

Synthesis and Studies on Anticonvulsant Activity of 8-Alkoxy-[1,2,4]triazolo[4,3-a]pyrazine

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In this study, a series of new 8-alkoxy-[1,2,4]triazolo[4,3-a]pyrazine derivatives were synthesized and their anticonvulsant activity and neurotoxicity were evaluated by the maximal electroshock test and the rotarod test, respectively. The most promising compounds **3d** (8-butoxy-[1,2,4]triazolo[4,3-a]pyrazine) and **3f** (8-hexyloxy-[1,2,4]triazolo[4,3-a]pyrazine) showed a median effective dose of 44 and 35.3 mg/kg and had protective index value of 3.2 and 4.8, respectively. To explain the possible mechanism of anticonvulsant activity, all compounds were tested in chemical induced model in anti pentylenetetrazol test.

Key Words: [1,2,4]Triazolo[4,3-a]pyrazine, Anticonvulsant activity, Maximal electroshock test, Pentylenetetrazol.

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 brain¹. Anti-convulsant 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 anemia²⁻⁴ and even life-threatening conditions⁵. Therefore the search for safer and more effective antiepileptic drugs is necessary and the development of these drugs has been important and challenging for medicinal chemists to develop new antiepileptic drugs with desirable therapeutic properties.

In the previous study, we reported the synthesis and anti-convulsant activities of 7-alkoxy-4*H*-[1,2,4]triazolo[4,3-d]-benzo[*b*]-[1,4]thiazines (**pre. 1'**)⁶ and 6-alkoxy-[1,2,4]triazolo[4,3-*b*]pyridazine (**pre. 2'**)⁷. From compounds **pre. 1** to compounds **pre. 1'** and from compounds **pre. 2** to compounds **pre. 2'**, in which we incorporated triazole ring at the first and second position for compounds **pre. 1** and at the second and third position for compounds **pre. 2** (Fig. 1). The pharmacology test showed the anticonvulsant activity increase when incorporated triazole ring. It may have higher affinity for the receptor and enhance anticonvulsant activity, after the triazole ring was introduced and there were some similar design reports⁸. In addition, Chau *et al.*⁹, carried out a similar docking experiment with the imidazobenzodiazepine and alprazolam. Alprazolam is an agonist benzodiazepine, like flunitrazepam,

but its activity was higher than that of flunitrazepam. With the introduction of a triazole ring to the first and second position of flunitrazepam, the physical space occupied by this imidazobenzodiazepine is similar to that found for flunitrazepam, but it has a significantly higher affinity for the recognition site (Fig. 1).

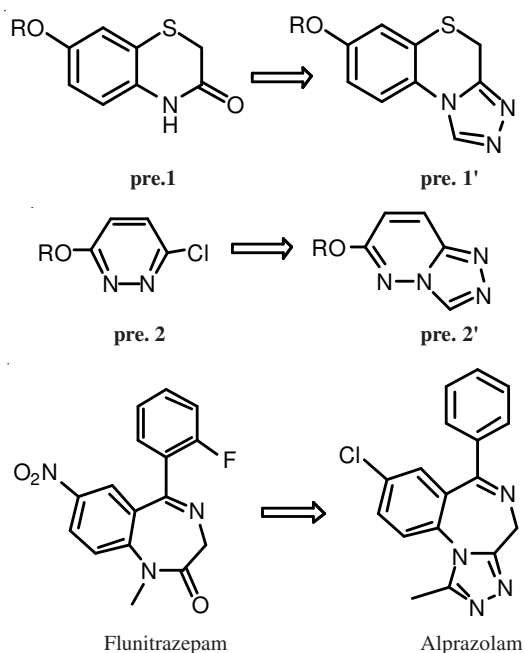


Fig. 1. Structure of previous studied compounds, **1**, **2**, flunitrazepam and alprazolam

In an attempt to discover a novel anticonvulsant compound with better activity, keeping the above-mentioned in view, compound **I** (8-phenoxy-[1,2,4]triazolo[4,3-a]pyrazine) was designed and synthesized as the leading compound to investigate the contribution of different alkoxy groups at position 8 of the 1,2,4-triazolo[4,3-a]pyrazine to obtain a series of 8-alkoxy-[1,2,4]triazolo[4,3-a]pyrazine derivatives (Fig. 2). Their structures were characterized using IR, ^1H NMR and MS. Their anticonvulsant activity was evaluated using the maximal electroshock (MES) test and reported for the first time. Their neurotoxicity was evaluated using the rotarod test in mice. In this contribution, to explain the possible mechanism of action, compound **3a-s** was tested in the pentylenetetrazol (PTZ) test. Under identical conditions, the anti pentylenetetrazol activity of the marketed agent carbamazepine was evaluated as a comparison.

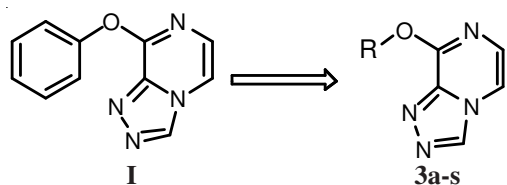


Fig. 2. Structure of compounds **I** and **3a-s**

EXPERIMENTAL

Melting points were determined in open capillary tubes and were uncorrected. IR spectra were recorded (in KBr) on a FT-IR1730 (Bruker, Switzerland), ^1H NMR was measured on an AV-300 (Bruker, Switzerland) and all chemical shifts were given in ppm relative to tetramethylsilane. Mass spectra were measured on an HP1100LC (Agilent Technologies, USA). The major chemicals were purchased from Alderich Chemical Corporation. All other chemicals were the analytical grade.

Synthesis of 1-hydrazino-4-chloropyrazine (1): To a solution of hydrazine hydrate (3.04 g, 62.8 mmol) in 20 mL of ethanol, the solution of 2,3-dichloropyrazine (2.50 g, 12.6 mmol) in 30 mL ethanol was added dropwise at room temperature. The mixture was stirred and refluxed for 1 h, then, the half of the solvent was removed under reduced pressure and the solution was poured into petroleum ether. The precipitate was filtered and washed with petroleum ether, then kept below 0°C . The resulting crude intermediates were used for the next step¹⁰.

Synthesis of 8-chloro-[1,2,4]triazolo[4,3-a]pyrazine (2): Compound **1** (4 mmol), formic acid (4 mmol) and ethanol 30 mL was placed in a 50 mL round-bottomed flask. The mixture was stirred for 2 h at room temperature to obtain the acid intermediate. The mixture was evaporated under reduced pressure. The acid intermediate was reacted in refluxing 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. Yield 47.6 %. ^1H NMR (DMSO) δ : 7.83 (1H, d, $J = 4.64$ Hz, pyrazine-H), 8.70 (1H, d, $J = 4.64$ Hz, pyrazine-H), 9.61 (1H, s, triazolo-H). MS ($M + 1$): 155.

General procedure for the synthesis of 8-alkoxy-[1,2,4]triazolo[4,3-a]pyrazine (3a-s): Compound **2** (0.5 g,

3.2 mmol) and appropriate 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 with stirring and refluxing for 3-5 h. After the solvent was removed under reduced pressure, the solid residue was purified by silica gel chromatography with dichloromethane:methanol (20:1). The yield, melting point, spectral data of each compound is given below⁷.

8-Methoxy-[1,2,4]triazolo[4,3-a]pyrazine (3a): Yield: 83 %; m.p.: $152-154^\circ\text{C}$. ^1H NMR (CDCl_3): δ 8.90 (1H, s, triazolo-H), 7.75 (1H, d, $J = 4.68$ Hz, pyrazine-H), 7.42 (1H, d, $J = 4.68$ Hz, pyrazine-H), 4.21 (3H, s, $-\text{OCH}_3$). IR (KBr, ν_{max} , cm^{-1}): 1623 (C=N), 1258, 1031 (C-O-C), 1128 (N-N). MS m/z : 151 ($M + 1$).

8-Ethoxy-[1,2,4]triazolo[4,3-a]pyrazine (3b): Yield: 78 %; m.p. $134-136^\circ\text{C}$. ^1H NMR (CDCl_3): δ 8.87 (1H, s, triazolo-H), 7.73 (1H, d, $J = 4.64$ Hz, pyrazine-H), 7.40 (1H, d, $J = 4.64$ Hz, pyrazine-H), 4.66 (2H, p, $-\text{OCH}_2$), 1.55 (3H, t, $-\text{CH}_3$). IR (KBr, ν_{max} , cm^{-1}): 1623 (C=N), 1254, 1030 (C-O-C), 1127 (N-N). MS m/z : 165 ($M + 1$).

8-Isopropoxy-[1,2,4]triazolo[4,3-a]pyrazine (3c): Yield: 75 %; m.p. $116-118^\circ\text{C}$. ^1H NMR (CDCl_3): δ 8.85 (1H, s, triazolo-H), 7.70 (1H, d, $J = 4.74$ Hz, pyrazine-H), 7.40 (1H, d, $J = 4.74$ Hz, pyrazine-H), 5.59 (1H, m, $-\text{OCH}$), 1.50 (3H, s, $-\text{CH}_3$), 1.52 (3H, s, $-\text{CH}_3$). IR (KBr, ν_{max} , cm^{-1}): 1623 (C=N), 1252, 1034 (C-O-C), 1126 (N-N). MS m/z : 179 ($M + 1$).

8-Butoxy-[1,2,4]triazolo[4,3-a]pyrazine (3d): Yield: 72 %; m.p. $104-106^\circ\text{C}$. ^1H NMR (CDCl_3): δ 8.88 (1H, s, triazolo-H), 7.72 (1H, d, $J = 4.71$ Hz, pyrazine-H), 7.40 (1H, d, $J = 4.71$ Hz, pyrazine-H), 4.58 (2H, t, $-\text{OCH}_2$), 1.51-1.94 (4H, m, $-(\text{CH}_2)_2$), 1.00 (3H, t, $-\text{CH}_3$). IR (KBr, ν_{max} , cm^{-1}): 1622 (C=N), 1253, 1033 (C-O-C), 1126 (N-N). MS m/z : 193 ($M + 1$).

8-Pentyloxy-[1,2,4]triazolo[4,3-a]pyrazine (3e): Yield: 71 %; m.p. $58-60^\circ\text{C}$. ^1H NMR (CDCl_3): δ 8.84 (1H, s, triazolo-H), 7.70 (1H, d, $J = 4.74$ Hz, pyrazine-H), 7.39 (1H, d, $J = 4.74$ Hz, pyrazine-H), 4.57 (2H, t, $-\text{OCH}_2$), 1.11-1.72 (6H, m, $-(\text{CH}_2)_3$), 0.95 (3H, t, $-\text{CH}_3$). IR (KBr, ν_{max} , cm^{-1}): 1622 (C=N), 1251, 1034 (C-O-C), 1127 (N-N). MS m/z : 207 ($M + 1$).

8-Hexyloxy-[1,2,4]triazolo[4,3-a]pyrazine (3f): Yield: 69 %; m.p. $102-104^\circ\text{C}$. ^1H NMR (CDCl_3): δ 8.93 (1H, s, triazolo-H), 7.81 (1H, d, $J = 4.71$ Hz, pyrazine-H), 7.40 (1H, d, $J = 4.71$ Hz, pyridazine-H), 4.57 (2H, t, $-\text{OCH}_2$), 1.32-1.94 (8H, m, $-(\text{CH}_2)_4$), 0.90 (3H, t, $-\text{CH}_3$). IR (KBr, ν_{max} , cm^{-1}): 1624 (C=N), 1252, 1034 (C-O-C), 1127 (N-N). MS m/z : 221 ($M + 1$).

8-Heptyloxy-[1,2,4]triazolo[4,3-a]pyrazine (3g): Yield: 76 %; m.p. $110-112^\circ\text{C}$. ^1H NMR (CDCl_3): δ 8.85 (1H, s, triazolo-H), 7.71 (1H, d, $J = 4.74$ Hz, pyrazine-H), 7.39 (1H, d, $J = 4.74$ Hz, pyrazine-H), 4.57 (2H, t, $-\text{OCH}_2$), 1.30-1.97 (10H, m, $-(\text{CH}_2)_5$), 0.89 (3H, t, $-\text{CH}_3$). IR (KBr, ν_{max} , cm^{-1}): 1622 (C=N), 1251, 1036 (C-O-C), 1127 (N-N). MS m/z : 235 ($M + 1$).

8-Octyloxy-[1,2,4]triazolo[4,3-a]pyrazine (3h): Yield: 80 %; m.p. $112-114^\circ\text{C}$. ^1H NMR (CDCl_3): δ 8.86 (1H, s, triazolo-H), 8.11 (1H, d, $J = 4.76$ Hz, pyrazine-H), 7.07 (1H, d, $J = 4.76$ Hz, pyrazine-H), 4.57 (2H, t, $-\text{OCH}_2$), 1.29-1.97 (12H, m, $-(\text{CH}_2)_6$), 0.88 (3H, t, $-\text{CH}_3$). IR (KBr, ν_{max} , cm^{-1}): 1623 (C=N), 1251, 1034 (C-O-C), 1124 (N-N). MS m/z : 249 ($M + 1$).

8-(2-Tolyloxy)-[1,2,4]triazolo[4,3-a]pyrazine (3i): Yield: 81 %; m.p. 136-138 °C. ¹H NMR (CDCl₃): δ 8.96 (1H, s, triazolo-H), 7.77 (1H, d, *J* = 4.77 Hz, pyrazine -H), 7.36 (1H, d, *J* = 4.77 Hz, pyrazine-H), 7.20-7.32 (4H, m, -C₆H₄), 2.24 (3H, s, -CH₃). IR (KBr, ν_{max}, cm⁻¹): 1623 (C=N), 1254, 1034 (C-O-C), 1126 (N-N). MS *m/z*: 227 (*M* + 1).

8-(3-Tolyloxy)-[1,2,4]triazolo[4,3-a]pyrazine (3j): Yield: 83 %; m.p. 142-144 °C. ¹H NMR (CDCl₃): δ 8.97 (1H, s, triazolo-H), 7.82 (1H, d, *J* = 4.70 Hz, pyrazine-H), 7.38 (1H, d, *J* = 4.70 Hz, pyrazine-H), 7.09-7.34 (4H, m, -C₆H₄), 2.42 (3H, s, -CH₃). IR (KBr, ν_{max}, cm⁻¹): 1622 (C=N), 1251, 1032 (C-O-C), 1126 (N-N). MS *m/z*: 227(*M* + 1).

8-(4-Tolyloxy)-[1,2,4]triazolo[4,3-a]pyrazine(3k): Yield: 82 %; m.p. 224-226 °C. ¹H NMR (CDCl₃): δ 8.94 (1H, s, triazolo-H), 7.78 (1H, d, *J* = 4.71 Hz, pyrazine-H), 7.37 (1H, d, *J* = 4.71 Hz, pyrazine-H), 7.18-7.30 (4H, m, -C₆H₄), 2.40 (3H, s, -CH₃). IR (KBr, ν_{max}, cm⁻¹): 1622 (C=N), 1251, 1032 (C-O-C), 1127 (N-N). MS *m/z*: 227(*M* + 1).

8-(2-Methoxyphenoxy)-[1,2,4]triazolo[4,3-a]pyrazine (3l): Yield: 77 %; m.p. 174-176 °C. ¹H NMR (CDCl₃): δ 8.03 (1H, s, triazolo-H), 7.87 (1H, d, *J* = 4.70 Hz, pyrazine-H), 7.35 (1H, d, *J* = 4.70 Hz, pyrazine-H), 7.02-7.30 (4H, m, -C₆H₄), 3.75 (3H, s, -OCH₃). IR (KBr, ν_{max}, cm⁻¹): 1623 (C=N), 1252, 1030 (C-O-C), 1126 (N-N). MS *m/z*: 243 (*M* + 1).

8-(4-Methoxyphenoxy)-[1,2,4]triazolo[4,3-a]pyrazine (3m): Yield: 74 %; m.p. 170-172 °C. ¹H NMR (CDCl₃): δ 8.96 (1H, s, triazolo-H), 7.80 (1H, d, *J* = 4.60 Hz, pyrazine-H), 7.38 (1H, d, *J* = 4.60 Hz, pyrazine-H), 6.98-7.27 (4H, m, -C₆H₄), 3.85 (3H, s, -OCH₃). IR (KBr, ν_{max}, cm⁻¹): 1623 (C=N), 1254, 1033 (C-O-C), 1127 (N-N). MS *m/z*: 243(*M* + 1).

8-(2-Chlorophenoxy)-[1,2,4]triazolo[4,3-a]pyrazine (3n): Yield: 61 %; m.p. 172-174 °C. ¹H NMR (CDCl₃): δ 9.00 (1H, s, triazolo-H), 7.86 (1H, d, *J* = 4.71 Hz, pyrazine-H), 7.35 (1H, d, *J* = 4.71 Hz, pyrazine-H), 7.25-7.54 (4H, m, -C₆H₄). IR (KBr, ν_{max}, cm⁻¹): 1622 (C=N), 1253, 1033 (C-O-C), 1126 (N-N). MS *m/z*: 247(*M* + 1).

8-(3-Chlorophenoxy)-[1,2,4]triazolo[4,3-a]pyrazine (3o): Yield: 65 %; m.p. 194-196 °C. ¹H NMR (CDCl₃): δ 8.97 (1H, s, triazolo-H), 7.83 (1H, d, *J* = 4.70 Hz, pyrazine-H), 7.38 (1H, d, *J* = 4.70 Hz, pyrazine-H), 7.23-7.45 (4H, m, -C₆H₄). IR (KBr, ν_{max}, cm⁻¹): 1622 (C=N), 1251, 1030 (C-O-C), 1127 (N-N). MS *m/z*: 247 (*M* + 1).

8-(4-Chlorophenoxy)-[1,2,4]triazolo[4,3-a]pyrazine (3p): Yield: 70 %; m.p. 250-252 °C. ¹H NMR (CDCl₃): δ 9.47 (1H, s, triazolo-H), 8.32 (1H, d, *J* = 4.74 Hz, pyrazine-H), 7.48 (1H, d, *J* = 4.74 Hz, pyrazine-H), 7.34-7.57 (4H, m, -C₆H₄). IR (KBr, ν_{max}, cm⁻¹): 1621 (C=N), 1250, 1031 (C-O-C), 1126 (N-N). MS *m/z*: 247 (*M* + 1).

8-(4-Fluorophenoxy)-[1,2,4]triazolo[4,3-a]pyrazine (3q): Yield: 58 %; m.p. 253-255 °C. ¹H NMR (CDCl₃): δ 9.46 (1H, s, triazolo-H), 8.31 (1H, d, *J* = 4.74 Hz, pyrazine-H), 7.42 (1H, d, *J* = 4.74 Hz, pyrazine-H), 7.32-7.40 (4H, m, -C₆H₄). IR (KBr, ν_{max}, cm⁻¹): 1622 (C=N), 1252, 1033 (C-O-C), 1127 (N-N). MS *m/z*: 231 (*M* + 1).

8-(4-Bromophenoxy)-[1,2,4]triazolo[4,3-a]pyrazine (3r): Yield: 72.1 %; m.p. 251-253 °C. ¹H NMR (CDCl₃): δ 8.98 (1H, s, triazolo-H), 8.13 (1H, d, *J* = 4.72 Hz, pyrazine-H), 7.37 (1H, d, *J* = 4.72 Hz, pyrazine-H), 7.21-7.62 (4H, m,

-C₆H₄). IR (KBr, ν_{max}, cm⁻¹): 1622 (C=N), 1250, 1032 (C-O-C), 1127 (N-N). MS *m/z*: 290 (*M* + 1).

8-(2,4-Dichlorophenoxy)-[1,2,4]triazolo[4,3-a]pyrazine (3s): Yield: 59 %; m.p. 255-257 °C. ¹H NMR (CDCl₃): δ 8.97 (1H, s, triazolo-H), 7.83 (1H, d, *J* = 4.68 Hz, pyrazine-H), 7.55 (1H, d, *J* = 4.68 Hz, pyrazine-H), 7.28-7.39 (3H, m, -C₆H₃). IR (KBr, ν_{max}, cm⁻¹): 1623 (C=N), 1254, 1030 (C-O-C), 1125 (N-N). MS *m/z*: 281 (*M* + 1).

Pharmacology: The maximal electroshock test and rotarod test were carried out according to procedures described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA)^{11,12}. All compounds, which were dissolved in polyethylene glycol-400, were evaluated for anticonvulsant activities in KunMing mice in the 18-25 g weight range. 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 plots of these data, the respective ED₅₀ and TD₅₀ values, 95 % confidence intervals, slopes of the regression line and the standard error of the slopes were calculated by computer program written by the National Institute of Neurological Disorders and Stroke.

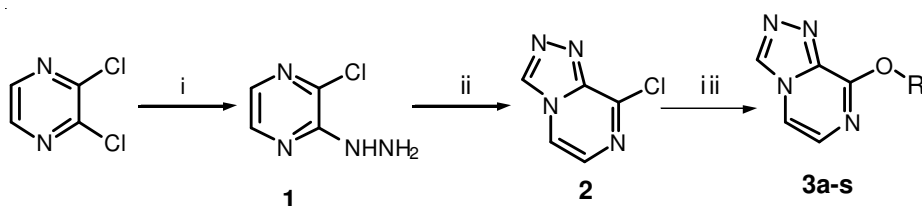
Maximal electroshock test: Seizures were elicited with a 60 Hz alternating current of 50 mA intensity in mice. The current was applied via corneal electrodes for 0.2 s. Protection against the spread of maximal electroshock-induced seizures was defined as the abolition of the hind leg and tonic maximal extension component of the seizure. The derivatives in maximal electroshock test were evaluated 15 min after administration of the compounds.

Rotarod test: After 15 min administration of the compounds (with different doses), the animals were tested on a 2.5 cm diameter knurled plastic rod rotating at 6 rpm for 1 min. Neurotoxicity was indicated by the inability of an animal to maintain equilibrium in each of three trials¹³.

Pentylenetetrazole-induced seizures: The doses used were: pentylenetetrazole, 85 mg/kg. The test compounds and standard AEDs were administered i.p. in a volume of 100 mg/kg to groups of 10 mice 0.5 h before s.c. injection of pentylenetetrazole. The mice were placed in individual cages and observed for 0.5 h, the number of clonic seizures, tonic seizures and the lethality were recorded¹⁴.

RESULTS AND DISCUSSION

Target compounds **3a-s** was prepared by three-step synthesis. As illustrated in **Scheme-I**, in the first step, the starting material 2,3-dichloropyrazine reacted with hydrazine hydrate in ethanol to afford¹⁰ compound **1**. The second step, compound **1** reacted further with formic acid in ethanol to obtain compound **2**. Finally, compound **2** reacted with appropriate alcohol and substituted phenol to produce the target compounds **3a-s**⁷. All the compounds were identified by spectral data. In general, IR spectra showed the C=N peak at 1624-1621 cm⁻¹ and the N-N peak at 1128-1124 cm⁻¹. In the nuclear magnetic resonance spectra (¹H 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 8.03-9.47 ppm.



R				
3a = -CH ₃	3e = -C ₅ H ₁₁	3i = 2-C ₆ H ₄ CH ₃	3m = 4-C ₆ H ₄ OCH ₃	3q = 4-C ₆ H ₄ F
3b = -C ₂ H ₅	3f = -C ₆ H ₁₃	3j = 3-C ₆ H ₄ CH ₃	3n = 2-C ₆ H ₄ Cl	3r = 4-C ₆ H ₄ Br
3c = -CH(CH ₃) ₂	3g = -C ₇ H ₁₅	3k = 4-C ₆ H ₄ CH ₃	3o = 3-C ₆ H ₄ Cl	3s = 2,4-C ₆ H ₃ Cl ₂
3d = -C ₄ H ₉	3h = -C ₈ H ₁₇	3l = 2-C ₆ H ₄ OCH ₃	3p = 4-C ₆ H ₄ Cl	–

Reagents:(i) NH₂NH₂·H₂O/C₂H₅OH, reflux; (ii) HCOOH/C₂H₅OH, reflux; (iii) alkanol or phenol, DMSO, NaOH or K₂CO₃.

Scheme-I: Synthesis route of compounds **3a-s**

Pharmacology: The anticonvulsant activity and neurotoxicity of the synthesized compounds **3a-s** were carried out according to procedures described in the Antiepileptic Drug Development Program (ADD) of the National Institutes of Health (USA)^{15,16}.

The initial evaluation of the anticonvulsant activity of synthesized compounds **3a-s** (Table-1) indicated that thirteen compounds displayed differently anticonvulsant activity, while compounds **3d**, **3f** possessed anticonvulsant activity against MES-induced seizure at 30 mg/kg, compound **3g** were active at 100 mg/kg. The rotarod toxicity test result indicated that all compounds did not show toxicity at 100 mg/kg.

In the phase-II pharmacology test, compounds **3d** and **3f** were quantitatively evaluated for anticonvulsant activity (indicated by ED₅₀) and neurotoxicity (indicated by TD₅₀) (Table-2) by intraperitoneal (i.p.) administration. The most promising compounds **3d** and **3f** showed a median effective dose of 44.0 and 35.3 mg/kg and had protective index value of 3.2 and 4.8, respectively, in which had better than the leading compound **I** (the dose of 300 mg/kg).

To further investigate the effect of anticonvulsant activity in chemical induced model, all compounds were tested against convulsions induced by chemical substances pentylenetetrazol. Compounds **3a-s** were administered (i.p.) into mice at 100 mg/kg. The reference drug carbamazepine was administered (i.p.) at 50 mg/kg.

As shown in Table-3, all compounds completely or partly inhibited the clonic seizures induced by sc-pentylenetetrazol, but the reference drug carbamazepine did not show any inhibition activity. Whereas compounds **3d**, **3i**, **3j** and **3o** inhibited the tonic seizures and death induced by pentylenetetrazol at values of 100 and 100 %, respectively, in which was similarly to the reference drug carbamazepine. Pentylenetetrazol have been reported to produce seizures by inhibiting

TABLE-1
PRIMARY EVALUATION OF COMPOUNDS **I**
AND **3a-s** IN ANTICONVULSANT ACTIVITY
(TEST DRUG ADMINISTERED i.p.)

Comp.	R	Dosage (mg/kg)	MES ^a		Rotarod ^b	
			0.5 h	4 h	0.5 h	4 h
I	-C ₆ H ₅	300	1/3	0/3	0/3	0/3 ^c
3a	-CH ₃	300	0/3	0/3	0/3	0/3
3b	-C ₂ H ₅	300	0/3	0/3	0/3	0/3
3c	-CH(CH ₃) ₂	300	0/3	0/3	0/3	0/3
3d	-C ₄ H ₉	30	1/3	0/3	0/3	0/3
3e	-C ₅ H ₁₁	300	1/3	0/3	0/3	0/3
3f	-C ₆ H ₁₃	30	1/3	0/3	0/3	0/3
3g	-C ₇ H ₁₅	100	1/3	0/3	0/3	0/3
3h	-C ₈ H ₁₇	300	1/3	0/3	0/3	0/3
3i	2-C ₆ H ₄ CH ₃	300	0/3	0/3	0/3	0/3
3j	3-C ₆ H ₄ CH ₃	300	0/3	0/3	0/3	0/3
3k	4-C ₆ H ₄ CH ₃	300	1/3	0/3	0/3	0/3
3l	2-C ₆ H ₄ OCH ₃	300	0/3	0/3	0/3	0/3
3m	4-C ₆ H ₄ OCH ₃	300	1/3	0/3	0/3	0/3
3n	2-C ₆ H ₄ Cl	300	1/3	0/3	0/3	0/3
3o	3-C ₆ H ₄ Cl	300	1/3	0/3	0/3	0/3
3p	4-C ₆ H ₄ Cl	300	1/3	0/3	0/3	0/3
3q	4-C ₆ H ₄ F	300	1/3	0/3	0/3	0/3
3r	4-C ₆ H ₄ Br	300	1/3	0/3	0/3	0/3
3s	2,4-C ₆ H ₃ Cl ₂	300	1/3	0/3	0/3	0/3

^aMaximal electroshock seizure test (number of animals protected/number of animals tested). ^bRotarod toxicity (number of animals exhibiting toxicity/number of animals tested). ^cCompounds are metabolized/excreted at 4 h.

aminobutyric acid (GABA) neurotransmission^{17,18}. GABA is the main inhibitory neurotransmitter in the brain and is widely

TABLE-2
PHASE-II QUANTITATIVE ANTICONVULSANT DATA IN MICE
(TEST DRUG ADMINISTERED VIA THE INTRAPERITONEAL ROUTE)

Compound	MES, ED ₅₀ ^a	TD ₅₀ ^b	PI ^c (TD ₅₀ /ED ₅₀)
3d	44.0 (36.7-52.8) ^d	141.4 (117.8-169.7)	3.2
3f	35.3 (28.3-44.1)	169.7 (141.4-203.7)	4.8
Phenobarbital	21.8 (21.8-25.5)	69 (62.8-72.9)	3.2

^aED₅₀: Median effective dose affording anticonvulsant protection in 50 % of animals, the dose is measured in mg/kg. ^bTD₅₀: Median toxic dose eliciting minimal neurological toxicity in 50 % of animals, the dose is measured in mg/kg. ^cPI: Protective index (TD₅₀/ED₅₀). ^d95 % confidence intervals given in parentheses.

TABLE-3
EFFECT OF COMPOUNDS **1** AND **2a-s** ON
PENTYLENETETRAZOL (PTZ) CONVULSION IN MICE

Compound	Dosage (mg/kg)	Test time (h)	Clonic seizures (%)	Tonic seizures (%)	Lethality (%)
1	100	0.5	0	100	100
3a	100	0.5	0	100	100
3b	100	0.5	0	100	60
3c	100	0.5	0	60	0
3d	100	0.5	0	0	0
3e	100	0.5	0	30	0
3f	100	0.5	60	100	0
3g	100	0.5	30	60	0
3h	100	0.5	30	60	0
3i	100	0.5	0	0	0
3j	100	0.5	0	0	0
3k	100	0.5	60	100	60
3l	100	0.5	0	60	60
3m	100	0.5	0	100	100
3n	100	0.5	0	60	60
3o	100	0.5	0	0	0
3p	100	0.5	0	100	100
3q	100	0.5	0	100	60
3r	100	0.5	0	100	60
3s	100	0.5	0	100	60
Control	—	0.5	100	100	40
Carbamazepine	50	0.5	100	0	0

implicated in epilepsy. Inhibition of GABAergic neurotransmission or activity has been shown to promote and facilitate seizures¹⁹, whereas enhancement of GABAergic neurotransmission is known to inhibit or attenuate seizures. The findings of the present study suggest that the newly synthesized compounds **3a-s** may have inhibited or attenuated pentylene-tetrazol-induced seizures in mice by enhancing GABAergic neurotransmission.

Conclusion

In the present study, we synthesized a series of 8-alkoxy-[1,2,4]triazolo[4,3-a]pyrazine derivatives and tested their anticonvulsant activity and neurotoxicity using the maximal

electroshock test, sc-PTZ. The only two compounds had better anticonvulsant activity. But all compounds showed antagonistic activity against seizures induced by pentylene-tetrazol. These experiments suggested that compound may activate GAD or inhibit GABA-T, thereby enhancing GABAergic neurotransmission.

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