Synthesis and Studies on the Anticonvulsant Activity of 5-alkoxy-[1,2,4]triazolo[4,3-*a*]pyridine Derivatives

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Key words
- [1,2,4]triazolo[4,3-a]pyridine
- synthesis
- anticonvulsant activity
- chemically induced model

Abstract

In this study, a series of new 5-alkoxy-[1,2,4]triazolo[4,3-*a*]pyridine derivatives was synthesized and their anticonvulsant activity and neurotoxicity was evaluated with the maximal electroshock and rotarod tests, respectively. The most promising compounds, **3p** (5-(4-chlorophenoxy)-[1,2,4]triazolo[4,3-*a*]pyridine) and **3r** (5-(4-bromophenoxy)-[1,2,4]triazolo[4,3-*a*]pyridine), showed a median effective dose of 13.2 and 15.8 mg/kg and had a protective index value of 4.8 and 6.9, respectively. For exploring the putative mechanism of action, compounds **3n, 3p** and **3r** were tested in chemically induced models.

received 31.12.2011
accepted 02.05.2012

Bibliography
DOI http://dx.doi.org/10.1055/s-0032-1314821
Published online: July 10, 2012
Arzneimittelforschung 2012; 62: 372–377

ISSN 0004-4172

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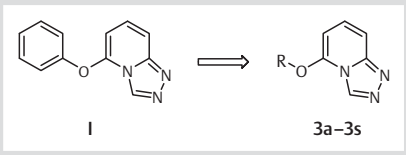
Introduction

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]. Anticonvulsant drugs currently on the market are of unsatisfactory effectiveness in seizure control and cause adverse reactions such as drowsiness, ataxia, gastrointestinal disturbance, hepatotoxicity and megaloblastic anemia [2–6] and even life-threatening conditions [7]. Therefore, the search for safer and more effective antiepileptic drugs [2, 3] is of high importance. The development of new antiepileptic drugs with the desired therapeutic properties, however, has been a challenge for medicinal chemists.

As part of our program directed towards the search for central nervous system (CNS) agents and in order to discover a new anticonvulsant compound with improved activity, we synthesized compound **I** (5-phenoxy-[1,2,4]-triazolo [4,3-*a*]pyridine) (**Fig. 1**), which showed anticonvulsant activity at an effective dose of 300 mg/kg in the maximal electroshock (MES) test. To obtain further compounds with better anticonvulsant activity, we designed and synthesized a new series of 5-alkoxy-[1,2,4]triazolo[4,3-*a*]pyridine derivatives (**3a–3s**) using compound **I** as the lead compound and investigated the contribution of different alkoxyl groups at position 5 of the [1,2,4]triazolo[4,3-*a*]pyridine to the anticonvulsant activity. A conjugation effect increased the electron cloud density at the triazole ring because of the lone-pair electrons of the oxygen atom at the 5-alkoxy moiety. This in turn increased the combination ability of compounds **3a–3s** to the receptor. The pharmacology results indicated that the most promising compounds **3n** (5-(2-chlorophenoxy)-[1,2,4]triazolo[4,3-*a*]pyridine), **3p** (5-(4-chlorophenoxy)-[1,2,4]triazolo[4,3-*a*]pyridine) and **3r** (5-(4-bromophenoxy)-[1,2,4]triazolo[4,3-*a*]pyridine) showed median effective doses of 19.0, 13.2 and 15.8 mg/kg$^-$ and had protective index values (PI) of 4.8, 4.8 and 6.9, respectively, which is much better than the PI value of the reference drug phenobarbital.

The structures of the new derivatives were characterized using IR, ^{1}H NMR, ^{13}C NMR, MS and elemental analysis techniques. Their anticonvulsant activity, reported for the first time, was evaluated using the MES test and the neurotoxicity was evaluated using the rotarod test in mice. In this contribution, in order to explain the possible mechanism of action, compounds **3n**, **3p** and **3r** were tested in the pentylenetetrazol (PTZ), isoniazid (ISN), 3-mercaptopropionic acid (3-MP) and thiosemicarbazide tests. The anti-MES activity and neurotoxicity of the marketed agent phenobarbital was evaluated as a positive control.



I 3a–3s

Fig. 1 The structure of compounds **I** and **3a–3s**.

Materials and Methods

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730 apparatus (Bruker), ^{1}H-NMR and ^{13}C-NMR were measured on an AV-300 spectrometer (Bruker). All chemical shifts were given in ppm relative to tetramethysilane. Mass spectra were measured on an HP1100LC spectrometer (Agilent Technologies). Elemental analysis (CHN) was performed on a Perkin Elmer 204Q CHN or a Heraeus CHN Rapid Analyzer. The majority of the chemicals was purchased from Aldrich Chemical Corporation. All other chemicals were of analytical grade.

Synthesis of 1-hydrazino-6-chroropyridine (1)

To a solution of hydrazine hydrate (3.04 g, 62.8 mmol) in 20 mL ethanol, a solution of 2,6-dichloropyridine (1.85 g, 12.6 mmol) in 30 mL ethanol was added dropwise at room temperature. The mixture was stirred and refluxed for 1 h. Then, half of the solvent was removed under reduced pressure and the solution was combined with petroleum ether. The precipitate was filtered and washed with petroleum ether, then kept below 0 °C. The resulting crude compound was purified by recrystallization in EtOH [8].

Synthesis of 5-chloro-[1,2,4]triazolo[4,3-*a*]pyridine (2)

Compound **1** (4 mmol), formic acid (4 mmol) and 30 mL ethanol were placed in a 50 mL round-bottom flask. The mixture was stirred for 2 h at room temperature to obtain the acid intermediate. The mixture was then evaporated under reduced pressure and the acid intermediate was reacted byrefluxing in DMF for 3 h. The solution was evaporated to dryness and the oily residue was filtered on a silica gel chromatographic column (ethyl acetate) to give a light yellow or white solid [8]. Yield = 87 %. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ: 8.97 (1H, s, triazolo-H), 7.77 (1H, d, J = 9.24 Hz, 7.05 Hz, pyridine-H), 7.28 (1H, dd, J = 7.05 Hz, 9.24 Hz, pyridine-H), 6.93 (1H, d, J = 6.99 Hz, 7.05 Hz, pyridine-H). MS (M+1): 154.

General protocol for the synthesis of 8-alkoxy-[1,2,4] triazolo[4,3-*a*]pyrazine (3a–3s)

Compound **2** (0.5 g, 3.2 mmol) and either alkanol or substituted phenol (3.2 mmol) were added to a solution of sodium hydroxide (3.2 mmol) or K_2CO_3 (3.2 mmol) in DMF followed by stirring and refluxing for 3–5 h. After removal of the solvent under reduced pressure, the solid residue was purified by silica gel column chromatography with dichloromethane: methanol (20:1). The yield, melting point and spectral data for each compound are given below [9, 10].

5-Methoxy-[1,2,4]triazolo[4,3-a]pyridine (3a)

mp 108–110 °C, yield = 80 %. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.88 (1H, s, triazolo-H), 7.41 (1H, d, J = 9.16 Hz, 7.24 Hz, pyridine-H), 7.29 (1H, dd, J = 7.24 Hz, 9.16 Hz, pyridine-H), 6.04 (1H, d, J = 7.21 Hz, 7.24 Hz, pyridine-H), 4.13 (3H, s, -OCH_3). IR (KBr) cm^{-1}: 1643 (C = N), 1284, 1061 (C-O-C), 1193 (N-N). MS *m/z*: 150 (M+1). Anal. calculated for $C_7H_7N_3O$: C, 56.37; H, 4.73; N, 28.17. Found: C, 56.25; H, 4.65; N, 28.25.

5-Ethoxy-[1,2,4]triazolo[4,3-a]pyridine (3b)

mp 94–96 °C, yield = 79 %. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.88 (1H, s, triazolo-H), 7.38 (1H, d, J = 9.09 Hz, 7.22 Hz, pyridine-H), 7.30 (1H, dd, J = 7.22 Hz, 9.09 Hz, pyridine-H), 6.02 (1H, d, J = 7.20 Hz, 7.22 Hz, pyridine-H), 4.36 (2H, p, -OCH_2), 1.59 (3H, t, -CH_3). IR (KBr) cm^{-1}: 1642 (C = N), 1294, 1060 (C-O-C), 1190 (N-N). MS *m/z*: 164 (M+1). Anal. calculated for $C_8H_9N_3O$: C, 58.88; H, 5.56; N, 25.75. Found: C, 58.75; H, 5.42; N, 25.60.

5-Isopropoxy-[1,2,4]triazolo[4,3-a]pyridine (3c)

mp 90–92 °C, yield = 81 %. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.86 (1H, s, triazolo-H), 7.42 (1H, d, J = 9.06 Hz, 7.29 Hz, pyridine -H), 7.32 (1H, dd, J = 7.29 Hz, 9.06 Hz, pyridine-H), 6.06 (1H, d, J = 7.26 Hz, 7.29 Hz, pyridine-H), 4.86 (1H, m, -OCH), 1.54 (3H, s, -CH_3), 1.52 (3H, s, -CH_3). IR (KBr) cm^{-1}: 1639 (C = N), 1282, 1045 (C-O-C), 1189 (N-N). MS *m/z*: 178 (M+1). Anal. calculated for $C_9H_{11}N_3O$: C, 61.00; H, 6.26; N, 23.71. Found: C, 59.87; H, 6.38; N, 23.62.

5-Butoxy-[1,2,4]triazolo[4,3-a]pyridine (3d)

mp 28–30 °C, yield = 74 %. ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 8.87 (1H, s, triazolo-H), 7.39 (1H, d, J = 9.09 Hz, 7.23 Hz, pyridine -H), 7.28 (1H, dd, J = 7.23 Hz, 9.09 Hz, pyridine-H), 6.02 (1H, d, J = 7.20 Hz, 7.23 Hz, pyridine-H), 4.28 (2H, t, -OCH_2), 1.56–1.96 (4H, m, -$(CH_2)_2$), 1.04 (3H, t, -CH_3). IR (KBr) cm^{-1}: 1643 (C = N), 1283, 1053 (C-O-C), 1193 (N-N). MS *m/z*: 192 (M+1). Anal. calculated for $C_{10}H_{13}N_3O$: C, 62.81; H, 6.85; N, 21.97. Found: C, 62.69; H, 6.77; N, 21.83.

5-Pentyloxy-[1,2,4]triazolo[4,3-a]pyridine (3e)

Oil, yield = 78 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.88 (1H, s, triazolo-H), 7.42 (1H, d, J = 9.16 Hz, 7.25 Hz, pyridine-H), 7.28 (1H, dd, J = 7.25 Hz, 9.16 Hz, pyridine-H), 6.02 (1H, d, J = 7.20 Hz, 7.25 Hz, pyridine-H), 4.31 (2H, t, -OCH_2), 1.43–1.96 (6H, m, -$(CH_2)_3$), 0.98 (3H, t, -CH_3). IR (KBr) cm^{-1}: 1641 (C = N), 1281, 1021 (C-O-C), 1189 (N-N). MS *m/z*: 206 (M+1). Anal. calculated for $C_{11}H_{15}N_3O$: C, 64.37; H, 7.37; N, 20.47. Found: C, 64.28; H, 7.26; N, 20.39.

5-Hexyloxy-[1,2,4]triazolo[4,3-a]pyridine (3f)

Oil, yield = 73 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.88 (1H, s, triazolo-H), 7.40 (1H, d, J = 9.18 Hz, 7.21 Hz, pyridine-H), 7.36 (1H, dd, J = 7.21 Hz, 9.18 Hz, pyridine-H), 6.02 (1H, d, J = 7.23 Hz, 7.21 Hz, pyridine-H), 4.28 (2H, t, -OCH_2), 1.25–1.99 (8H, m, -$(CH_2)_4$), 0.95 (3H, t, -CH_3). IR (KBr) cm^{-1}: 1639 (C = N), 1283, 1054 (C-O-C), 1193 (N-N). MS *m/z*: 220 (M+1). Anal. calculated for $C_{12}H_{17}N_3O$: C, 65.73; H, 7.81; N, 19.16. Found: C, 65.62; H, 7.76; N, 19.01.

5-Heptyloxy-[1,2,4]triazolo[4,3-a]pyridine (3g)

mp 30–32 °C, yield = 77 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.87 (1H, s, triazolo-H), 7.39 (1H, d, J = 9.19 Hz, 7.21 Hz, pyridine -H), 7.28 (1H, dd, J = 7.21 Hz, 9.19 Hz, pyridine-H), 6.01 (1H, d, J = 7.22 Hz, 7.23 Hz, pyridine-H), 4.27 (2H, t, -OCH_2), 1.34–1.97 (10H, m, -$(CH_2)_5$), 0.91 (3H, t, -CH_3). IR (KBr) cm^{-1}: 1642 (C = N), 1282, 1054 (C-O-C), 1191 (N-N). MS *m/z*: 234 (M+1). Anal. calculated for $C_{13}H_{19}N_3O$: C, 66.92; H, 8.21; N, 18.01. Found: C, 66.82; H, 8.15; N. 18.16.

5-Octyloxy-[1,2,4]triazolo[4,3-a]pyridine (3h)

mp 46–48 °C, yield = 81 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 8.87 (1H, s, triazolo-H), 7.39 (1H, d, J = 9.09 Hz, 7.14 Hz, pyridine -H), 7.28 (1H, dd, J = 7.14 Hz, 9.09 Hz, pyridine-H), 6.01 (1H, d, J = 7.20 Hz, 7.14 Hz, pyridine-H), 4.27 (2H, t, -OCH_2), 1.30–1.99 (12H, m, -$(CH_2)_6$), 0.90 (3H, t, -CH_3). IR (KBr) cm^{-1}: 1639 (C = N), 1281, 1061 (C-O-C), 1193 (N-N). MS *m/z*: 248 (M+1). Anal. calculated for $C_{14}H_{21}N_3O$: C, 67.98; H, 8.56; N, 16.99. Found: C, 67.80; H, 8.45; N, 16.78.

5-(2-Tolyloxy)-[1,2,4]triazolo[4,3-a]pyridine (3i)

mp 116–118 °C, yield = 79 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.10 (1H, s, triazolo-H), 7.60 (1H, d, J = 9.15 Hz, 6.93 Hz, pyridine-H), 7.17 (1H, dd, J = 6.93 Hz, 9.15 Hz, pyridine-H), 5.85 (1H, d, J = 7.35 Hz, 6.93 Hz, pyridine -H), 7.26–7.39 (4H, m, -C_6H_4), 2.24 (3H, s, -CH_3). IR (KBr) cm^{-1}: 1643 (C = N), 1284, 1044 (C-O-C), 1153 (N-N). MS *m/z*: 226(M+1). Anal. calculated for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.41; H, 4.81; N, 18.78.

5-(3-Tolyloxy)-[1,2,4]triazolo[4,3-a]pyridine (3j)

mp 120–122 °C, yield = 75 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.04 (1H, s, triazolo-H), 7.57 (1H, d, J = 9.09 Hz, 7.21 Hz, pyridine-H), 7.11 (1H, dd, J = 7.21 Hz, 9.09 Hz, pyridine-H), 6.00 (1H, d, J = 7.35 Hz, 7.65 Hz, pyridine-H), 7.03–7.41 (4H, m, -C_6H_4), 2.42 (3H, s, -CH_3). IR (KBr) cm^{-1}: 1642 (C = N), 1281, 1054 (C-O-C), 1151 (N-N). MS *m/z*: 226 (M+1). Anal. calculated for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.23; H, 4.85; N, 18.55.

5-(4-Tolyloxy)-[1,2,4]triazolo[4,3-a]pyridine (3k)

mp 124–126 °C, yield = 83 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.06 (1H, s, triazolo-H), 7.58 (1H, d, J = 9.14 Hz, 7.41 Hz, pyridine-H), 7.33 (1H, dd, J = 7.41 Hz, 9.14 Hz, pyridine-H), 5.98 (1H, d, J = 7.40 Hz, 7.41 Hz, pyridine-H), 7.18–7.34 (4H, m, -C_6H_4), 2.39 (3H, s, -CH_3). IR (KBr) cm^{-1}: 1642 (C = N), 1281, 1054 (C-O-C), 1153 (N-N). MS *m/z*: 226(M+1). Anal. calculated for $C_{13}H_{11}N_3O$: C, 69.32; H, 4.92; N, 18.66. Found: C, 69.25; H, 4.87; N, 18.49.

5-(2-Methoxyphenoxy)-[1,2,4]triazolo[4,3-a]pyridine (3l)

mp 118–120 °C, yield = 85 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.09 (1H, s, triazolo-H), 7.53 (1H, d, J = 9.15 Hz, 7.50 Hz, pyridine-H), 7.23 (1H, dd, J = 7.50 Hz, 9.15 Hz, pyridine-H), 5.83 (1H, d, J = 7.22 Hz, 7.50 Hz, pyridine -H), 7.06–7.35 (4H, m, -C_6H_4), 3.77 (3H, s, -OCH_3). IR (KBr) cm^{-1}: 1638 (C = N), 1282, 1040 (C-O-C), 1152 (N-N). MS *m/z*: 242 (M+1). Anal. calculated for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.83; H, 4.51; N, 17.34.

5-(4-Methoxyphenoxy)-[1,2,4]triazolo[4,3-a]pyridine (3m)

mp 128–130 °C, yield = 82 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.06 (1H, s, triazolo-H), 7.53 (1H, d, J = 9.09 Hz, 7.59 Hz, pyridine-H), 7.24 (1H, dd, J = 7.59 Hz, 9.09 Hz, pyridine-H), 5.90 (1H, d, J = 7.35 Hz, 7.59 Hz, pyridine-H), 7.01–7.28 (4H, m, -C_6H_4), 3.86 (3H, s, -OCH_3). IR (KBr) cm^{-1}: 1639 (C = N), 1294, 1043 (C-O-C), 1151 (N-N). MS *m/z*: 242 (M+1). Anal. calculated for $C_{13}H_{11}N_3O_2$: C, 64.72; H, 4.60; N, 17.42. Found: C, 64.65; H, 4.54; N, 17.35.

5-(2-Chlorophenoxy)-[1,2,4]triazolo[4,3-a]pyridine (3n)

mp 150–152 °C, yield = 74 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.11 (1H, s, triazolo-H), 7.53 (1H, d, J = 9.17 Hz, 7.19 Hz, pyridine-H), 7.27 (1H, dd, J = 7.19 Hz, 9.17 Hz, pyridine-H), 5.81 (1H, d, J = 7.31 Hz, 7.19 Hz, pyridine-H), 7.20–7.59 (4H, m, -C_6H_4). IR (KBr) cm^{-1}: 1642 (C = N), 1283, 1051 (C-O-C), 1153 (N-N). MS *m/z*: 246(M+1). Anal. calculated for $C_{12}H_8ClN_3O$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.54; H, 3.19; N, 17.01.

5-(3-Chlorophenoxy)-[1,2,4]triazolo[4,3-a]pyridine (3o)

mp 138–140 °C, yield = 69 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.02 (1H, s, triazolo-H), 7.56 (1H, d, J = 9.18 Hz, 8.04 Hz, pyridine-H), 7.16 (1H, dd, J = 8.04 Hz, 9.18 Hz, pyridine-H), 6.02 (1H, d, J = 7.29 Hz, 8.04 Hz, pyridine-H), 7.24–7.48 (4H, m, -C_6H_4). IR (KBr) cm^{-1}: 1642 (C = N), 1281, 1060 (C-O-C), 1152 (N-N). MS *m/z*: 246(M+1). Anal. calculated for $C_{12}H_8ClN_3O$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58, 59; H, 3.33; N, 17.20.

5-(4-Chlorophenoxy)-[1,2,4]triazolo[4,3-a]pyridine (3p)

mp 155–157 °C, yield = 76 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.03 (1H, s, triazolo-H), 7.53 (1H, d, J = 9.18 Hz, 7.17 Hz, pyridine-H), 7.23 (1H, dd, J = 7.17 Hz, 9.18 Hz, pyridine-H), 5.95 (1H, d, J = 7.29 Hz, 7.17 Hz, pyridine-H), 7.19–7.50 (4H, m, -C_6H_4). ^{13}C NMR ($CDCl_3$): δ151.0, 150.6, 146.8, 132.3, 131.9, 130.7, 128.8, 122.0, 121.8, 118.7, 109.5, 92.5. IR (KBr) cm^{-1}: 1641 (C = N), 1284, 1024 (C-O-C), 1153 (N-N). MS *m/z*: 246(M+1). Anal. calculated for $C_{12}H_8ClN_3O$: C, 58.67; H, 3.28; N, 17.10. Found: C, 58.71; H 3.20; N 17.19.

5-(4-Fluorophenoxy)-[1,2,4]triazolo[4,3-a]pyridine (3q)

mp 168–170 °C, yield = 60 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.05 (1H, s, triazolo-H), 7.53 (1H, d, J = 9.12 Hz, 7.47 Hz, pyridine-H), 7.21 (1H, dd, J = 7.47 Hz, 9.12 Hz, pyridine-H), 5.89 (1H, d, J = 7.35 Hz, 7.47 Hz, pyridine-H), 7.17–7.27 (4H, m, -C_6H_4). IR (KBr) cm^{-1}: 1643 (C = N), 1294, 1050 (C-O-C), 1152 (N-N). MS *m/z*: 230 (M+1). Anal. calculted for $C_{12}H_8FN_3O$: C, 62.88; H, 3.52; N, 18.33. Found: C, 62.79, H 3.46, N, 18.38.

5-(4-Bromophenoxy)-[1,2,4]triazolo[4,3-a]pyridine (3r)

mp 154–156 °C, yield = 74 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.03 (1H, s, triazolo-H), 7.52 (1H, d, J = 9.30 Hz, 7.50 Hz, pyridine-H), 7.24 (1H, dd, J = 7.50 Hz, 9.30 Hz, pyridine-H), 5.94 (1H, d, J = 7.20 Hz, 7.50 Hz, pyridine-H), 7.13–7.66 (4H, m, -C_6H_4). ^{13}C NMR ($CDCl_3$): δ151.0, 150.6, 146.8, 132.3, 131.9, 130.7, 128.8, 122.0, 121.8, 118.7, 109.5, 92.5. IR (KBr) cm^{-1}: 1642 (C = N), 1282, 1060 (C-O-C), 1151 (N-N). MS *m/z*: 289 (M+1). Anal. calculated for $C_{12}H_8BrN_3O$: C, 49.68; H, 2.78; N, 14.48. Found: C, 49.57; H, 2.65; N, 14.35.

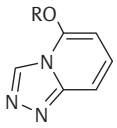
5-(2,4-Dichlorophenoxy)-[1,2,4]triazolo[4,3-a]pyridine (3s)

mp 144–146 °C, yield = 62 %. ^{1}H-NMR ($CDCl_3$, 300 MHz): δ 9.09 (1H, s, triazolo-H), 7.54 (1H, d, J = 9.18 Hz, 7.35 Hz, pyridine-H), 7.23 (1H, dd, J = 7.35 Hz, 9.18 Hz, pyridine-H), 5.81 (1H, d, J = 7.29 Hz, 7.35 Hz, pyridine-H), 7.19–7.60 (3H, m, -C_6H_3). IR (KBr) cm^{-1}: 1643 (C = N), 1284, 1020 (C-O-C), 1153 (N-N). MS *m/z*: 280 (M+1). Anal. calculated for $C_{12}H_7Cl_2N_3O$: C, 51.45; H, 2.52; N, 15.00. Found: C, 51.34; H, 2.40; N, 15.09.

Pharmacology

The MES and rotarod tests were carried out according to the standard described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA) [11, 12]. All compounds were tested for anticonvulsant activity in KunMing mice (weight 18–25 g) purchased from the Laboratory of Animal Research, College of Pharmacy, Yanbian University. The tested compounds were dissolved in DMSO.

Table 1 Phase-I evaluation of anticonvulsant activity in mice (i.p.).



Compound	R	Dosage (mg/kg)	MES[a]	
			0.5 h	4 h
I	-C_6H_5	300	1/3	0/3
3a	*n*-CH_3	300	2/3	0/3
3b	*n*-C_2H_5	300	2/3	0/3
3c	*n*-$CH(CH_3)_2$	100	3/3	0/3
3d	*n*-C_4H_9	30	2/3	0/3
3e	*n*-C_5H_{11}	100	3/3	0/3
3f	*n*-C_6H_{13}	100	3/3	0/3
3g	*n*-C_7H_{15}	100	3/3	0/3
3h	*n*-C_8H_{17}	100	3/3	0/3
3i	2-$C_6H_4CH_3$	100	3/3	0/3
3j	3-$C_6H_4CH_3$	100	3/3	0/3
3k	4-$C_6H_4CH_3$	300	2/3	0/3
3l	2-$C_6H_4OCH_3$	100	3/3	0/3
3m	4-$C_6H_4OCH_3$	100	3/3	0/3
3n	2-C_6H_4Cl	30	3/3	0/3
3o	3-C_6H_4Cl	30	3/3	0/3
3p	4-C_6H_4Cl	30	3/3	0/3
3q	4-C_6H_4F	100	3/3	0/3
3r	4-C_6H_4Br	30	3/3	0/3
3s	2,4-$C_6H_3Cl_2$	30	2/3	0/3

[a] Maximal electroshock test (number of animals protected/number of animals tested), the number of mice was 3

Table 2 Phase-II based, quantitative anticonvulsant data in mice (test drug administered i.p.).

Compound	MES, ED^a_{50}	Rotarod Toxicity $TD_{50}{}^b$	PI[c] (TD_{50}/ED_{50})
3d	32.9 (28.0–38.6)[d]	126.8 (108.1–148.8)	3.9
3n	19.0 (16.2–22.3)	91.3 (77.8–107.1)	4.8
3o	22.8 (19.4–26.8)	93.0 (79.2–109.2)	4.1
3p	13.2 (11.3–15.5)	63.4 (54.0–74.4)	4.8
3r	15.8 (13.5–18.5)	109.5 (93.3–128.5)	6.9
3s	27.4 (23.4–32.1)	76.1 (64.9–89.3)	2.8
Phenobarbital	21.8 (21.8–25.5)	69 (62.8–72.9)	3.2

[a] ED_{50}-median effective dose required to assure anticonvulsant protection in 50 % of the animals, the number of mice was 10

[b] TD_{50}-median toxic dose eliciting minimal neurological toxicity in 50 % of the animals

[c] PI protective index (TD_{50}/ED_{50})

[d] 95 % confidence limits given in parentheses

For the phase-I screening (**Table 1**), each compound was administered at 30, 100, 300 mg/kg for evaluating the anticonvulsant activity. The neurotoxicity was measured at 30 min and 4 h after administration. The anticonvulsant activity was measured with the MES test for which seizures were elicited in mice with a 60 Hz alternating current of 50 mA. The current was applied via corneal electrodes for 0.2 s. Protection against the propagation of the MES-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. Neurologic deficits caused by the compounds were detected with the rotorod ataxia test.

The pharmacologic parameters estimated from the phase-I screening were then quantified in the phase-II screening for compounds **3d**, **3n–3p** and **3r–3 s** (**Table 2**). The anticonvulsant activity was expressed as the median effective dose (ED_{50}) and neurotoxicity as the median toxic dose (TD_{50}). For determination of the ED_{50} and TD_{50} values, a dose range of the test compounds was administered intraperitoneally (i.p.) to groups of 10 mice until at least three points with 10–90 % seizure protection or minimal observed neurotoxicity were measured. By plotting these 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 the National Institute of Neurological Disorders and Stroke.

In the case of chemically induced seizures (**Table 3**), mice were given doses of the compounds that could induce seizures in at least 97 % of the animals. The doses used were: PTZ, 85 mg/kg; ISN, 250 mg/kg; 3-MP, 40 mg/kg and thiosemicarbazide, 100mg/kg. The test compounds and standard AEDs were administered i.p. at 50 mg/kg to groups of 10 mice 1 h before either i.p. administration of ISN and thiosemicarbazide or s.c. injection of PTZ and 3-MP. The mice were placed in individual cages and observed for 30 min. The number of clonic seizures, tonic seizures and the lethality were recorded [13, 14]. Thiosemicarbazide was also administered i.p., and in this case the test compounds were given to mice i.p. 30 min prior to thiosemicarbazide administration. The mice were placed in individual cages and observed for 2 h and the number of clonic seizures, tonic seizures and the lethality were noted [15].

Results and Discussion

Synthesis of the new derivatives was carried out starting from 2,6-dichloropyridine according to **Fig. 2**. 2,6-Dichloropyridine reacted with hydrazine hydrate in ethanol to afford compound **1**[8]. In a next step, compound **1** reacted with formic acid in ethanol to obtain compound **2**, which in turn reacted with the appropriate alcohol or substituted phenol to yield the target compounds **3a–3 s** [9, 10]. All compounds were identified based on their spectral data. In general, the IR spectra showed the C = N peak at 1 638–1 643 cm^{-1} and the N-N peak at 1 151–1 193 cm^{-1}. In the nuclear magnetic resonance spectrum (^{1}H-NMR), the signals of the respective protons of the synthesized compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed the triazolo-H proton as a singlet at 8.86–9.10 ppm.

The anticonvulsant activity and neurotoxicity of the synthesized compounds **3a–3s** were measured according to the procedures described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA) [11, 12]. The results from the pharmacology tests for all synthesized compounds are shown in **Table 1**. All compounds showed anticonvulsant activity. The lead compound **I** (5-phenoxy-[1,2,4] triazolo[4,3-*a*]pyridine) was active in the MES test only at the high dose of 300 mg/kg. 3 of the synthesized compounds (**3a**, **3b**, **and 3k**) exhibited an anti-MES effect at 300 mg/kg, ten compounds (**3c**, **3e–3j**, **3l**, **3m**, and **3q**) at 100 mgkg, and 6 compounds (**3d**, **3n–3p**, **3r**, and **3s**) at 30 mg/kg (**Table 1**). However, none of these compounds was active for more than 4 h following administration. The higher activity may be explained by the

Table 3 Effect of compounds **3n, 3p** and **3r** on PTZ-, ISN-, 3-MP-, and thiosemicarbazide-induced convulsion in mice.

Chemical substances	Compound	Doses (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
PTZ	DMSO	–	1.0	100	100	40
	carbamazepine	50	1.0	70	0	0
	3n	50	1.0	40	30	0
	3p	50	1.0	30	20	0
	3r	50	1.0	10	30	0
ISN	DMSO	–	1.0	100	100	75
	carbamazepine	50	1.0	50	0	0
	3n	50	1.0	30	50	0
	3p	50	1.0	10	30	0
	3r	50	1.0	50	50	0
3-MP	DMSO	–	1.0	100	100	100
	carbamazepine	50	1.0	60	0	0
	3n	50	1.0	60	50	0
	3p	50	1.0	30	20	0
	3r	50	1.0	100	0	0
thiosemicarbazide	DMSO	–	2.0	100	100	100
	carbamazepine	50	2.0	50	0	0
	3n	50	2.0	50	80	0
	3p	50	2.0	70	60	0
	3r	50	2.0	60	60	0

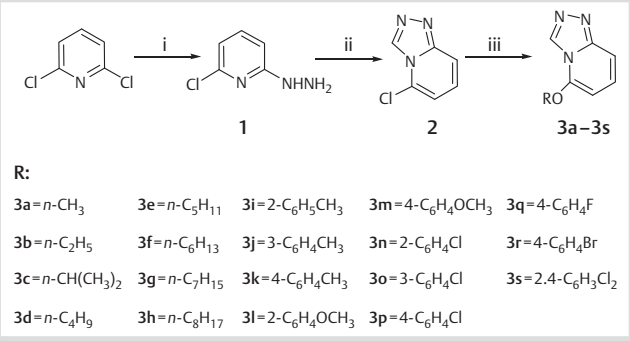


Fig. 2 The synthesis of compounds **3a–3s**. **Reagents:** (i) $NH_2NH_2{\cdot}H_2O/C_2H_5OH$, reflux; (ii) $HCOOH/C_2H_5OH$, reflux; (iii) alkanol/DMSO, NaOH or K_2CO_3.

presence of the triazole ring, which enhances the affinity of the pyridine ring for the recognition site [16].

In the phase-II pharmacology test, 6 compounds were quantitatively evaluated for their anticonvulsant activity (indicated by ED_{50}) and neurotoxicity (indicated by TD_{50}) (**Table 2**) following i.p. administration. Based on the activities of compounds **3d, 3n–3p** and **3r–3s**, the following structure activity relationship (SAR) was gained.

Generally, the anticonvulsant activity of an organic compound may be increased significantly by introducing a halogen atom. Therefore, some halogen substituted derivatives were designed and synthesized in this study. Comparison of the activity of the various halogen substituted derivatives indicates that the halogen atoms contribute to the anticonvulsant activity in the order 4-Cl > 4-Br > 4-F (**Table 2**). The ED_{50} and PI for phenobarbital were 21.8 mg/kg and 3.2. Remarkably, three compounds, **3n**, **3p** and **3r**, namely, had lower ED_{50} (19.0, 13.2 and 15.8 mg/kg, respectively) values than phenobarbital and one of the compounds, **3r**, also had a higher PI of 6.9 than the control substance. Compared with phenobarbital, compound **3r** showed a better anticonvulsant activity and a decreased neurotoxicity, which indicates a certain safety margin for this compound.

When assessing the influence of the position of the atomic Cl in the phenyl ring, it was found that compounds with Cl in position 4 were the most active. Cl in position 2 rendered the compound more active than Cl in position 3. A double-chlorinated compound (positions 2 and 4) showed the weakest activity. Introducing the Cl atom into the benzyl ring resulted in increased activity (**Table 2**).

When comparing the influence of the electron-donor group on the anticonvulsant activity, it was found that, from the 8 alkyl chain substituted derivatives **3a–3h**, only compound **3d** exhibited increased anticonvulsant activity, with an ED_{50} value of 32.9 mg/kg and decreased neurotoxicity (TD_{50} value of 126.8 mg/kg). In addition, the results show the superior activity of aryloxy derivatives in comparison to alkoxy ones (**Table 2**).

To further investigate the anticonvulsant activity in different models, compounds **3n**, **3p** and **3r** were tested against convulsions induced by chemical substances, i.e., PTZ, ISN, 3-MP, and thiosemicarbazide. Compounds **3n**, **3p** and **3r** were administered i.p. to mice at 50 mg/kg,which corresponded to their $3\times ED_{50}$, but was far below their TD_{50}. The reference drug carbamazepine was administered i.p. at 50 mg/kg. Compounds **3n**, **3p** and **3r** inhibited PTZ induced clonic seizures by 60%, 70% and 90%, tonic seizures by 70%, 80% and 70% and death by 100%, 100% and 100%, respectively (**Table 3**). While carbamazepine showed inhibition values of 30%, 100% and 100%. Carbamazepine inhibited ISN induced clonic seizures, tonic seizures and death by 50%, 100% and 100%, respectively, while inhibition values of 70%, 90% and 50% (clonic seizures), of 50%, 70% and 50% (tonic seizures) and 100%, 100% and 100% (death) were measured for compounds **3n**, **3p** and **3r** (**Table 3**). PTZ and ISN were reported to produce seizures by inhibiting aminobutyric acid (GABA) neurotransmission [17, 18]. GABA is the main inhibitory neurotransmitter in the brain and has been widely implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures [19], whereas enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study suggest that the newly synthesized compounds

3n, **3p** and **3r** may inhibit or attenuate PTZ- and ISN-induced seizures in mice by enhancing GABAergic neurotransmission.

In the 3-MP-induced seizure model, carbamazepine inhibited clonic seizures, tonic seizures and death by 40%, 100%, and 100%, respectively, while compounds **3n**, **3p** and **3r** showed inhibition values of 40%, 70% and 0% (clonic seizures), of 50%, 80% and 100% (tonic seizures) and of 100%, 100% and 100% (death) (**Table 3**). When assessing the activity of the compounds on thiosemicarbazide-induced convulsion, the effect was similar to that observed in the 3-MP-induced seizure model. Compound **3n** showed a lower degree of inhibition of clonic seizures, a stronger inhibition of tonic seizures, and a similar inhibition of death (50%, 20% and 100%, respectively) if compared to the reference drug. Compound **3p** inhibited clonic seizures less, tonic seizures more and death to a similar extent (30%, 40% and 100%, respectively) than the control substance. The same tendency was observed for compound **3r** (40%, 40% and 100%, respectively) (**Table 3**). 3-MP and thiosemicarbazide are known to be competitive inhibitors of glutamate decarboxylase (GAD), an enzyme involved in GABA synthesis; they inhibit GABA synthesis, resulting in a decreased level of GABA in the brain [20]. Compounds **3n**, **3p** and **3r** were moderately active against 3-MP- and thiosemicarbazide-induced seizures. This suggests that compounds **3n**, **3p** and **3r** may activate GAD or inhibit aminotransferase (GABA-T) in the brain.

Conclusion

In conclusion, compounds **3n** (5-(2-chlorophenoxy)-[1,2,4]triazolo[4,3-*a*]pyridine) and **3p** (5-(4-chlorophenoxy)-[1,2,4]triazolo[4,3-*a*]pyridine) were found to possess the most potent anticonvulsant activity among the synthetic derivatives whereas compound **3r** (5-(4-bromophenoxy)-[1,2,4]triazolo[4,3-*a*]pyridine) possessed strong anticonvulsant activity combined with the highest PI value of the assayed compounds. Compounds **3n**, **3p** and **3r** showed antagonistic activity against seizures induced by PTZ, ISN, 3-MP and thiosemicarbazide. These experiments suggest that compounds **3n**, **3p** and **3r** may activate GAD or inhibit GABA-T, thereby enhancing GABAergic neurotransmission.

Acknowledgement

This work was supported by the National Natural Science Foundation of China (No. 30960458) and the Natural Science Foundation of Zhejiang Province of China (No. LY12C19005).

Conflicts of Interest

We declare that we have no conflict of interest with respect to this study.

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