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Synthesis and Anticonvulsant Activity of Some New 6,8-*Bis*(aryl)-5-methyl-6,7,8,9-tetrahydro-5*H*-tetrazolo [1,5-*d*][1,4]diazepines

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ABSTRACT Some new 6,8-*bis*(aryl)-5-methyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepines have been synthesized starting from piperidin-4-ones. Piperidin-4-ones were reacted with sodium azide to give seven-membered diazepan-5-ones, which on refluxing with sodium azide and phosphorous oxychloride yielded the desired tetrazolodiazepine derivatives. The structures of the compounds were confirmed by spectral (infrared, ^{1}H nuclear magnetic resonance [^{1}H NMR], ^{13}C NMR, and mass) and elemental analytical data. The anticonvulsant activity of the tetrazolodiazepines was tested by maximal electroshock method using phenytoin as the standard drug.

KEYWORDS Anticonvulsant activity, Diazepine, Maximal electroshock, Tetrazolodiazepines.

INTRODUCTION

The heterocyclic nuclei have remarkable ability to serve both as biomimetics and reactive pharmacophores. This property has contributed them to have unique value as traditional key elements of numerous drugs.[1] Piperidine compounds are reported to possess various biological activities such as antiviral, anti-tumor, analgesic, local anesthetic, antimicrobial, bactericidal, fungicidal, herbicidal, insecticidal, central nervous system (CNS) stimulant, and depressant activities.[2] The skeletal ring of piperidine nucleus is often found in the molecular framework of many synthetic and natural medicaments.[3] Furthermore, the significance of piperidin-4-ones as intermediates in the synthesis of a variety of physiologically active compounds has also been reviewed by Prostakov and Gaivoronskaya.[4] It has been recently reported that the hydrogen bonding capability of tetrazolic anions with receptor recognition sites is responsible for the key interaction of enhanced binding affinity. It has been found that the tetrazole substrates form two hydrogen bonds with peptide residues in a biological target site which is responsible for the strong binding interaction. Researchers[5] have proved that the tetrazole groups in drug molecules could improve the interaction of drugs with the receptors in cell membrane. From this view point, tetrazole is expected to be an alternative group to carboxylic acid in cation exchanger and the resulting exchanger might be used to investigate the interaction between tetrazole moieties in drugs and receptor.

Epilepsy is a common disorder affecting approximately 40 million people.[6] Specific cause cannot be determined for who are all have an epilepsy problem. Although there is no recognizable cause for this disease, it may develop as a consequence of various kinds of brain damage such as trauma, inhibition, or tumor growth. It is a neurological

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condition characterized by recurrent, paroxysmal abnormal episodes of motor, sensory, autonomic, or physic function. Epilepsy can affect anyone at any stage. However, the disease is more common in children than adults, with a prevalence rate between 4 and 8 per thousand children's below the age of 7 years. The characteristic event in epilepsy is seizure. Seizure is associated with the episodic high-frequency discharge of impulses by a group of neurons in the brain. It initially starts as a local abnormal discharge and then spread to other areas of the brain. The particular symptoms are produced depending on the function of the region of the brain that is affected. Thus, the involvement of the motor cortex causes convulsions, the involvement of the hypothalamus causes peripheral autonomic discharge, and the involvement of the reticular formation in the upper brain stem leads to loss of consciousness. Phenytoin[7] is the primary drug for all types of epilepsy except the absence of seizures. It exerts a selective antiepileptic action without causing drowsiness. The onset of action is low even on intravenous injection but the action persists for a considerable time after sedation or therapy.

RESULTS AND DISCUSSION

Chemical synthesis

New 6,8-*bis*(aryl)-5-methyl-6,7,8,9-tetrahydro-5*H*-tetrazolo[1,5-*d*][1,4]diazepines (**3a-g**) were synthesized in two steps starting from piperidin-4-ones (**1a-g**) as outlined in **Scheme 1**. The first step involved ring expansion of the piperidin-4-ones with sodium azide in the presence of H_2SO_4, thereby affording corresponding diazepan-4-ones (**2a-g**). Subsequently, **2a-g** underwent cyclization with $NaN_3/POCl_3$ to yield the target compounds. The structures of the compounds were confirmed by infrared, ^{1}H nuclear magnetic resonance (^{1}H NMR), ^{13}C NMR, mass, and elemental analyses.

Anticonvulsant activity

The results of the anticonvulsant activity of the tetrazolodiazepine derivatives are summarized in **Table 1.** The results of the study indicated that the synthesized compounds possess fairly good anticonvulsant activity. Introduction of electron donor group, such as methoxy group reduced the anticonvulsant activity, whereas the introduction of electron acceptor groups, such as chlorine, bromine, nitro, and hydroxyl to the phenyl ring has shown to increase the anticonvulsant activity. The chlorosubstituted derivative of tetrazolodiazepine (**3c**) showed maximum activity compared to the other tested compounds.

EXPERIMENTAL SECTION

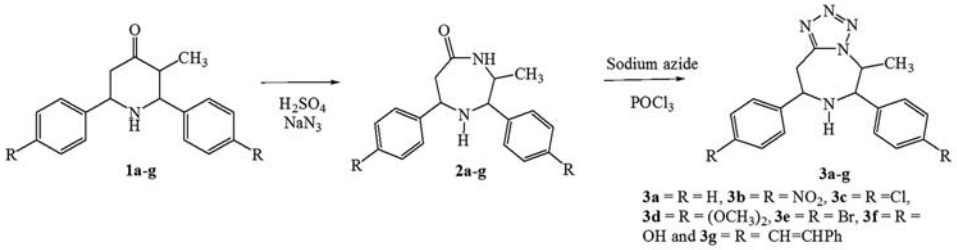
Infrared spectra were taken on a PerkinElmer Fourier transform IR (FTIR) 1600 spectrometer using KBr disks. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker spectrometer. Chemical shifts are given in ppm (δ) relative to tetramethylsilane as internal standard and coupling constants (*J*) are given in Hz. Melting points were measured by a digital melting point apparatus and were uncorrected. All reactions were followed by thin layer chromatography (TLC). The mass spectra were recorded on JEOL GCmate spectrometer. Synthesis of 2,6-*bis*-(substituted phenyl)-3-methyl-piperidin-4-ones (**1a-1g**) was effected according to the reported procedure.[8]

2,7-*Bis*(phenyl)-3-methyl-1,4-diazepan-4-one (2a)

The powdered 2,6-*bis*-(phenyl)-3-methyl-piperidin-4-one (**1a**) (2.65 g and 0.01 mol) was added in small portions to ice-cold concentrated H_2SO_4 (20 ml) in a conical flask equipped with a magnetic stirrer. After the addition was complete, the solution was allowed to equilibrate at room temperature. Sodium azide (0.64 g and 0.01 mol) was added in portions over a period of 1 h. After the addition was over, the solution was poured into crushed ice. The pH of the mixture was adjusted to ≈8.0 using 2N NaOH solution. The white solid separated was isolated by filtration. The crude product was recrystallized from ethanol. The same experimental procedure [9] was followed for the preparation of remaining target compounds **2b-2g**. Melting points of compound **2a-2g** are 90–92°C, 156–158°C, 165–167°C, 116–118°C, 186–188°C, 92–94°C, and 110–112°C.

6,8-*Bis*(phenyl)-5-methyl-6,7,8,9-tetrahydro-5*H*-tetrazolo [1,5-*d*][1,4]diazepine (3a)

A mixture of 2,7-*Bis*(phenyl)-3-methyl-1,4-diazepan-4-one (**2a**) (2.80 g and 0.01 mol), sodium azide (0.64 g and 0.01 mol), and phosphorus oxychloride (1.64 ml and 0.01 mol) in acetonitrile (20 ml) was heated under reflux for 7–10 h. After the completion of the reaction which was monitored by TLC (Hexane:Ethyl acetate; 2:8), the solvent was evaporated *in vacuo*. The residue was dissolved in water and subsequently neutralized with sodium bicarbonate. The precipitated crude product was filtered, dried, and recrystallized from ethanol.



Scheme 1: Synthesis of tetrazolodiazepine derivatives

Table 1: Anticonvulsant activity of tetrazolodiazepine derivatives against maximal electroshock seizures in rats

Groups	Flexion (Sec)	Extensor (Sec)	Clonus (Sec)	Stupor (Sec)
(a) ½ h after drug administration				
3a	3.4±0.2	7.5±1.01	13.5±2.1	95.6±10.1
3b	3.8±0.04	7.5±0.16	4.2±0.6	86.4±6.9
3c	3.5±0.08	6.8±0.8	16.4±1.4	99.4±12.3
3d	2.3±0.02	8.1±0.2	13.7±1.2	97.6±10.9
3e	4.2±0.3	7.3±0.06	12.5±1.7	135.2±13.6
Control	2.6±0.1	8.6±0.41	17.5±1.2	123.8±12.4
Standard	2.4±0.12	-----	5.8±0.9	58.5±3.6
(b) 1 h after drug administration				
3a	3.2±0.1	7.4±1.8	8.4±2.1	137.6±10.2
3b	2.6±0.3	7.6±1.2	11.4±1.9	93.4±9.6
3c	3.5±0.04	7.1±2.1	16.2±1.6	99.5±11.4
3d	3.1±0.2	8.2±0.98	14.6±1.3	128.6±10.7
3e	6.2±1.3	7.2±1.7	20.7±2.9	125.8±11.6
Control	2.4±0.22	8.3±0.64	7.8±2.2	107.6±8.5
Standard	2.1±0.14	----	4.6±1.1	46.8±4.6

6,8-Bis(phenyl)-5-methyl-6,7,8,9-tetrahydro-5H-tetrazolo [1,5-d][1,4]diazepine (3a)

Yield 86 %, m.p. 162-164 ºC. FTIR (KBr, cm^{-1}) ν_{max}: 3425 (-NH), 2922 (Ar-CH), 1643 (-C=N), 1450(-N=N), 1237 (N-N=N), 1095, 1023 (Tetrazole ring). ^{1}H NMR (500 MHz, dimethyl sulfoxide [DMSO-d_6]): δ 1.5 (d, *J* = 7 Hz, 3H, -CH_3), 3.35–3.40 (dd, 1H, H6b), 3.65 (dd, *J* = 2 Hz, 1H, H6a), 3.83 (d, *J* = 8.5 Hz, 1H, H2), 4.1 (dd, *J* = 2 Hz, 1H, H7), 4.81 (m, 1H, H3), 7.30–7.44 (m, 10H, Ar-H). ^{13}C NMR (DMSO-d_6, 125 MHz): 17.69, 32.03, 59.11, 63.06, 65.23, 126.49-129.36, 141.53, 143.80, 153.73. MS: m/z 305 [M+1]. Elemental analysis: Calculated: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.12; H, 6.94; N, 20.78%. Compounds **3b**-**3g** were prepared using similar method.

6,8-Bis-(3-nitrophenyl)-5-methyl-6,7,8,9-tetrahydro-5H-tetrazolo [1,5-d][1,4]diazepine (3b)

Yield: 75 %, m.p. 78–80 °C. FTIR (KBr, cm^{-1}) ν_{max}: 3415 (NH), 2923 (Ar-CH), 1649 (-C=N), 1383 (-N=N), 1097, 1022 (Tetrazole ring). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.5 (d, *J* = 1.5 Hz, 3H, CH_3), 3.7 (dd, *J* = 2 Hz, 1H, H6a,), 3.45 (dd, *J* = 10 Hz, 1H, H6b), 4.1 (d, *J* = 2 Hz, 1H, H2), 4.95 (m, 1H, H3), 4.3 (dd, *J* = 2 Hz, 1H, H7), 7.5 (m, 8H, Ar-H). ^{13}C NMR (DMSO-d_6, 125 MHz): 148.88, 148.29, 140.24, 139.36, 136.74, 135.94, 134.59, 134.28, 133.75, 131.17, 130.86, 130.59, 130.51, 124.92, 123.96, 123.60, 123.21, 122.60, 121.66, 22.25, 14.81. MS: m/z 395 [M+1]. Elemental analysis: Calculated: C, 55.86; H, 5.30; N, 22.66. Found: C, 55.83; H, 5.32; N, 22.68%.

6,8-Bis-(3-chlorophenyl)-5-methyl-6,7,8,9-tetrahydro-5H-tetrazolo [1,5-d][1,4]diazepine (3c)

Yield: 74 %, m.p. 110–112 °C. FTIR (KBr, cm^{-1}) ν_{max}: 3430 (-NH), 2923 (Ar-CH), 1598 (-C=N), 1468 (-N=N), 1128, 1023 (Tetrazole ring). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.5 (d, *J* = 7 Hz, 3H, CH_3), 3.61 (dd, *J* = 2 Hz, 1H, H6a), 3.31 (dd, *J* = 10 Hz, 1H, H6b), 3.38 (d, *J* = 8.5 Hz, 1H, H2), 4.04 (dd, *J* = 2 Hz, 1H, H7), 4.75 (m, 1H, H3), 7.3 (m, 8H, Ar-H). ^{13}C NMR (DMSO-d_6, 125 MHz): 153.90, 142.95, 141.08, 134.44, 134.11, 130.68, 130.25, 129.93, 128.27, 127.70, 127.55, 126.43, 125.92, 63.45, 62.26, 59.48, 32.01, 16.58. MS: m/z 476 [$M^{+\cdot}$], Elemental analysis: Calculated: C, 58.35; H, 5.54; N, 17.75. Found: C, 58.38; H, 5.61; N, 17.67 %.

6,8-Bis-(3,4-dimethoxyphenyl)-5-methyl-6,7,8,9-tetrahydro-5H-tetrazolo [1,5-d][1,4]diazepine (3d)

Yield: 72 %, m.p. 78–80 °C. FTIR (KBr, cm^{-1}) ν_{max}: 3437 (NH), 2921 (Ar-CH), 1615 (-C=N), 1420 (-N=N), 1091, 1025 (Tetrazole ring). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.5 (d, *J* = 6.5 Hz, 3H, CH_3), 3.62 (dd, *J* = 2 Hz 1H, H6a), 3.33 (dd, *J* = 10 Hz, 1H, H6b), 3.76 (d, *J* = 8.5 Hz, 1H, H2), 4.76 (m 1H, H3), 4.02 (dd, *J* = 2 Hz, 1H, 7H), 6.82–6.97 (m, 6H, Ar-H). ^{13}C NMR (DMSO-d_6, 125 MHz): 154.28, 149.44, 148.90, 135.74, 133.24, 119.84, 118.46, 111.36, 109.58, 68.62, 64.06, 61.29, 56.08, 55.98, 37.12, 17.42. MS: m/z 425 [M+1]. Elemental analysis: Calculated: C, 61.82; H, 6.92; N, 16.02.Found: C, 61.84; H, 6.96; N, 16.14%.

6,8-Bis-(4-bromophenyl)-5-methyl-6,7,8,9-tetrahydro-5H-tetrazolo [1,5-d][1,4]diazepine (3e)

Yield: 79 %, m.p. 88–90 °C. FTIR (KBr, cm^{-1}) ν_{max}: 3399 (NH), 2922 (Ar-CH), 1654 (-C=N), 1402 (-N=N), 1071, 1011 (Tetrazole ring), 821 (C-Br). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.5 (d, *J* = 7 Hz, 3H, CH_3), 3.82 (dd, *J* = 8.5 Hz 1H, H6a), 3.65 (dd, *J* = 1.5 Hz, 1H, H6b), 4.07 (dd, *J* = 2 Hz, 1H, H2), 4.3 (m, 1H, H3), 4.7 (m, 1H, H7), 7.5 (m, 8H, Ar-H). ^{13}C NMR (DMSO-d_6, 125 MHz): 142.49, 131.39, 131.18, 130.89, 129.58, 129.21, 128.69, 121.20, 120.34, 64.74, 59.57, 52.71, 14.73. MS: m/z 463 [$M^{+\cdot}$], Elemental analysis: Calculated: C, 49.13; H, 4.66; N, 14.95. Found: C, 49.04; H, 4.63; N, 14.98.

4,4'-(5-Methyl-6,7,8,9-tetrahydro-5H-tetrazolo[1,5-d][1,4] diazepine-6,8-diyl)diphenol (3f)

Yield: 69 %, m.p. 243–245 °C. FTIR (KBr, cm^{-1}) ν_{max}: 3449 (NH), 2931 (Ar-CH), 1642 (-C=N), 1392 (-N=N), 1095, 1010 (Tetrazole ring). ^{1}H NMR (125 MHz, DMSO-d_6): δ 1.5 (d, *J* = 7 Hz, 3H, CH_3), 3.62 (dd, *J* = 2 Hz 1H, H6a), 3.31 (dd, *J* = 10 Hz, 1H, H6b), 3.8 (d, *J* = 8.5 Hz, 1H, H2), 4.05 (dd, *J* = 2 Hz, 1H, 7H), 4.75 (m, 1H, H3), 7.3(m, 4H, *meta* to –OH), 7.52 (m, 4H, *ortho* to -OH). MS: m/z 337 [M+1]. Elemental analysis: Calculated: C, 63.29; H, 6.47; N, 19.25. Found: C, 63.13; H, 6.51; N, 19.22%.

6,8-Bis(styryl)-5-methyl-6,7,8,9-tetrahydro-5H-tetrazolo [1,5-d][1,4]diazepine (3g)

Yield: 70 %, m.p. 239–241 °C. FTIR (KBr, cm^{-1}) ν_{max}: 3411 (-NH) 2921 (Ar-CH), 1638 (-C=N), 1383 (-N=N), 1120, 1031 (Tetrazole ring). ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.24 (s, 3H, CH_3), 3.82 (s, 1H, H6a), 3.52 (s, 1H, H6b), 7.26 (m, 10H, Ar-H). MS: m/z 357 [M+1]. Elemental analysis: Calculated: C, 63.29; H, 6.47; N, 19.25. Found: C, 63.13; H, 6.51; N, 19.22%.

PHARMACOLOGY

Albino rats weighing 150–250 g and mice weighing 25–30 g of either sex were used for the study. They were housed six per cage under controlled temperature (22 ± 2°C) and humidity (55 ± 10%). The room was lighted from 7:00 a.m. to 7:00 p.m. and during the behavioral test. Food and water were available *ad libitum*. Animal experiments were conducted after obtaining animal ethical committee approval from the Institutional Animal Ethical Committee bearing the Ref. No. 265/CPCSEA given by the Government of India. Acute toxicity study was carried out as per the Organization of Economic Cooperation and Development (OECD) 423 guidelines.

Statistical analysis

The results were represented as mean ± standard error of mean. The difference between the control and test group was estimated by Student's *t*-test. The results were considered significantly when $P < 0.001$.

Acute toxicity study – OECD 423[10]

Three healthy young adult rats of either sex were fasted before dosing overnight. The test substance was administered in a single dose by gavage using an oral tube. 300 mg/kg body weight was chosen as the starting dose for all the test compounds. Animals were observed individually after dosing at least once during the first 30 min. Periodical observation was done for first 24 h and for a period of 14 days. Observations include changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and CNSs, somatomotor activity, and behavior pattern. Attention was given to the observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep, and coma.

Anticonvulsant activity by maximal electroshock method

Electroshock method[11] was followed to study the antiepileptic activity. Wistar rats of either sex (150–180 g) were used in this study. The positive control used was phenytoin (25 mg/kg orally). The control group was fed with the normal saline (5 ml/kg) orally. The remaining groups received 100 mg/kg of the test compounds (**3a, 3b, 3c, 3d,** and **3f**) orally. Supramaximal electroshock of 150 mA for 0.2 s by a Techno Convulsiometer was given to the rats. The animals which showed positive hind limb tonic extensor response during pre-screening were selected and the test and the standard drugs were injected intraperitoneally ½ h before the supramaximal shock. The abolition or reduction of the hind limb tonic extension component of the seizure was noted. The mean value for each group was calculated and compared with the control. The results were expressed as mean standard error. The test of significance was analyzed by Student's *t*-test. The percentage reduction in extensor phase was calculated.

CONCLUSION

Seven new tetrazolodiazepines were prepared from piperidones through diazepine derivatives. The anticonvulsant activity of the representative tetrazolodiazepine compounds was tested by maximal electroshock method. The results of the study indicated that the synthesized compounds possess fairly good anticonvulsant activity. The chlorosubstituted derivative of tetrazolodiazepine (**3c**) showed maximum activity compared to the other tested compounds.

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