respectively.

Key Words: 1,2,4-triazolo[4,3-*a*] quinoline, Anticonvulsant, MES, sc-PTZ, Isoniazid

Introduction

Epilepsy, a ubiquitous disease characterized by recurrent seizures, inflicts more than sixty million people worldwide, according to epidemiological studies.[1] Nearly 95% of the clinically available drugs used to treat epilepsy were approved before 1985 and provide satisfactory seizure control in only 60%-70% of patients. These drugs, however, also cause notable adverse side effects, such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity, and megaloblastic anemia,[2] and even life-threatening conditions.[3] The search for more effective and safer antiepileptic drugs is, therefore, an imperative challenge for medicinal chemistry.

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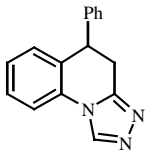
In an earlier study[4] we reported the chemical and biological analyses of a series of 5-substituted-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinolines. Some of these compounds have shown differential anticonvulsant activity, among which 5-phenyl-4,5-dihydro-1,2,4-triazolo[4,3-*a*]quinoline (compound **1**) showed a slight positive anticonvulsant activity with an ED_{50} value of 54.8 mg kg^{-1} in the maximal electroshock test (MES), while the reference drug phenytoin had an ED_{50} of 9.9 mg kg^{-1}. In order to obtain compounds with better anticonvulsant activity, we made 2 structural modifications, i.e. the introduction of a double bond into the 4th and 5th positions, and then a methoxy group in the 7th position on the phenyl ring of compound **1**, which in turn gave compound **2** and **4a**, respectively.



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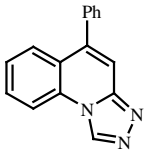

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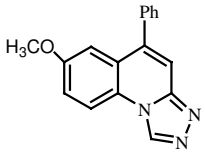


4a

Furthermore, to search for better compounds and elucidate 1-substituted structure-activity relationships, we prepared a novel series of derivatives of 1-substituted-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-*a*]quinolines and tested them as anticonvulsants. Their structures were characterized using IR, ^{1}H-NMR, MS, and elemental analysis techniques. The new compounds were evaluated as anticonvulsant agents in the MES experimental epilepsy model. The most active compound (**4a**) was further tested in the rotarod neurotoxicity test (Tox.), pentylenetetrazole (sc-PZT) test, and isoniazid test, and the possible mechanism of action is discussed.

Experimental

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded on an FT-IR-1730 spectrometer as potassium bromide disks. ^{1}H-NMR spectra were measured on a Bruker AV-300 spectrometer in $CDCl_3$. All chemical shifts are reported as δ(ppm) values. Mass spectra were measured on an Agilent Technologies HP-1100LC. Elemental analyses were performed on a Perkin-Elmer 204Q CHN. 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 analytical grade. The synthesis of N-(4-methoxyphenyl)-3-oxo-3-phenylpropanamide[9] **(1)** and 6-methoxy-4-phenyl-quinoline-2(1*H*)-one[9] **(2)** was as previously reported.

Synthesis of 5-phenyl-1,2,4-triazolo[4,3-a]quinoline (Compound 2)

4-Phenyl-quinoline (0.04 mol) and acyl hydrazide (0.04 mol) were dissolved in n-butanol in a round-bottomed flask, and the mixture was refluxed for 20-40 h in a nitrogen atmosphere. Solvents were removed under reduced pressure and the residue was extracted twice with 30 mL of dichloromethane. The dichloromethane layer was washed 3 times with water (3 × 30 mL) and dried over anhydrous $MgSO_4$. After removing the solvents the product was purified by silica gel column chromatography (dichloromethane: methanol = 20:1). Yield: 78.2%; mp: 160-162 °C. IR (KBr) cm^{-1}: 1613 (C=N), 1297 (C-N), 1243, 1024 (C-O-C), 1151 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 7.52 (s, 1H, =CH), 7.64-8.01 (m, 4H, C_6H_4), 7.55-7.78 (m, 5H, C_6H_5), 9.31 (s, 1H,

H-1); MS m/z: 246 (M + 1). Anal. for $C_{16}H_{11}N_3$: Calc. C: 78.35, H: 4.52, N: 17.13. Found C: 78.21, H: 4.39, N: 17.05.

General Procedure for the synthesis of compounds 4a-o

Synthesis of 2-Chloro-6-methoxy-4-phenyl-quinoline (3)

2-Chloro-6-methoxy-4-phenyl-quinoline (0.04 mol) was placed in a round-bottomed flask, to which 10 mL of $POCl_3$ was added. The mixture was refluxed for 7 h in a nitrogen atmosphere. After removing the solvent under reduced pressure, the residue was dissolved in 30 mL of water and then extracted with CH_2Cl_2. The CH_2Cl_2 layer was dried over anhydrous $MgSO_4$. After removing the solvents the product was purified by silica gel column chromatography (petroleum benzene: acetic ether = 5:1) and a yellow solid was obtained. Yield: 81%; mp: 103-105 °C. ^{1}H-NMR ($CDCl_3$): δ = 4.01 (s, 3H, OCH_3), 7.95(s, 1H, =CH), 8.08-8.13 (m, 3H, C_6H_3), 7.41-7.56 (m, 5H, C_6H_5); MS m/z: 269(M + 1). Anal. for $C_{16}H_{12}ClNO$: Calc. C: 71.25, H: 4.48, N: 5.19. Found C: 71.18, H: 4.54, N: 4.93.

General Procedure A for the Synthesis of 1-Ethyl-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a] quinoline (4c)

2-Chloro-6-methoxy-4-phenyl-quinoline (1.4 mmol) was dissolved in 4 mL of anhydrous pyridine in a round-bottomed flask under nitrogen. The flask was warmed to 70 °C and 1.5 mL of anhydrous hydrazine was added, and then the mixture was refluxed for 2 h. The pyridine and excess hydrazine were then removed under reduced pressure to a yellow solid. Next, the different carboxylic ester (4.7 mmol) and n-butanol were added to the flask in a nitrogen atmosphere, and the mixture was stirred for 7 h at 120 °C. Solvents were removed under reduced pressure and the residue was extracted twice with 30 mL of dichloromethane. The dichloromethane layer was washed 3 times with water (3 × 30 mL) and dried over anhydrous $MgSO_4$. After removing the solvents the product was purified by silica gel column chromatography (dichloromethane: methanol = 20:1). Yield: 66.0%; mp: 107-109 °C. IR (KBr) cm^{-1}: 1608 (C=N), 1291 (C-N), 1247, 1026 (C-O-C), 1138 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 1.24 (t, 3H, J = 6.0 Hz, CH_3), 2.69 (t, 2H, J = 6.0 Hz, CH_2), 3.78 (s, 3H, OCH_3), 7.49 (s, 1H, =CH), 7.29-8.09 (m, 3H, C_6H_3), 7.02-7.26 (m, 5H, C_6H_5); MS m/z: 304 (M + 1). Anal. for $C_{19}H_{17}N_3O$: Calc. C: 75.23, H: 5.65, N: 13.85. Found C: 75.12, H: 5.46, N: 13.69.

7-Methoxy-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4a)

General procedure A. Yield: 64.5%; mp: 120-122 °C. IR (KBr) cm^{-1}: 1610 (C=N), 1300 (C-N), 1250, 1025 (C-O-C), 1146 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 3.75 (s, 3H, OCH_3), 7.46 (s, 1H, =CH), 7.36-8.01 (m, 3H, C_6H_3), 6.96-7.30 (m, 5H, C_6H_5), 9.22 (s, 1H, H-1); MS m/z: 276 (M + 1). Anal. for $C_{17}H_{13}N_3O$: Calc. C: 74.17, H: 4.76, N: 15.26. Found C: 74.32, H: 4.64, N: 15.12.

7-Methoxy-1-methyl-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4b)

General procedure A. Yield: 65.3%; mp: 102-104 °C. IR (KBr) cm^{-1}: 1611 (C=N), 1293 (C-N), 1240, 1029 (C-O-C), 1138 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 2.37 (s, 3H, CH_3), 3.72 (s, 3H, OCH_3), 7.47 (s, 1H, =CH), 7.35 – 8.03 (m, 3H, C_6H_3), 7.00-7.27 (m, 5H, C_6H_5); MS m/z: 290 (M + 1). Anal. for $C_{18}H_{15}N_3O$: Calc. C: 74.72, H: 5.23, N: 14.52. Found C: 74.64, H: 5.15, N: 14.42.

7-Methoxy-5-phenyl-1-propyl-1,2,4-triazolo[4,3-a]quinoline (4d)

General procedure A. Yield: 64.3%; mp: 118-120 °C. IR (KBr) cm^{-1}: 1612 (C=N), 1294 (C-N), 1244, 1027 (C-O-C), 1138 (N-N); ^{1}H-NMR ($CDCl_3$): $\delta = 1.06$ (t, 3H, $J = 7.2$ Hz, CH_3), 1.25-1.66 (m, 2H , CH_2), 2.56 (t, 2H, $J = 6.8$ Hz, CH_2), 3.79 (s, 3H, OCH_3), 7.46 (s, 1H, =CH), 7.36-8.05 (m, 3H, C_6H_3), 6.99-7.34 (m, 5H, C_6H_5); MS m/z: 318 (M + 1). Anal. for $C_{20}H_{19}N_3O$: Calc. C: 75.69, H: 6.03, N: 13.24. Found C: 75.46, H: 5.75, N: 13.38.

1-Butyl-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4e)

General procedure A. Yield: 63.1%; mp: 142-144 °C. IR (KBr) cm^{-1}: 1618 (C=N), 1289 (C-N), 1181, 1043 (C-O-C), 1123 (N-N);^{1}H-NMR ($CDCl_3$): $\delta = 0.92$ (t, 3H,$J = 7.4$ Hz, CH_3), 1.25-1.65 (m, 4H, CH_2), 2.54 (t, 2H,$J = 7.5$ Hz, CH_2), 3.78 (s, 3H, OCH_3), 7.47 (s, 1H, =CH), 7.42-8.07 (m, 3H, C_6H_3), 6.98-7.35 (m, 5H, C_6H_5); MS m/z: 332 (M + 1). Anal. for $C_{21}H_{21}N_3O$: Calc. C: 76.11, H: 6.39, N: 12.68. Found C: 76.02, H: 6.17, N: 12.43.

7-Methoxy-1-pentyl-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4f)

General procedure A. Yield: 61.3%; mp: 132-134 °C. IR (KBr) cm^{-1}: 1659 (C=N), 1302 (C-N), 1243, 1027 (C-O-C), 1124 (N-N); ^{1}H-NMR ($CDCl_3$): $\delta = 0.91$ (t, 3H,$J = 7.2$ Hz, CH_3), 1.26-1.76 (m, 6H, CH_2), 2.75 (t, 2H,$J = 6.6$ Hz, CH_2), 3.73 (s, 3H, OCH_3), 7.48 (s, 1H, =CH), 7.45-8.08 (m, 3H, C_6H_3), 6.99-7.38 (m, 5H, C_6H_5); MS m/z: 346 (M + 1). Anal. for $C_{22}H_{23}N_3O$: Calc. C: 76.49, H: 6.71, N: 12.16. Found C: 76.28, H: 6.57, N: 12.01.

1-Hexyl-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4g)

General procedure A . Yield: 58.4%; mp: 118-120 °C. IR (KBr) cm^{-1}: 1597 (C=N), 1300 (C-N), 1250, 1030 (C-O-C), 1123 (N-N); ^{1}H-NMR ($CDCl_3$): $\delta = 0.88$ (t, 3H,$J = 7.0$ Hz, CH_3), 1.26-1.75 (m, 8H , CH_2), 2.74 (t, 2H,$J = 7.5$ Hz, CH_2), 3.73 (s, 3H, OCH_3), 7.47 (s, 1H, =CH), 7.40-8.02 (m, 3H, C_6H_3), 6.99-7.34 (m, 5H, C_6H_5); MS m/z: 359 (M + 1). Anal. for $C_{24}H_{29}N_3O$: Calc. C: 76.85, H: 7.01, N: 11.69. Found C: 76.61, H: 6.71, N: 11.47.

1-Heptyl-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4h)

General procedure A. Yield: 62.5%; mp: 122-124 °C. IR (KBr) cm^{-1}: 1590 (C=N), 1269 (C-N), 1234, 1039 (C-O-C), 1123 (N-N);^{1}H-NMR ($CDCl_3$): $\delta = 0.97$ (t, 3H,$J = 7.2$ Hz, CH_3), 1.26-1.75 (m, 10H , CH_2), 2.57 (t, 2H,$J = 7.4$ Hz, CH_2), 3.73 (s, 3H, OCH_3), 7.47 (s, 1H, =CH), 7.42-8.09 (m, 3H, C_6H_3), 6.97-7.38 (m, 5H, C_6H_5); MS m/z: 374 (M + 1). Anal. for $C_{24}H_{27}N_3O$: Calc. C: 77.18, H: 7.29, N: 11.25. Found C: 77.09, H: 7.35, N: 11.02.

General procedure B for the Synthesis of 7-Methoxy-1-(4-methoxyphenyl)-5-phenyl-1,2,4-triazolo[4,3-a] quinoline (4k)

2-Chloro-6-methoxy-4-phenyl-quinoline (0.04 mol) and substituted acyl hydrazide (0.04 mol) were dissolved in n-butanol in a round-bottomed flask, and the mixture was refluxed for 20-40 h in a nitrogen atmosphere. Solvents were removed under reduced pressure and the residue was extracted twice with 30 mL of

dichloromethane. The dichloromethane layer was washed 3 times with water (3 × 30 mL) and dried over anhydrous $MgSO_4$. After removing the solvents the product was purified by silica gel column chromatography (dichloromethane: methanol = 20:1). Yield: 68.5%; mp: 112-114 °C. IR (KBr) cm^{-1}: 1624 (C=N), 1295 (C-N), 1241, 1025 (C-O-C), 1153 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 3.63 (s, 3H, OCH_3), 3.73 (s, 3H, OCH_3), 7.48 (s, 1H, =CH), 7.37-8.05 (m, 3H, C_6H_3), 6.98-7.34 (m, 9H, C_6H_5 , C_6H_4); MS m/z: 382 (M + 1). Anal. for $C_{24}H_{19}N_3O_2$: Calc. C: 75.57, H: 5.02, N: 11.02. Found C: 75.36, H: 4.89. N: 11.15.

7-Methoxy-1,5-diphenyl-1,2,4-triazolo[4,3-a]quinoline (4i)

General procedure B. Yield: 59.6%; mp: 178-180 °C. IR (KBr) cm^{-1}: 1619 (C=N), 1292 (C-N), 1251, 1044 (C-O-C), 1138 (N-N);^{1}H-NMR ($CDCl_3$): δ = 3.73 (s, 3H, OCH_3), 7.49 (s, 1H, =CH), 7.41-8.04 (m, 3H, C_6H_3), 6.93-7.38 (m, 10H, 2 × C_6H_5); MS m/z: 352 (M + 1). Anal. for $C_{23}H_{17}N_3O$: Calc. C: 78.61, H: 4.88, N: 11.96. Found C: 78.42, H: 4.67, N: 11.84.

7-Methoxy-5-phenyl-1-p-tolyl-1,2,4-triazolo[4,3-a]quinoline (4j)

General procedure B. Yield: 69.6%; mp: 113-115°C. IR (KBr) cm^{-1}: 1623 (C=N), 1295 (C-N), 1237, 1041 (C-O-C), 1145 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 2.43 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 7.48 (s, 1H, =CH), 7.34-8.02 (m, 3H, C_6H_3), 6.98-7.32 (m, 9H, C_6H_5 , C_6H_4); MS m/z: 366 (M + 1). Anal. for $C_{24}H_{19}N_3O$: Calc. C: 78.88, H: 5.24, N: 11.50. Found C: 78.63, H: 5.12, N: 11.34.

1-(4-Chlorophenyl)-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4l)

General procedure B. Yield: 47.3%; mp: 192-194 °C. IR(KBr) cm^{-1}: 1624 (C=N), 1292 (C-N), 1231, 1028 (C-O-C), 1131 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 3.74 (s, 3H, OCH_3), 7.47 (s, 1H, =CH), 7.40-8.03 (m, 3H, C_6H_3), 6.97-7.36 (m, 9H, C_6H_5 , C_6H_4); MS m/z: 386 (M + 1). Anal. for $C_{23}H_{16}ClN_3O$: Calc. C: 71.59, H: 4.18, N: 10.89. Found C: 71.34, H: 4.02, N: 10.64.

1-(4-Fluorophenyl)-7-methoxy-5-phenyl-[1,2,4]triazolo[4,3-a]quinoline (4m)

General procedure B. Yield: 46.4%; mp: 176-178 °C. IR (KBr) cm^{-1}: 1623 (C=N), 1290 (C-N), 1226, 1029 (C-O-C), 1135 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 3.72 (s, 3H, OCH_3), 7.47 (s, 1H, =CH), 7.41-8.02 (m, 3H, C_6H_3), 6.98-7.23 (m, 9H, C_6H_5 , C_6H_4); MS m/z: 370 (M + 1). Anal. for $C_{23}H_{16}FN_3O$: Calc. C: 74.78, H: 4.37, N: 11.38. Found C: 74.57, H: 4.28, N: 11.11.

1-(2,5-Dichlorophenyl)-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4n)

General procedure B. Yield: 49.8%; mp: 188-190 °C. IR (KBr) cm^{-1}: 1622 (C=N), 1273 (C-N), 1228, 1040 (C-O-C), 1121 (N-N); ^{1}H-NMR ($CDCl_3$): δ = 3.73 (s, 3H, OCH_3), 7.49 (s, 1H, =CH), 7.37-8.05 (m, 3H, C_6H_3), 6.99-7.36 (m, 8H, C_6H_5 , C_6H_3); MS m/z: 420 (M + 1). Anal. for $C_{23}H_{15}Cl_2N_3O$: Calc. C: 65.73, H: 3.60, N: 10.00. Found C: 65.57, H: 3.48, N: 9.81.

1-Benzyl-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a]quinoline (4o)

General procedure B. Yield: 72.4%; mp: 122-124 °C. IR (KBr) cm^{-1}: 1613 (C=N), 1302 (C-N), 1246, 1029 (C-O-C), 1140 (N-N);^{1}H-NMR ($CDCl_3$): δ = 3.72 (s, 3H, OCH_3), 4.30 (s, 2H, CH_2), 7.48 (s, 1H, =CH),

7.38-8.08 (m, 3H, C_6H_3), 6.94-7.32 (m, 10H, C_6H_5 , C_6H_5); MS m/z: 366 (M + 1). Anal. for $C_{24}H_{19}N_3O$: Calc. C: 78.88, H: 5.24, N: 11.50. Found C: 78.69, H: 5.18, N: 11.41.

Anticonvulsant activity

All compounds were tested for anticonvulsant activity with C57B/6 mice in the 18-25-g weight range, which were purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University. The tested compounds were dissolved in polyethylene glycol-400. All compounds were tested with the MES test. In addition, compound **4a** was tested by the rotarod neurotoxicity test (Tox.), sc-PZT test, and sc-isoniazid test.

Anticonvulsant Effects in the Maximal Electroshock Seizure (MES) Test[5,6]

Seizures in mice were elicited with an alternating current of 60 Hz and 50 mA intensity. 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.

sc-PZT-induced Seizures Test[5,7]

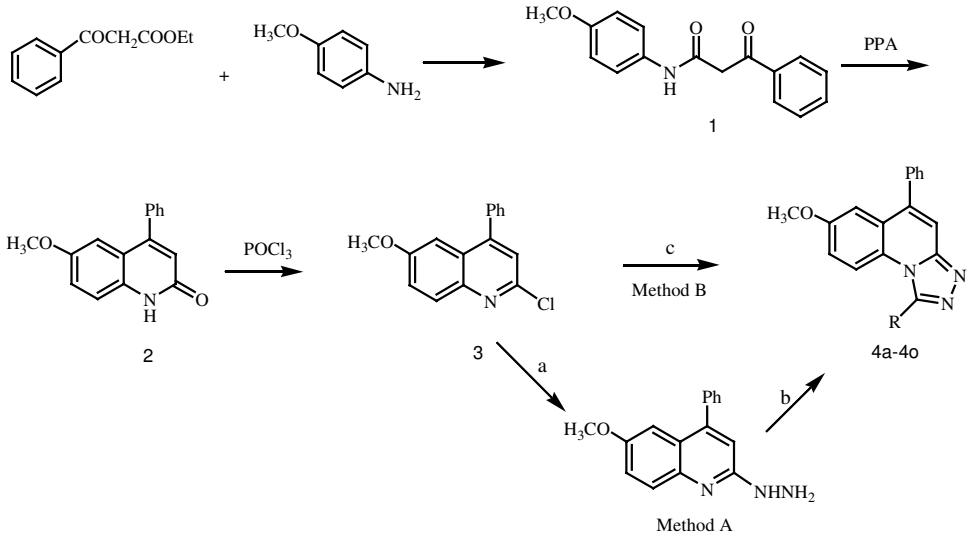
At 30 min after the administration of the test compound, 85 mg kg^{-1} pentylenetetrazole dissolved in saline was administered sc. The animals were placed in individual cages and observed for 30 min. The number of clonic and tonic seizures, as well as the number of deaths, was noted.

sc-Isoniazid-induced Seizures Test[8]

At 30 min after the administration of the test compound, the animals were given an sc. dose of isoniazid (250 mg kg^{-1}), which induced a convulsive reaction in 100% of the animals. The mice were placed in individual cages and observed for 1 h. The test compound dose that protected 50% of the treated animals from tonic convulsions (ED_{50}) was calculated.

Results and Discussion

The target compounds **4a-o** were synthesized according to Scheme 1. Compound **1** was prepared according to a previously reported procedure.[9] Briefly, it was obtained by the acylation of ethyl-3-oxo-3-phenylpropanoate and 4-methoxybenzenamine. Compound **2** was prepared from compound **1** using polyphosphoric acid as the catalyst.[9] Compound **3** was obtained[9] by the chlorization of compound **2** with phosphorus oxychloride ($POCl_3$). 1-Substituted-7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a]quinolines (**4a-o)** were prepared by 2 routes.[10−11] The first route started with compound **3** and hydrazine in pyridine at 70 °C, followed by the addition of the appropriate different carboxylic ester, and the mixture was refluxed. In the second route compound **3** was cyclized with suitable acyl hydrazine and the mixture was heated to 140-150 °C. The second route was developed due to the limitation of commercially available hydrazides.



Method A. Reagents: (a) anhydrous pyridine; (b) anhydrous hydrazine, 120 °C, 7h.

Method B. Reagents: (c) $RCONHNH_2$, n-butanol, 140-150 °C, 20-40 h.

Scheme 1. The synthesis route of compounds **4a-o**.

The MES test results of all the synthesized compounds and reference drugs are shown in Table 1. Most of the compounds showed positive anticonvulsant activity. Compound **2** (ED_{50} = 28.4 mg kg^{-1}) showed higher activity than compound **1** (ED_{50} = 54.8 mg kg^{-1}). We reason that the increase in anticonvulsant activity might have been due to the conjugation of the phenyl ring in the 5th position and the triazole nucleus through the introduction of the double bond into the 4th and 5th positions of compound **1**, which increased the electron cloud density at the triazole nucleus and then enhanced the combining power with the acceptor.

Remarkably, compound **4a** (ED_{50} = 9.2 mg kg^{-1}) showed the most potent anticonvulsant activity among all the synthesized compounds and its potency was comparable with that of the reference drug, phenytoin (ED_{50} = 9.9 mg kg^{-1}). This may have been due to the introduction of methoxyl into the 7th position of compound **2**, which further increased the electron cloud density at the triazole ring and significantly enhanced the anticonvulsant activity.

As seen in Table 1, alkyl, particularly bulky alkyl or aryl substitution of C-1, caused a decrease in anticonvulsant activity. Overall, their anticonvulsant activity was markedly lower than the unsubstituted compound **4a**, which might have been due to the electron-absorb/electron-donor effect of the aryl-substitution at the first position.

Next, the most active compound (**4a)** was evaluated in the neurotoxicity test where its protective index (PI) was calculated as an indicator of safety. Furthermore, in order to understand its mechanism of action, the activity of the compound against convulsions induced by pentylenetetrazole or isoniazid was determined. As shown in Table 2, compound **4a** showed lower neurotoxicity than phenytoin; its TD_{50} was 151 mg kg^{-1} and its PI in the MES test was 16.6, which was higher than the PI value of phenytoin. Compound **4a** also antagonized pentylenetetrazole and isoniazid-induced seizures with an ED_{50} of 21.1 mg kg^{-1} and 83.3 mg kg^{-1}, respectively.

Table 1. Quantitative anticonvulsant data for mice (test drug administered i.p.).

Compound	R	MES[a)], ED_{50}
I	–	54.8 (46.3-64.8)
II	–	28.4 (21.1-38.5)
4a	-H	9.2 (7.0-12.1)[b)]
4b	-CH_3	56.2 (46.8-67.6)
4c	-C_2H_5	81.5 (67.9-109.1)
4d	-n-C_3H_7	90.6 (76.2-109.2)
4e	-n-C_4H_9	91.0 (75.9-109.7)
4f	-n-C_5H_{11}	> 200
4g	-n-C_6H_{13}	> 200
4h	-n-C_7H_{15}	> 200
4i	-C_6H_5	91.9 (76.1-110.9)
4j	-C_6H_5(*p*-CH_3)	101.9 (84.9-122.2)
4k	-C_6H_5(*p*-OCH_3)	53.4 (43.7-62.9)
4l	-C_6H_5 (*p*-Cl)	94.9 (78.0-116.9)
4m	-C_6H_5(*p*-F)	44.0 (36.2-52.5)
4n	-C_6H_4(2,5-Cl_2)	> 200
4o	-$CH_2C_6H_5$	81.1 (67.8-109.4)
Phenytoin	–	9.9 (8.3-11.8)

[a]Maximal electroshock test.

[b]95% confidence limits.

Table 2. Effect of compound **4a** on chemical substance-induced convulsion and rotarod neurotoxicity test.

Compound	ED_{50}^{a}			Tox, TD_{50}^{c}	PI (TD_{50} / ED_{50})[b]		
	MES	PTZ	Isoniazid		MES	PTZ	Isoniazid
4a	9.2 (7.0-12.1)	21.1 (17.4-25.0)	83.3 (69.3-100.2)	152.1 (127.6-181.2)	16.6	7.2	1.8
Phenytoin	9.9 (8.3-11.8)			69.3	7.0		

[a]Dose measured in mg kg^{-1}.

[b]PI = TD_{50}/ED_{50}.

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

Pentylenetetrazole and isoniazid have been reported to produce seizures by inhibiting gamma-amino-butyric acid (GABA) neurotransmission.[12,13] GABA is the main inhibitory neurotransmitter substance in the brain and is widely implicated in epilepsy. Inhibition of GABA-ergic neurotransmission or activity has been shown to promote and facilitate seizures,[14] while enhancement of GABA-ergic 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 and isoniazid-induced seizures in mice by enhancing GABA-ergic neurotransmission.

Conclusion

In the present study, through a series of substitutions at the first position of 7-methoxy-5-phenyl-1,2,4-triazolo[4,3-a] quinoline, the compound without substitution **(4a)** was found to posses the most potent anticonvulsant activity in the MES test, which was comparable to phenytoin. Compound **4a** had a higher protective index than phenytoin. Compound **4a** antagonized pentylenetetrazole and isoniazid-induced seizures, which suggest that compound **4a** might effect GABA-ergic neurotransmission and the glycine system.

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References

1. W. Loscher, **Eur. J. Pharmacol. 1,** 1-13 (1998).
2. I. E. Leppik, **Epilepsia. 35 (S4),** 29-40 (1994).
3. Y. A. Al-Soud, N. A.Al-Masoudi and Ael-R. Ferwanah, **Bioorg. Med. Chem. 11,** 1701-1708 (2003).
4. L. P. Guan, Q. H. Jin, G. R. Tian, K. Y. Chai and Z. S. Quan, **J. Pharm. Pharm. Sc. 3,** 254-262 (2007).
5. R. J. Krall, J. K. Penry, B. G. White and H. J. Kupferberg, **Epilepsia. 19,** 409-428 (1978).
6. R. J. Poter, J. J. Cereghino, G. D. Gladding, B. J. Hessie, H. J. Kupferberg and B. Scoville, **Clev. Clin. J. Med. 51,** 293-305 (1984).
7. M. Geurts, J. H. Poupaert, G. K. Scriba and D. M. Lambert, **J. Med. Chem. 41,** 24-30 (1998).
8. R. Bernasconi, M. Klein, P. Martin, P. Christen, T. Hafner, C. Portet and M. Schmutz, **J. Neural. Transm. 72,** 213-233 (1988).
9. K. Hino, K. Kawashima, M. Oka, H. Uno, and J. I. Matsumoto, **Chem. Pharm. Bull. 1**,190-192 (1989).
10. B. Bouteau, J. C. Lancelot and M. Robba, **J. Heterocycl. Chem. 6**, 1649-1651 (1990).
11. D. B. Moran, G. O. Morton and J. D. Albright, **J. Heterocycl. Chem. 23**, 1071-1077 (1986).
12. R. Okada and H. Negishi, **Brain Res. 480,** 383-387 (1989).
13. R.W. Olsen, **J. Neurochem. 27,** 1-3 **(**1981).
14. K. Gale, **Epelepsia. 33,** S3-S12 (1992).