Synthesis and Anticonvulsant Activity of 1-Substituted-7-Benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline

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Starting from 6-hydroxy-3,4-dihydro-1*H*-quinoline-2-one, a series of 1-substituted-7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinolines was synthesized and their structures were characterized using IR, ^{1}H-NMR, MS, and elemental analysis techniques. Anticonvulsant activity was evaluated in the maximal electroshock (MES) test, subcutaneous pentylenetetrazol (scMet) test, and rotarod neurotoxicity test. The most active compound was 7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline 4a. Its ED_{50} in the MES and scMet tests was 17.3 and 24 mg·kg^{-1}, respectively. The safest compound was 4g, 1-phenyl-7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline, with TD_{50} and protective index (PI) (PI=TD_{50}/ED_{50}) values of greater than 300 mg·kg^{-1} and 13, respectively. The PI value of compound 4g was better than that of most marketed drugs. Structure–activity relationships are also described in this paper.

Key words [1,2,4]triazolo[4,3-a]quinoline; anticonvulsant; maximal electroshock test; pentylenetetrazole; neurotoxicity

Epilepsy, one of the most common neurologic diseases, is characterized by epileptic seizures, which are evoked by unexpected, high-level neuronal discharges in the brain.[1)] Since the anticonvulsant agents currently used in the treatment of epilepsy have certain disadvantages such as notable side effects and inefficient therapy in some seizure types, a clear need for safer and more effective antiepileptic drugs is well known.[1—3)] Therefore the development of new antiepileptic drugs with approved therapeutic properties is an important challenge for medicinal chemists.

The derivatives of triazole exhibit a variety of activities such as antitumor,[4)] antiinflammatory,[5)] antimicrobial,[6,7)] antifugal,[8)] antithrombotic,[9)] antiplatelet,[10)] antiviral,[11)] and anticonvulsant activities.[12—14)] In our search for new compounds with anticonvulsant activity, 6-benzyloxy-3,4-dihydro-1*H*-quinoline-2-one was found although its activity is low. To increase its anticonvulsant activity and taking into account the anticonvulsant activity of triazole, we incorporated triazole with 6-benzyloxy-3,4-dihydro-1*H*-quinolinone at the first and second position of the latter and obtained 7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline with more potent activity. To search for better compounds and elucidate structure–activity relationships, a series of 1-substituted-7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinolines was synthesized and their structures were characterized using IR, ^{1}H-NMR, MS, and elemental analysis techniques. The anticonvulsant activity was evaluated using the maximal eletroshock (MES) test and the subcutaneous pentylenetetrezole (sc-Met) test. Neurotoxicity was evaluated using the rotarod test.

CHEMISTRY

Melting points were determined in open capillary tubes and are uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730, ^{1}H-NMR spectra were measured on a BRUKER-300, and all chemical shifts are given in ppm relative to tetramethysilane. Mass spectra were measured on an AP12000 (EIS, 70 eV). Elemental analyses were performed on a Pekin-Elmer 204Q. Microanalyses of C, N, and H were performed using a Heraeus CHN Rapid Analyzer.

6-Hydroxy-3,4-dihydro-1*H*-quinoline-2-one 1 6-Hydroxy-3,4-dihydro-2-(1*H*)-quinoline-2-one was prepared from phenylamine and 3-chloro-propionylchloride using the method described previously,[15)] the total yield was 59%, mp 234—236 °C (value in the literature, 235—236 °C).

6-Benzyloxy-3,4-dihydro-1*H*-quinoline-2-one 2 Sodium hydroxide (0.09 mol), 60 ml of absolute ethanol, and 6-hydroxy-3,4-dihydro-(1*H*)-quinoline-2-one (0.03 mol) were added to a 100 ml round-bottomed flask equipped with a reflux condenser, and chloromethyl–benzene (0.36 mol) was added dropwise to the mixture. The reaction mixture was refluxed for 2.5 h and cooled. After being poured into 100 ml of ice-water, a white solid was obtained through filtration, which was dried in a vacuum and recrystallized in hexane–ethannol (5 : 1), with a yield of 72%. mp 159—161 °C. IR (KBr) cm^{-1}: 3432 (N–H), 1675 (C=O), 1238, 1056 (C–O–C). ^{1}H-NMR ($CDCl_3$) δ: 2.39 (t, 2H, J=7.1 Hz, CH_2), 2.82 (t, 2H, J=7.1 Hz, CH_2), 5.02 (s, 2H, CH_2), 6.75—6.87 (m, 3H, C_6H_3–), 7.31—7.46 (m, 5H, C_6H_5–), 9.94 (s,

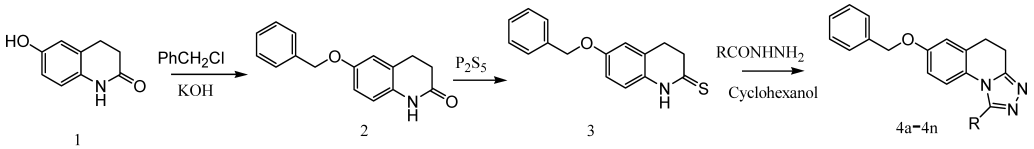

Fig. 1. Synthesis of Compounds **4a**—**n**

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1H, NH). 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, 5.73; N, 5.38.

6-Benzyloxy-3,4-dihydro-1*H*-quinoline-2-thione 3 Some improvements were made based on the method described previously.[16] Acelonitrile 30 ml and triethylamine 20 ml were placed in a three-necked round-bottomed flask, to which P_2S_5 1.3 g was added slowly in an ice bath and stirred until dissolved. Then **2** was added with stirring. The mixture was refluxed for 3 h in a nitrogen atmosphere. After removing the solvent under reduced pressure, the residue was dissolved in 30 ml of dichloromethane, washed with water (30×3), and dried over anhydrous $MgSO_4$. Evaporation of the solvents gave a crude product that was purified by recrystallization in hexane–ethanol (10 : 1) and a yellow solid was obtained in a yield of 68%. mp 156—159 °C. IR (KBr) cm^{-1}: 3437 (N–H), 1237, 1052 (C–O–C). ^{1}H-NMR ($CDCl_3$) δ: 2.74 (t, 2H, J=7.4 Hz, CH_2), 2.89 (t, 2H, J=7.4 Hz, CH_2), 5.05 (s, 2H, CH_2), 6.85—7.03 (m, 3H, C_6H_3), 7.32—7.44 (m, 5H, C_6H_5), 12.14 (s, 1H, NH). MS: (M+1) 270. *Anal*. Calcd for $C_{24}H_{21}N_3O$: C, 71.34; H, 5.61; N, 5.20. Found: C, 71.74; H, 5.82; N, 5.33.

General Procedure for the Synthesis of 1-Substituted-7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinolines 4a—n The method described previously was used with some improvements. Compound **3**, suitable acylhydrazine, and cyclohexanol 60 ml were added to a 100 ml three-necked round-bottomed flask, and the mixture was refluxed for 6 h in a nitrogen atmosphere. Solvents were removed under reduced pressure, and the residue was extracted twice with dichloromethane 30 ml. The dichloromethane layer was washed three times with water (30×3) and dried over anhydrous $MgSO_4$. After removing the solvents, products was purified by silica gel column chromatography (dichloromethane : methanol=10 : 1).

7-Benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4a**: Yield=64.5%, mp 174—176 °C. IR (KBr) cm^{-1}: 1614 (C=N), 1311 (C–N), 1255, 1022 (C–O–C), 1155 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 3.03 (t, 2H, J=7.2 Hz, CH_2), 3.22 (t, 2H, J=7.4 Hz, CH_2), 5.09 (s, 2H, CH_2), 6.94—7.24 (m, 3H, H-6, H-8, H-9), 7.26—7.42 (m, 5H, C_6H_5), 8.67 (s, 1H, H-1). MS: (M+1) 278. *Anal*. Calcd for $C_{17}H_{15}N_3O$: C, 73.63; H, 5.45; N, 15.15. Found: C, 73.41; H, 5.63; N, 15.38.

7-Benzyloxy-1-methyl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4b**: Yield=71%, mp 116—118 °C. IR (KBr) cm^{-1}:1620 (C=N), 1290 (C–N), 1244, 1026 (C–O–C), 1134 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 2.74 (s, 3H, CH_3), 2.96 (t, 2H, J=6.8 Hz, CH_2), 3.11 (t, 2H, J=6.6 Hz, CH_2), 5.03 (s, 1H, OCH_2), 6.94 (dd, 1H, J=2.8, 8.8 Hz, H-8), 6.98 (d, 1H, J=2.8 Hz, H-6), 7.39 (d, 1H, 9.0 Hz, H-9), 7.35—7.44 (m, 5H, C_6H_5). MS: (M+1) 292. *Anal*. Calcd for $C_{18}H_{17}N_3O$: C, 74.20; H, 5.88; N, 14.42. Found: C, 74.52; H, 5.62; N, 14.10.

7-Benzyloxy-1-propyl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4c**: Yield=75%, yellow oil. IR (KBr) cm^{-1}: 1612 (C=N), 1295 (C–N), 1254, 1026 (C–O–C), 1134 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 1.07 (t, 3H, J=7.2 Hz, CH_3), 1.88—1.96 (m, 2H, CH_2), 2.95 (t, 2H, J=6.8 Hz, CH_2), 3.01 (t, 2H, J=7.6 Hz, CH_2), 3.11 (t, 2H, J=7.5 Hz, CH_2), 5.09 (S, 2H, OCH_2), 6.94 (dd, 1H, J=2.8, 9.2 Hz, H-8), 6.98 (d, 1H, J=2.8 Hz, H-6), 7.39 (d, 1H, 9.2 Hz, H-9), 7.26—7.44 (m, 5H, C_6H_5). MS: (M+1) 320. *Anal*. Calcd for $C_{20}H_{21}N_3O$: C, 75.21; H, 6.63; N, 13.16. Found: C, 75.54; H, 6.87; N, 13.39.

7-Benzyloxy-1-pentyl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4d**: Yield=72%, mp 88—90 °C. IR (KBr) cm^{-1}: 1615 (C=N), 1292 (C–N), 1242, 1028 (C–O–C), 1140 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 0.91 (t, 3H, J=7.2 Hz, CH_3), 1.25—1.47 (m, 6H, CH_2), 1.88—1.92 (m, 2H, CH_2), 2.96 (t, 2H, J=6.6 Hz, CH_2), 3.04 (t, 2H, J=7.6 Hz, CH_2), 3.11 (t, 2H, J=7.6 Hz, CH_2), 5.10 (s, 2H, OCH_2), 6.95 (d, 1H, J=9.2 Hz, H-8), 6.98 (s, 1H, H-6), 7.38 (d, 1H, J=9.2 Hz, H-9), 7.26—7.45 (m, 5H, C_6H_5) MS: (M+1) 348.1. *Anal*. Calcd for $C_{22}H_{25}N_3O$: C, 76.05; H, 7.25; N, 12.09. Found: C, 75.78; H, 7.54; N, 12.32.

1-Benzyl-7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4e**: Yield=78%, mp 94—96 °C. IR (KBr) cm^{-1}:1610 (C=N), 1296 (C–N), 1246, 1026 (C–O–C), 1132 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 2.95 (t, 2H, J=7.0 Hz, CH_2), 3.14 (t, 2H, J=7.0 Hz, CH_2), 4.42 (s, 2H, CH_2), 5.05 (s, 2H, OCH_2), 6.79 (dd, 1H, J=2.4, 8.8 Hz, H-8), 6.95 (d, 1H, J=2.0 Hz, H-6), 7.14 (d, 1H, J=8.8 Hz, H-9), 7.16—7.38 (m, 10H, 2×C_6H_5) MS: (M+1) 368. *Anal*. Calcd for $C_{24}H_{21}N_3O$: C, 78.45; H, 5.76; N, 11.44. Found: C, 78.74; H, 5.82; N, 11.83.

7-Benzyloxy-1-(4-chloro-benzyl)-4,5dihydro-[1,2,4]triazolo[4,3-a]quinoline **4f**: Yield=80%, mp 176—178 °C. IR (KBr) cm^{-1} 1612 (C=N), 1308 (C–N), 1242, 1022 (C–O–C), 1126 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 2.98 (t, 2H, J=7.6 Hz, CH_2), 3.12 (t, J=7.6 Hz, 2H, CH_2), 4.44 (s, 2H, CH_2), 5.03 (s, 2H, CH_2), 6.77 (dd, J=2.6, 8.8 Hz , 1H, H-8), 6.93 (d, J=2.4 Hz, 1H, H-6), 7.17 (d, J=8.8 Hz, 1H, H-9), 7.13—7.40 (m, 9H, C_6H_5, C_6H_4) MS: (M+1) 403. *Anal*. Calcd for $C_{24}H_{20}ClN_3O$: C, 71.73; H, 5.02; N, 10.46. Found: C, 71.48; H, 5.13; N, 10.17.

7-Benzyloxy-1-phenyl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4g**: Yield=72%, mp 147—150 °C. IR (KBr) cm^{-1}: 1610 (C=N), 1306 (C–N), 1250, 1012 (C–O–C), 1097 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 3.04 (t, 2H, J=6.8 Hz, CH_2), 3.18 (t, 2H, J=6.8 Hz, CH_2), 5.05 (s, 2H, CH_2), 6.66 (dd, 1H, J=2.8, 8.8 Hz, H-8), 6.81 (d, 1H, J=8.8 Hz, H-9), 6.97 (d, 1H, J=2.4 Hz, H-6), 7.34—7.63 (m, 10H, 2×C_6H_5). MS: (M+1) 354.2. *Anal*. Calcd for $C_{23}H_{19}N_3O$: C, 78.16; H, 5.42; N, 11.89. Found: C, 77.81; H, 5.61; N, 11.57.

7-Benzyloxy-1-(3-methoxy-phenyl)-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4h**: Yield=92%, mp 126—128 °C. IR (KBr) cm^{-1}: 1614 (C=N), 1290 (C–N), 1252, 1028 (C–O–C), 1144 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 3.04 (t, 2H, J=6.8 Hz, CH_2), 3.18 (t, 2H, J=7.0 Hz, CH_2), 3.82 (s, 3H, CH_3), 5.04 (s, 2H, CH_2), 6.67 (dd, 1H, J=2.4, 8.8 Hz, H-8), 6.86 (d, 1H, J=8.8 Hz, H-9), 6.97 (d, 1H, J=2.4 Hz, H-6), 7.06—7.41 (m, 9H, C_6H_5, C_6H_4). MS: (M+1) 384. *Anal*. Calcd for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.29; H, 5.35; N, 11.14.

7-Benzyloxy-1-*m*-tolyl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4i**: Yield=78%, mp 138—140 °C. IR (KBr) cm^{-1}: 1610 (C=N), 1290 (C–N), 1250, 1045 (C–O–C), 1142 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 2.40 (s, 3H, CH3), 3.06 (t, 2H, J=7.0 Hz, CH_2), 3.23 (t, 2H, J=6.8 Hz, CH_2), 5.05 (s, 2H, CH_2), 6.67 (dd, 1H, J=2.4, 8.8 Hz, H-8), 6.86 (d, 1H, J=8.8 Hz, H-9), 6.98 (d, 1H, J=2.4 Hz, H-6), 7.26—7.49 (m, 9H, C_6H_5, C_6H_4). MS: (M+1) 368. *Anal*. Calcd for $C_{24}H_{21}N_3O$: C, 78.45; H, 5.76; N, 11.44. Found: C, 78.22; H, 5.94; N, 11.44.

7-Benzyloxy-1-(3-chloro-phenyl)-4,5-dihydro-[1,2,4]tri-

azolo[4,3-a]quinoline **4j**: Yield=80%, mp 174—176 °C. IR (KBr) cm^{-1}: 1620 (C=N), 1294 (C–N), 1248, 1043 (C–O–C), 1151 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 3.04 (t, 2H, J=6.6 Hz, CH_2), 3.18 (t, 2H, J=6.8 Hz, CH_2), 5.05 (s, 2H, CH_2), 6.70 (dd, 1H, J=2.4, 8.8 Hz, H-8), 6.82 (d, 1H, J=8.8 Hz, H-9), 6.99 (d, 1H, J=2.4 Hz, H-6), 7.26—7.66 (m, 9H, C_6H_5, C_6H_4) MS: (M+1) 389. *Anal*. Calcd for $C_{23}H_{18}ClN_3O$: C, 71.22; H, 4.68; N, 10.83. Found: C, 71.39; H, 4.36; N, 11.12.

7-Benzyloxy-1-(2,5-dichloro-phenyl)-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4k**: Yield=60%, mp 172—174 °C. IR (KBr) cm^{-1}: 1626 (C=N), 1296 (C–N), 1242, 1026 (C–O–C), 1155 (N–N), 600 (C–Cl). ^{1}H-NMR ($CDCl_3$) δ: 3.06 (t, 2H, J=6.8 Hz, CH_2), 3.38 (t, 2H, J=6.8 Hz, CH_2), 5.03 (s, 2H, CH_2), 6.60 (d, 1H, J=9.2 Hz, H-9), 6.66 (dd, 1H, J=2.4, 9.2 Hz, H-8), 6.97 (d, 1H, J=2.4 Hz, H-6), 7.26—7.68 (m, 8H, C_6H_5, C_6H_4). MS: (M+1) 425. *Anal*. Calcd for $C_{23}H_{17}Cl_2N_3O$: C, 65.41; H, 4.06; N, 9.95. Found: C, 65.27; H, 4.38; N, 9.75.

7-Benzyloxy-1-pyridin-4-yl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4l**: Yield=77%, mp 176—179 °C. IR (KBr) cm^{-1}: 1606 (C=N), 1309 (C–N), 1248, 1018 (C–O–C), 1152 (N–N). ^{1}H-NMR ($CDCl_3$) δ: 3.05 (t, 2H, J=8 Hz, CH_2), 3.17 (t, 2H, J=8 Hz, CH_2), 5.05 (s, 2H, CH_2), 6.71 (d, 1H, J=8 Hz, H-8), 6.83 (d, 1H, J=8 Hz, H-9), 7.00 (s, 1H, H-6), 7.33—7.40 (m, 5H, C_6H_5), 7.58 (d, 2H, J=4 Hz, H-2′, H-6′), 8.75 (d, 2H, J=4 Hz, H-3′, H-5′) MS: (M+1) 355. *Anal*. Calcd for $C_{22}H_{18}N_4O$: C, 74.56; H, 5.12; N, 15.81. Found: C, 74.69; H, 5.01; N, 16.11.

7-Benzyloxy-1-phenoxymethyl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4m**: Yield=90%, mp 136—138 °C. IR (KBr) cm^{-1}: 1608 (C=N), 1309 (C–N), 1252, 1020 (C–O–C), 1120 (N–N). ^{1}H-MNR ($CDCl_3$) δ: 2.98 (t, 2H, J=7.0 Hz, CH_2, 3.18 (t, 2H, J=7.2 Hz, CH_2), 5.09 (s, 2H, CH_2), 5.38 (s, 2H, CH_2), 6.91 (dd, 1H, J=9.0, 2.2 Hz, H-8), 7.00 (d, 1H, J=2.2 Hz, H-6), 7.13 (d, 1H, J=9.0 Hz, H-9), 7.02—7.77 (m, 10H, 2×C_6H_5). MS: (M+1) 384. *Anal*. Calcd for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.02; H, 5.80; N, 11.24.

7-Benzyloxy-1-(4-chloro-phenoxymethyl)-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline **4n**: Yield=88%, mp 104—106 °C. IR (KBr) cm^{-1}: 1610 (C=N), 1294 (C–N), 1236, 1020 (C–O–C), 1140 (N–N). ^{1}H-MNR ($CDCl_3$) δ: 2.98 (t, 2H, J=6.8 Hz, CH_2), 3.16 (t, 2H, J=7.0 Hz, CH_2), 5.09 (s, 2H, CH_2), 5.36 (s, 2H, CH_2), 6.93 (dd, 1H, J=2.8, 8.8 Hz, H-8), 6.98 (d, 1H, J=2.8 Hz, H-6), 7.07 (d, 1H, J=8.8 Hz, H-9), 7.24—7.71 (m, 9H, C_6H_5, C_6H_4). MS: (M+1) 419. *Anal*. Calcd for $C_{24}H_{20}ClN_3O_2$: C, 68.98; H, 4.82; N, 10.06. Found: C, 69.24; H, 4.68; N, 10.21.

PHARMACOLOGY

The MES test, scMet test, and rotarod test were carried out by the Antiepileptic Drug Development Program (ADD), Epilepsy Branch, National Institutes of Health, Bethesda, MD, U.S.A.[17,18] All compounds were tested for anticonvulsant activity with C57B/6 mice in the 18—25 g 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 screeng (Table 1) each compound was administered at three dose levels (30, 100, and 300 mg/kg i.p., to a total of 6 mice, using 2 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 scMet 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 scMet test involved subcutaneous injection of a convulsant dose (CD_{97}) of pentylenetetrazol (85 mg/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.

Table 1. Phase I Anticonvulsant and Toxicity Data in Mice (i.p.)[a]

Compd.	MES[b]		ScMet[c]		Rotarod toxicity	
	0.5 h	4 h	0.5 h	4 h	0.5 h	4 h
4a	30[d]	—	100	—	100	—[e]
4b	30	—	100	—	100	—
4c	100	—	300	—	100	100
4d	300	300	—	—	300	300
4e	100	100	300	—	—	—
4f	—	—	—	—	—	—
4g	30	100	100		—	—
4h	100	—	100	—	300	—
4i	100	—	100	—	300	—
4j	—	—	—	—	—	—
4k	—	—	—	—	—	—
4l	100	100	30	—	300	—
4m	—	—	—	—	—	—
4n	—	—	—	—	—	—

a) All tested compounds were dissolved in polyethylene glycol-400. *b*) The maximal electroshock test was induced 30 min post administration of the tested compounds. *c*) Subcutaneous pentylenetetrazol (85 mg·kg^{-1}) 30 min after the tested compounds were administered for 30 min. *d*) Doses are denoted in mg·kg^{-1}. *e*) —=no activity at 300 mg·kg^{-1}.

The pharmacologic parameters estimated in phase I screening were quantified for compounds **4a**—**c**, **4e**, **4g**—**i**, and **4l** 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 Institue of Neurological Disorders and Stroke.

RESULTS AND DISCUSSION

Based on previous reports,[12—14] we knew that trazoles have activity against both major and minor seizures. The MES test is regarded as the pharmacologic model of grand mal, and the sc-Met test as the pharmacologic model of petit mal seizures. Therefore we carried out those two tests to

Table 2. Phase II Quantitative Anticonvulsant Data in Mice (Test Drug Administered i.p.)

Compd.	ED_{50} [a]		Rotarod toxicity	PI [b]	
	MES	scMet	TD_{50} [c]	MES	scMet
4a	17.3 (14.8—20.4) [e]	24.0 (21.6—26.7)	61.4 (51.4—73.3)	3.5	2.6
4b	30.7 (26.1—36.1)	42.5 (35.4—50.9)	85.2 (78.1—92.9)	2.8	2.0
4c	97.7 (84.0—113.7)	>100	264	2.7	<2.6
4e	67.9 (59.6—77.4)	>100	212 (177—254)	3.1	<2.1
4g	23.0 (18.8—28.0)	61.0 (56.5—65.9)	>300	>13	>5
4h	60.6 (52.9—69.4)	>100	237 (200—280)	3.9	<2.4
4i	30.4 (27.2—34.1)	58.9 (49.1—70.7)	183 (155—217)	6.0	3.1
4l	58.5 (45.9—74.5)	>100	176 (151—206)	3.0	<1.8
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	<0.76
Phenobarbital [d]	21.8 (21.8—25.5)	13.2 (5.8—15.9)	69 (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 $mg \cdot kg^{-1}$. *b*) $PI = TD_{50}/ED_{50}$. *c*) Minimal neurotoxiciity was determined using the rotarod test after the tested compounds were administered for 30 min. *d*) Data from Huseyin *et al*., 1998.[19] *e*) 95% confidence limits.

evaluate the anticonvulsant activity of the synthesized compounds.

The compounds were tested for anticonvulsant activity using the procedures described previously.[17,18] The initial evaluation (phase I) of anticonvulsant activity of synthesized compounds is presented in Table 1. The compounds were administered intraperitoneally at three doses (30, 100, 300 $mg \cdot kg^{-1}$). Three tests were performed for each compound: MES-induced convulsions, sc-Met-induced convulsions, and rotarod neurotoxicity test.

As a result of preliminary screening , compounds **4a**—**c**, **4e**, **4g**—**i**, and **4l** were subjected to phase II trials for quantification of their anticonvulsant activity and neurotoxicity in mice. This phase provides an evaluation of the ED_{50} and TD_{50} values. The 95% confidence interval, slope of the regression line, and SE of the slope were then calculated. These data are shown in Table 2, which also includes comparisons with marketed antiepileptic drugs such as phenytoion, carbamazepine, phenobarbital, and valproate. Some of these derivatives showed a high degree of protection against MES-induced seizures, although they were less effective against scMet-induced seizures.

The following structure–activity relationships were observed. In a series of alkyl substitutions at the first position, the increase in the carbon number at the first position decreased the anticonvulsant activity markedly. The ED_{50} values of compounds **4a**—**c**, which were H, CH_3 and C_3H_7 at the first position, were 17.3, 30.7, and 97.7 $mg \cdot kg^{-1}$, respectively. The same tendency was seen in the rotarod neurotoxicty test. The values of their corresponding TD_{50} were 61.4, 85.2, and 264 $mg \cdot kg^{-1}$, respectively. Compound **4a** was the most active among the 14 compounds synthesized, with an ED_{50} value of 17.3 $mg \cdot kg^{-1}$ in the MES test. Its activity was more potent than that of valproate ($ED_{50} = 272\ mg \cdot kg^{-1}$), comparable to that of phenobarbital ($ED_{50} = 21.8\ mg \cdot kg^{-1}$), and less effective than that of phenytoin ($ED_{50} = 9.5\ mg \cdot kg^{-1}$). Among the aryl-substituted derivatives at the first position, the potency of compounds containing a substituted phenyl at the first position was less than that of those with an unsubstituted phenyl. Compounds **4g**, **4h**, and **4j**, which were -Ph, 3-CH_3Ph, or 3-OCH_3Ph at the first position, respectively, had corresponding ED_{50} value in the MES test of 23, 30.4, and 60.6 $mg \cdot kg^{-1}$, respectively. Introduction of a chlorine atom into the phenyl at the first position of the derivatives lead to a complete loss of potency, as seen in compounds **4f**, **4j**, **4k**, and **4n**, which did not exhibit any anticonvulsant activity in the MES test even at a dose of 300 $mg \cdot kg^{-1}$. Compounds substituted with a pyridine at the first position exhibited less activity than those substituted with a phenyl. Compound **4l**, which was substituted with pyridine at the first position, with an ED_{50} value of 58.5 $mg \cdot kg^{-1}$ in the MES test, was less active than compound **4g** ($ED_{50} = 23.0\ mg \cdot kg^{-1}$) which was substituted with a phenyl at the first position.

The neurotoxicity of compounds with an unsubstituted phenyl was less than that of those with a substituted phenyl. Therefore compound **4g** with a TD_{50} value of greater than 300 $mg \cdot kg^{-1}$, ED_{50} of 23.0 $mg \cdot kg^{-1}$, and protective index ($PI = TD_{50}/ED_{50}$) of more than 13 was the safest among the compounds synthesized. Compared with the marketed drugs, its ED_{50} value was comparable to that of phenobarbital in the MES test but its PI value was higher than that of phenytoin, carbamazepine, phenobarbital, and valproate. No dose-resistance tendency was seen. With the increase in dose, activity against both types of seizure improved, although their toxicity also became greater.

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