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

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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. 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. 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, antiinflammatory, antimicrobial, antifungal, antithrombotic, antiplatelet, antiviral, and anticonvulsant activities. In our search for new compounds with anticonvulsant activity, 6-benzyloxy-3,4-dihydro-1H-quinoline-2-one was prepared from phenylamine and 3-chloro-propionylchloride using the method described previously, the total yield was 59%, mp 234—236°C (value in the literature, 235—236°C). 6-Benzylamoxy-3,4-dihydro-1H-quinoline-2-one was prepared in open capillary tubes and are uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730. 1H-NMR spectra were measured on a BRUKER-300, and all chemical shifts are given in ppm relative to tetramethylsilane. 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-1H-quinoline-2-one 1

6-Hydroxy-3,4-dihydro-1H-quinoline-2-one was prepared from phenylamine and 3-chloro-propionylchloride using the method described previously, the total yield was 59%, mp 234—236°C (value in the literature, 235—236°C). 6-Benzyloxy-3,4-dihydro-1H-quinoline-2-one was synthesized and their structures were characterized using IR, 1H-NMR, MS, and elemental analysis techniques. Anticonvulsant activity was evaluated in the maximal electroshock (MES) test, subcutaneous pentylenetetrazol (scMet) test, and rotorod neurotoxicity test. The most active compound was 7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline 4a. Its ED<sub>50</sub> in the MES and scMet tests was 17.3 and 24 mg·kg<sup>−1</sup>, respectively. The safest compound was 4g, 1-phenyl-7-benzyloxy-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline, with TD<sub>50</sub> and protective index (PI) (PI = TD<sub>50</sub>/ED<sub>50</sub>) values of greater than 300 mg·kg<sup>−1</sup> 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
7-Benzyl-1-pentyl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline 4d: Yield = 78%, mp 88—90°C. IR (KBr) cm⁻¹: 1615 (C=N), 1292 (C–N), 1242, 1028 (C–O–C), 1140 (N–N). ¹H-NMR (CDCl₃) δ: 0.91 (t, 3H, J = 7.2 Hz, CH₃), 1.25—1.47 (m, 6H, CH₂), 1.88—1.92 (m, 2H, CH₂), 2.96 (t, 2H, J = 6.6 Hz, CH₃), 3.04 (t, 2H, J = 7.6 Hz, CH₂), 3.11 (t, 2H, J = 7.6 Hz, CH₂), 5.10 (s, 2H, OCH₂), 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, C₆H₅) MS: (M+1) 348.1. Anal. Calc. for C₂₅H₂₅N₃O: 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). ¹H-NMR (CDCl₃) δ: 2.95 (t, 2H, J = 7.0 Hz, CH₂), 3.14 (t, 2H, J = 7.0 Hz, CH₂), 4.42 (s, 2H, CH₂), 5.05 (s, 2H, OCH₂), 6.79 (dd, 1H, J = 2.4, 8.8 Hz, H-8), 6.95 (d, 1H, J = 2.0 Hz, H-6), 7.14—7.38 (m, 10H, 2xC₆H₅) MS: (M+1) 368. Anal. Calc. for C₂₅H₂₅N₃O: C, 78.45; H, 7.56; N, 11.44. Found: C, 78.74; H, 5.82; N, 11.83.

Benzyl-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). ¹H-NMR (CDCl₃) δ: 2.98 (t, 2H, J = 7.6 Hz, CH₂), 3.12 (t, J = 7.6 Hz, 2H, CH₃), 4.44 (s, 2H, CH₂), 5.03 (s, 2H, CH₂), 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, C₆H₅, C₆H₅) MS: (M+1) 403. Anal. Calc. for C₂₅H₂₅ClN₃O: C, 71.73; H, 5.02; N, 10.46. Found: C, 71.48; H, 5.13; N, 10.17.

Benzyl-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). ¹H-NMR (CDCl₃) δ: 3.04 (t, 2H, J = 6.8 Hz, CH₂), 3.18 (t, 2H, J = 6.8 Hz, CH₂), 5.05 (s, 2H, CH₂), 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, 2xC₆H₅) MS: (M+1) 354.2. Anal. Calc. for C₂₅H₂₅N₃O: C, 78.16; H, 5.42; N, 11.89. Found: C, 77.81; H, 5.61; N, 11.57.

Benzyl-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). ¹H-NMR (CDCl₃) δ: 3.04 (t, 2H, J = 6.8 Hz, CH₂), 3.18 (t, 2H, J = 7.0 Hz, CH₂), 3.82 (s, 3H, CH₃), 5.04 (s, 2H, CH₂), 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, C₆H₅, C₆H₅) MS: (M+1) 384. Anal. Calc. for C₂₅H₂₅O₂N₃O: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.29; H, 5.35; N, 11.14.

Benzyl-1-(n-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). ¹H-NMR (CDCl₃) δ: 2.40 (s, 3H, CH₃), 3.06 (t, 2H, J = 7.0 Hz, CH₂), 3.23 (t, 2H, J = 6.8 Hz, CH₂), 5.05 (s, 2H, CH₂), 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, C₆H₅, C₆H₅) MS: (M+1) 368. Anal. Calc. for C₂₅H₂₅N₃O: C, 78.45; H, 5.76; N, 11.44. Found: C, 78.22; H, 5.94; N, 11.44.

Benzyl-1-(3-chloro-phenyl)-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline 4j: Yield = 70%, mp 103—105°C. IR (KBr) cm⁻¹: 1610 (C=N), 1290 (C–N), 1250, 1045 (C–O–C), 1142 (N–N). ¹H-NMR (CDCl₃) δ: 3.04 (t, 2H, J = 6.8 Hz, CH₂), 3.18 (t, 2H, J = 7.0 Hz, CH₂), 3.82 (s, 3H, CH₃), 5.04 (s, 2H, CH₂), 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, C₆H₅, C₆H₅) MS: (M+1) 384. Anal. Calc. for C₂₅H₂₅O₂N₃O: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.29; H, 5.35; N, 11.14.
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). ¹H-NMR (CDCl₃) δ: 3.04 (t, 2H, J = 6.6 Hz, CH₂), 3.18 (t, 2H, J = 6.8 Hz, CH₂), 5.05 (s, 2H, CH₂), 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, C₆H₅, C₆H₄) MS: (M+1) 389. Anal. Calcd for C₂₃H₁₇Cl₂N₃O: C, 71.22; H, 4.68; N, 10.83. Found: C, 71.39; H, 4.36; N, 11.12.

7-Benzoyloxy-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). ¹H-NMR (CDCl₃) δ: 3.06 (t, 2H, J = 6.8 Hz, CH₂), 3.38 (t, 2H, J = 6.8 Hz, CH₂), 5.03 (s, 2H, CH₂), 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, C₆H₅, C₆H₄) MS: (M+1) 425. Anal. Calcd for C₂₄H₂₀ClN₃O₂: C, 75.02; H, 5.80; N, 11.24.

1H-MNR (CDCl₃): 7.68 (m, 8H, C₆H₅, C₆H₄). MS: (M+1) 309.

1H-MNR (CDCl₃): 6.83 (d, 1H, J = 9.0 Hz, H–9), 7.00 (d, 1H, J = 8.8 Hz, H–8), 7.07 (d, 1H, J = 8.8 Hz, H–9), 7.24—7.71 (m, 10H, 2 C₆H₅, 2 C₆H₄). MS: (M+1) 391.

1H-MNR (CDCl₃): 6.93 (d, 1H, J = 2.8 Hz, H–6), 7.00 (d, 1H, J = 7.0 Hz, CH₂), 7.33—7.40 (m, 5H, C₆H₅), 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 C₂₃H₁₇Cl₂N₃O: C, 74.69; H, 4.06; N, 9.95. Found: C, 74.95; H, 4.38; N, 9.75.

7-Benzoyloxy-1-phenoxymethyl-4,5-dihydro-[1,2,4]triazolo[4,3-a]quinoline 4l: Yield = 90%, mp 136—138 °C. IR (KBr) cm⁻¹: 1608 (C=C=N), 1309 (C–N), 1248, 1018 (C–O–C), 1152 (N–N). ¹H-NMR (CDCl₃) δ: 2.98 (t, 2H, J = 7.0 Hz, CH₂), 3.18 (t, 2H, J = 7.2 Hz, CH₂), 5.09 (s, 2H, CH₂), 5.38 (s, 2H, CH₂), 6.91 (dd, 1H, J = 9.0 Hz, H–9), 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 C₆H₅, 2 C₆H₄) MS: (M+1) 384. Anal. Calcd for C₂₃H₂₃N₃O: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.02; H, 5.80; N, 11.24.

7-Benzoyloxy-1-(4-chloro-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=C=N), 1309 (C–N), 1252, 1020 (C–O–C), 1120 (N–N). ¹H-NMR (CDCl₃) δ: 2.98 (t, 2H, J = 7.0 Hz, CH₂), 3.18 (t, 2H, J = 7.2 Hz, CH₂), 5.09 (s, 2H, CH₂), 5.38 (s, 2H, CH₂), 6.91 (dd, 1H, J = 9.0 Hz, H–9), 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 C₆H₅, 2 C₆H₄) MS: (M+1) 384. Anal. Calcd for C₂₃H₂₃N₃O: C, 75.18; H, 5.52; N, 10.96. Found: C, 75.02; H, 5.80; N, 11.24.

7-Benzoyloxy-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=C=N), 1294 (C–N), 1236, 1020 (C–O–C), 1140 (N–N). ¹H-NMR (CDCl₃) δ: 2.98 (t, 2H, J = 6.8 Hz, CH₂), 3.16 (t, 2H, J = 7.0 Hz, CH₂), 5.09 (s, 2H, CH₂), 5.36 (s, 2H, CH₂), 6.93 (dd, 1H, J = 9.0 Hz, 2.8 Hz, 8.8 Hz), 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, C₆H₅, C₆H₄) MS: (M+1) 419. Anal. Calcd for C₂₃H₁₇Cl₂N₃O: 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 Program Development (ADD), Epilepsy Branch, National Institutes of Health, Bethesda, MD, U.S.A. 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

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
evaluate the anticonvulsant activity of the synthesized compounds. The compounds were tested for anticonvulsant activity using the procedures described previously. 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·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 ED₅₀ and TD₅₀ 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 phenytoin, 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 ED₅₀ values of compounds 4a—c, which were H, CH₃ and C₃H₇ at the first position, were 17.3, 30.7, and 97.7 mg·kg⁻¹, respectively. The same tendency was seen in the rotarod neurotoxicity test. The values of their corresponding TD₅₀ were 61.4, 85.2, and 264 mg·kg⁻¹, respectively. Compound 4a was the most active among the 14 compounds synthesized, with an ED₅₀ value of 17.3 mg·kg⁻¹ in the MES test. Its activity was more potent than that of valproate (ED₅₀=272 mg·kg⁻¹), comparable to that of phenobarbital (ED₅₀=21.8 mg·kg⁻¹), and less effective than that of phenytoin (ED₅₀=9.5 mg·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-CH₃Ph, or 3-OCH₃Ph at the first position, respectively, had corresponding ED₅₀ value in the MES test of 23, 30.4, and 60.6 mg·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·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 ED₅₀ value of 58.5 mg·kg⁻¹ in the MES test, was less active than compound 4g (ED₅₀=23.0 mg·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 TD₅₀ value of greater than 300 mg·kg⁻¹, ED₅₀ of 23.0 mg·kg⁻¹, and protective index (PI=TD₅₀/ED₅₀) of more than 13 was the safest among the compounds synthesized. Compared with the marketed drugs, its ED₅₀ 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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REFERENCES

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

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<tr>
<th>Compd.</th>
<th>MES</th>
<th>scMet</th>
<th>Rotarod toxicity</th>
<th>Pl(⁻)</th>
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<th>scMet</th>
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<td>4a</td>
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<td>4b</td>
<td>30.7 (26.1—36.1)</td>
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<td>4c</td>
<td>97.7 (84.0—113.7)</td>
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<td>4e</td>
<td>67.9 (59.6—77.4)</td>
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<td>4f</td>
<td>23.0 (18.8—28.0)</td>
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<td>4h</td>
<td>60.6 (52.9—69.4)</td>
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<td>4i</td>
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<td>4l</td>
<td>58.5 (45.9—74.5)</td>
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a) Dose measured in mg·kg⁻¹. b) PI=TD₅₀/ED₅₀. c) Minimal neurotoxicity was determined using the rotarod test after the tested compounds were administered for 30 min. d) Data from Huseyin et al., 1998. e) 95% confidence limits.


